MACQUARIE University
SYDNEY·AUSTRALIA

Hypothalamic control of blood pressure in polycystic kidney disease

Conor Francis Underwood, B.Med.Sci, M.Res

Faculty of Medicine and Health Sciences

Macquarie University

A thesis submitted to Macquarie University in fulfilment of the requirement for the degree of Doctor of Philosophy

Principle supervisor: Dr. Cara Hildreth

Secondary supervisor: Prof. Jacqueline Phillips

May 2019

Table of Contents

Abstract

Polycystic kidney disease (PKD) is characterised by the progressive accumulation of multiple bilateral renal cysts that threaten body-fluid homeostasis and glomerular filtration. In PKD patients, the development of hypertension and baroreflex dysfunction both contribute to a high risk of cardiovascular mortality, but the underlying mechanisms are poorly understood. This thesis examined the hypothesis that altered signalling within the hypothalamus and elevations in extracellular fluid osmolality contribute to hypertension and baroreflex dysfunction in a rat model of PKD, the Lewis Polycystic Kidney (LPK) rat.

The first goal of this thesis was to determine whether the ongoing activity of neurons in the hypothalamic paraventricular nucleus (PVN), a structure that regulates arterial pressure through multiple neural and humoral outputs, contributes to the maintenance of hypertension and baroreflex dysfunction in LPK rats in early and advanced stages of the disease. Acute pharmacological experiments in anaesthetised animals showed that an early increase in the glutamatergic excitation of PVN neurons maintains hypertension, but not baroreflex dysfunction, in LPK animals. This study also suggested that vasopressin release maintains a component of the hypertension in anaesthetised LPK rats but is independent of PVN neuronal activity and might therefore involve the supraoptic nucleus. However, additional work showed that while supraoptic nucleus vasopressin neurons are more active in LPK rats, chronic systemic inhibition of V_{1A} receptors is not anti-hypertensive.

Further studies examined the source of elevated PVN glutamatergic tone in LPK rats. The first hypothesis examined was that the local signalling of angiotensin II, a critical regulator of PVN glutamatergic tone, is enhanced in LPK rats. It was shown that the sensitivity but not tonicity of the angiotensin II type 1 receptor (AT1R) is enhanced in LPK rats and that its activation preferentially drives vasopressin release rather than sympathetic outflow via an indirect interaction with PVN astrocytes. Subsequent work determined whether heightened PVN glutamatergic tone is produced by a greater ongoing discharge of glutamatergic afferents in LPK rats. It was demonstrated that the subfornical organ (SFO), a sensory structure that detects plasma angiotensin II and osmolality levels, contains more activated PVN-projecting neurons in LPK animals and is the primary source of enhanced glutamatergic drive to the PVN in this disease model. However, PVN glutamatergic tone was not reduced by chronic suppression of AT1R (with pharmacology) or plasma hyperosmolality (via high-

water intake). High-water intake nevertheless provided novel cardiovascular benefits in LPK rats, including an improvement in hypertension and cardiac baroreflex function.

These experiments further our understanding of the origins of cardiovascular dysfunction in PKD, highlighting a SFO-PVN neuronal pathway as a novel therapeutic target to manage hypertension. The work encourages the clinical investigation into whether a prescribed increase in water consumption protects against the development of cardiovascular disease in PKD patients.

Declaration of Originality

I hereby declare that the work presented in this thesis has not been submitted for a higher degree to any other university or institution. To the best of my knowledge, the thesis contains no material previously published or written by another person except where due reference is stated. The contents of this thesis are the original experimental and written work of the candidate except where due acknowledgments are made. All animal experiments presented in this thesis were conducted with the approval of the Macquarie University Animal Ethics Committee (Authority Research Authority No.: 2015/001, 2017/015, 2018/016).

Conor Francis Underwood

Department of Biomedical Sciences
Faculty of Medicine and Health Sciences
Macquarie University

May 2019

Declaration of Contributions

Chapter 2

The candidate contributed to the conception and design of the experiments, performed all in-vivo experiments, analysed the data, interpreted the results and was the major contributor to the manuscript. Rochelle Boyd and Jacqueline Phillips performed the PCR experiments. Dr. Cara Hildreth and Prof. Jacqueline Phillips contributed to the conception and design of the experiments, interpretation of the results and revision of the manuscript.

Chapter 3

The candidate contributed to the conception and design of the experiments, performed all experiments, analysed the data, interpreted the results and was the major contributor to the manuscript. Dr. Ahmed Rahman assisted with in-vivo electrophysiological experiments. Dr. Natasha Kumar contributed to the design and optimisation of the in-situ hybridisation experiments. Dr. Simon McMullan assisted with the retrograde tracing experiments. Dr. Cara Hildreth and Prof. Jacqueline Phillips contributed to the conception and design of the experiments, interpretation of the results and revision of the manuscript. Assoc. Prof. Ann Goodchild contributed to the revision of the manuscript.

Chapter 4

The candidate contributed to the conception and design of the experiments, performed all in-vivo electrophysiological experiments, anatomical experiments, animal treatment procedures, analysed the data, interpreted the results and was the major contributor to the manuscript. Dr. Cara Hildreth implanted the radiotelemetry probes. Dr. Cara Hildreth, Prof. Jacqueline Phillips, Assoc. Prof. Simon McMullan and Assoc. Prof. Ann Goodchild contributed to the conception and design of the experiments, interpretation of the results and revision of the manuscript.

Chapter 5

The candidate contributed to the conception and design of the experiments, performed all experiments, analysed the data, interpreted the results and was the major contributor to the manuscript. Dr. Cara Hildreth implanted radiotelemetry probes and performed radiotelemetry

analysis. Dr. Cara Hildreth and Prof. Jacqueline Phillips contributed to the conception and design of the experiments, interpretation of the results and revision of the manuscript.

Acknowledgments

The completion of this thesis would not have been possible without the support provided by my supervisors, colleagues, friends and family.

First and foremost, I would like to thank my supervisor, Dr. Cara Hildreth. I consider myself extremely lucky to have had the opportunity acquire wisdom from you. Thank you for all the time you have dedicated to me over the years and for always being interested in my project and my ideas no matter how ludicrous.

I am extremely grateful for the efforts of my secondary supervisor, Prof. Jacqueline Phillips. Thank you firstly for stimulating my interest in this research area as an undergraduate and secondly for providing me with a range of diverse experiences throughout my candidature that will stick with me forever.

I would also like to extend my gratitude to departmental colleagues Assoc. Prof. Simon McMullan and Assoc. Prof. Ann Goodchild for their technical and intellectual inputs that greatly enhanced this thesis. I also greatly appreciate the technical support provided by my colleagues at UNSW, Dr. Natasha Kumar and Miss. Ayse Dereli – thank you for being so accommodating.

I am deeply thankful for my incredibly supportive family who pulled me through this journey. I am incredibly lucky to have been born to parents that appreciate the importance of following passions – you are my inspiration. To my partner, Caitlyn, thank you for tolerating me and all the encouragement that you have provided me over the years. Finally, thank you Master Otto Underwood – while you have deprived me of sleep in the past few months, you have enabled me to visualise a post-PhD life.

Publications

Publications arising from this thesis

Underwood CF, Boyd R, Phillips JK & Hildreth CM (2018). Increased excitatory regulation of the hypothalamic paraventricular nucleus and circulating vasopressin results in the hypertension observed in polycystic kidney disease. *Journal of Hypertension. **37**(1), 109-115.*

Underwood CF, Phillips JK & Hildreth CM (2018). Osmoregulation in polycystic kidney disease: relationship with cystogenesis and hypertension. *Annals of Nutrition and Metabolism. **72**(Suppl 2), 33-38.*

Publications during the period of candidature

Underwood CF, Hildreth CM, Wyse BF, Boyd R, Goodchild AK & Phillips JK (2016). Uraemia: an unrecognised driver of central neurohumoral dysfunction in chronic kidney disease? *Acta Phyiologica (Oxford).* **219**(1), 305-323.

Underwood CF, & Lynch EA (2017). Under what circumstances do rostral ventrolateral medulla neurons support blood pressure? *Journal of Neuroscience.* **37**(34), 8048-8050.

Communications

Underwood CF, Tallapragada VJ, Phillips JK & Hildreth CM (2016). Excitatory drive of the hypothalamic paraventricular nucleus maintains hypertension but not baroreflex dysfunction in chronic kidney disease, The 36th Annual Meeting of The Australasian Neuroscience Society, Hobart. [Poster Presentation]

Underwood CF, Boyd R, Phillips JK & Hildreth CM (2017). Increased excitatory regulation of the hypothalamic PVN and circulating vasopressin underlie the high blood pressure observed in polycystic kidney disease, Inter-university Neuroscience and Mental Health Conference, Sydney. [Oral Presentation]

Underwood CF, Phillips JK & Hildreth CM (2017). Increased excitatory regulation of the hypothalamic PVN and circulating vasopressin underlie the high blood pressure observed in polycystic kidney disease, Encourage, Macquarie University, Sydney. [Oral Presentation]

Underwood CF, Phillips JK & Hildreth CM (2017). Hypertension in polycystic kidney disease depends on vasopressin and the paraventricular nucleus: possible role of hydration-

sensing areas of the brain, The Hydration for Health 9th Annual Scientific Conference, Evian, FRANCE. [Oral Presentation]

Underwood CF, Phillips JK & Hildreth CM (2017). Elucidation of the central origins of enhanced vasopressin release in a rat model of polycystic kidney disease, Australia and New Zealand Society of Nephrology Annual Scientific Conference, Sydney. [Oral Presentation]

Underwood CF, Rahman AA, Phillips JK & Hildreth CM (2017). Angiotensin II regulation of blood pressure and sympathetic nerve activity at the level of the PVN in a rodent model of polycystic kidney disease, Central Cardiorespiratory Control: Future Directions conference, Sydney. [Poster Presentation]

Underwood CF, Rahman AA, Phillips JK & Hildreth CM (2017). Angiotensin II acts in the hypothalamic paraventricular nucleus to differentially regulate blood pressure and sympathetic nerve activity in a rodent model of polycystic kidney disease, Experimental Biology 2018, San Diego, USA. [Oral and Poster Presentation]

Underwood CF, McMullan S, Phillips JK & Hildreth CM (2019). Thirst centres in the brain contribute to hypertension in a rodent model of polycystic kidney disease. World Congress of Nephrology, Melbourne, Australia. [Oral Presentation]

Awards arising during the period of candidature

2017: Young Researcher Award, Hydration for Health 9th Annual Scientific Conference.

2017: 1st Place and People's Choice Award, Three-minute Thesis Competition, Faculty of Medicine and Health Sciences, Macquarie University.

2017: Postgraduate Research Fund Award, Macquarie University.

List of Figures

List of Tables

Abbreviations

ACE	Angiotensin converting enzyme
ACTH	Adrenocorticotropic hormone
ADPKD	Autosomal dominant polycystic kidney disease
Ang	Angiotensin
ARPKD	Autosomal recessive polycystic kidney disease
AT1R	AT1 receptor
BRS	Baroreflex sensitivity
BUN	Blood urea nitrogen
CKD	Chronic kidney disease
CNS	Central nervous system
CRH	Corticotrophin-releasing hormone
CTB	Cholera toxin subunit B
CVLM	Caudal ventrolateral medulla
DAB	3,3’-diaminobenzidine
DBH	Dopamine beta-hydroxylase
DBP	Diastolic blood pressure
DHK	Dihydrokainic acid
DMSO	Dimethyl sulfoxide
DOCA	Deoxycorticosterone
EPSCs	Excitatory post-synaptic currents
FISH	Fluorescent in-situ hybridisation
GFAP	Glial fibrillary acidic protein
GFR	Glomerular filtration rate
GLT-1	Glutamate transporter-1
HPA	Hypothalamic-pituitary-adrenal
HR	Heart rate
HWI	High-water intake
IP3	Inositol triphosphate
LPK	Lewis polycystic kidney
lSNA	Lumbar sympathetic nerve activity
LVI	Left ventricular mass index

MAP	Mean arterial pressure
MAPK	Mitogen-activated protein kinase
MnPO	median preoptic nucleus
Nek8	Never in mitosis gene A-related kinase 8
NTS	Nucleus of the solitary tract
OVLT	organum vasculosum of the lamina terminalis
PBS	Phosphate-buffered saline
PKD	Polycystic kidney disease
PP	Pulse pressure
PSPs	Post-synaptic potentials
PVN	Hypothalamic paraventricular nucleus
qPCR	Real time quantitative polymerase chain reaction
RAS	Renin-angiotensin system
ROS	Reactive oxygen species
rSNA	Renal sympathetic nerve activity
RVLM	Rostral ventrolateral medulla
SBP	Systolic blood pressure
SFO	Subfornical organ
SHR	Spontaneous hypertensive rat
SNA	Sympathetic nerve activity
SON	Supraoptic nucleus
sSNA	Splanchnic sympathetic nerve activity
TRH	Thyrotropin-releasing hormone

1.
Introduction

Part of this literature review has been published in Annals of Nutrition and Metabolism: Underwood CF, Phillips JK & Hildreth CM (2018). Osmoregulation in polycystic kidney disease: relationship with cystogenesis and hypertension. *Annals of Nutrition and Metabolism. **72**(Suppl 2), 33-38.*

Regulation of arterial pressure

Homeostasis requires that blood flow through different vascular beds is continuously adjusted according to the current and anticipated metabolic demands of all tissues. Two variables determine blood flow within a particular vascular bed: local resistance to flow and arterial blood pressure (Dampney et al., 2002). These variables are interrelated since arterial pressure is in turn the product of the total resistance to flow within the systemic circulation and cardiac output. The local resistance to flow is regulated by altering the luminal diameter of the blood vessels, principally the small arteries and arterioles, by adjusting the state of smooth muscle contraction (henceforth referred to as vasomotor tone) (Secomb, 2016). Vasomotor tone is autoregulated in response to fluctuations in local flow or metabolism, allowing for the vasculature to steal cardiac output as required in order to maintain adequate capillary perfusion over a wide range of arterial pressure and local metabolism (Clifford, 2011). Superimposing autoregulation is the integrative control of both regional vascular resistance and arterial pressure provided by various neurohumoral systems that act in the 'best interests' of the organism (De Hert, 2012). Chief among them is a class of noradrenergic post-ganglionic sympathetic neuron that maintain and continually adjust vasomotor tone whilst also influencing all determinants of cardiac output, including intravascular volume (Dampney et al., 2002). The neural control of the circulation is supported by several humoral systems, such as those originating from the kidneys (renin-angiotensin system [RAS]) and hypothalamus (vasopressin and hypothalamic-pituitary-adrenal [HPA] axis), that can be engaged to facilitate increases in vasomotor tone and cardiac output through direct actions or indirectly by modulating the gain of the sympathetic nervous system (Sapolsky et al., 2000; Sharshar et al., 2003; Mehta and Griendling, 2007).

Ambulatory recordings in healthy humans highlight that arterial pressure fluctuates significantly throughout the course of a day in relation to arousal, but the twenty-four hour average is constant over time (Drayer et al., 1985; Pickering, 1988). Arterial pressure is therefore thought to be regulated around a reference value or set-point according to metabolic requirements (Dampney et al., 2002). However, as noted by Kotas and Medzhitov (2015) physiological variables with adjustable set-points, such as arterial pressure, are particularly vulnerable to maladaptation precisely because they are adjustable and the variables that they control (e.g., tissue oxygenation) are not. The maladaptiveness of the arterial pressure set-point is exemplified by the high prevalence of hypertension, the single biggest contributor to

the global burden of disease (Collaborators, 2018). It is therefore paramount to understand what determines the set-point of arterial pressure.

Initial work of Guyton postulated that the arterial pressure set-point resided within the kidneys (Guyton, 1989). The central tenets of Guyton's model are that long-term arterial pressure is buffered by expansions or contractions in blood volume, and that sustained increases in arterial pressure can *only* result from an expansion of blood volume due to a shift in the renal set-point. Historically this model has been widely accepted and provides a simple explanation for why increases in dietary-salt consumption produce hypertension in some individuals (Weinberger, 1996). However, more recent work suggests that Guyton's model is incomplete as it does not consider the important role of the brain in the long-term control of vasomotor tone via its neural and humoral outflows (Averina et al., 2015). Indeed, there is compelling evidence that sympathetic outflow to muscle vascular beds, kidneys and heart is increased in hypertensive patients (Esler et al., 1989; Greenwood et al., 1999; Grassi et al., 2018), and that the sympathetic activation is casually-related to the hypertension because sympatholytic agents produce exaggerated decreases in arterial pressure in these individuals (Goldstein et al., 1985; Diedrich et al., 2003). Similarly, but less commonly, circulating vasopressin tends to be higher in hypertensives (Crofton et al., 1986; Bursztyn et al., 1990; Bakris et al., 1997; Zhang et al., 1999) and may contribute via its vasoconstrictor action to a component of the hypertension observed in individuals of certain racial backgrounds (i.e. African-Americans) (Bakris et al., 1997). Furthermore, work in experimental rodents demonstrates that during salt-sensitive hypertension, the brain detects plasma hypertonicity and/or hypernatremia and in turn increases arterial pressure via sympathetic and vasopressin outflows (O'Donaughy et al., 2006; Nomura et al., 2019). Importantly, this body of work also shows that if the brains capacity to survey the ionic concentrations of the blood is removed – i.e., via destruction of sensory forebrain structures (Berecek et al., 1982; Goto et al., 1982; Collister et al., 2013) or genetic deletion of the brain's sodium sensor (Nomura et al., 2019) – the development of salt-sensitive hypertension is either greatly attenuated or prevented altogether. Thus, the brain plays a fundamental role in setting the long-term level of arterial pressure. Increasing evidence suggests that the central nervous system (CNS) participates not only in salt-sensitive hypertension, but also in the hypertension associated with adiposity (Harlan et al., 2011; Lim et al., 2013) and inflammation (Shi et al., 2010; Korim et al., 2018). This thesis examines the CNS origins of hypertension in genetic (polycystic) kidney disease.

The CNS has several advantageous properties that make it an ideal regulator of long-term arterial pressure. Firstly, unlike the kidneys, which according to Guyton's model has blood volume as its sole controller, the brain has multiple neural and humoral outflows at its disposal to control various aspects of cardiovascular function (e.g., vasomotor tone, venous capacitance, cardiac function, renin section and volume status via urinary output but also consumption of water and salt) (Osborn, 2005). Secondly, only the CNS is capable of integrating diverse sensory inputs from internal and external sources and in turn deciding the 'appropriate' level of arterial pressure and/or distribution of cardiac output. Importantly, these stimulus-response pathways have flexible gains that enables the fine-tuning or sensitisation of responses to defend against recurring challenges (Johnson and Xue, 2018); for example, increases in circulating angiotensin II, such as that encountered during extracellular volume depletion (Leenen and Stricker, 1974), produces greater increases in arterial pressure if the CNS has been exposed to a low-dose of angiotensin II challenge previously (Xue et al., 2012). While no doubt beneficial in our evolutionary past, CNS plasticity almost certainly contributes to the development of hypertension (Johnson and Xue, 2018).

The following section will consider the neural and humoral mechanisms through which the brain, particularly the hypothalamus, regulates arterial pressure in normal and hypertensive conditions.

Neural control of arterial pressure

Organisation of the sympathetic nervous system

The sympathetic nervous system comprises cholinergic pre-ganglionic neurons and noradrenergic post-ganglionic neurons. The cell bodies of sympathetic pre-ganglionic neurons are topographically distributed in the thoracic and upper lumbar segments of the spinal cord, with most located in the intermediolateral cell group (Strack et al., 1989; Schramm et al., 1993). The axons of sympathetic pre-ganglionic neurons exit the ipsilateral ventral root of the most proximal spinal cord segment and form white rami on route to their paravertebral ganglia. Upon entry into the paravertebral ganglia, sympathetic pre-ganglionic neurons either synapse with post-ganglionic neurons or pass through and synapse in prevertebral ganglia proximal to the visceral target or in the adrenal gland (Langley, 1893; Wehrwein et al., 2016).

Sympathetic ganglia do not merely serve as a relay for sympathetic signals on route to the viscera. Rather, the anatomical and electrophysiological properties of sympathetic ganglia support the amplification and distribution of sympathetic outflow. In that regard, anatomical data suggest that there is high level of divergence in ganglionic neurotransmission, with post-ganglionic neurons greatly out-numbering preganglionic neurons (by a factor of ~15 in rat and ~200 in human) (Purves et al., 1986; McLachlan, 2003). Nevertheless, the fidelity of neurotransmission in sympathetic ganglia is high as intracellular recordings show that post-ganglionic neurons typically discharge in response to one strong excitatory input, whereas the summation of subthreshold inputs is relatively uncommon and fast-acting inhibitory input is absent (Ivanov and Purves, 1989; McLachlan et al., 1997). Nonetheless, the discharge of post-ganglionic sympathetic neurons is governed not only by cholinergic inputs (via post-synaptic nicotinic receptors) (Wehrwein et al., 2016), but also by various other neuromodulators (e.g. angiotensin II, enkephalin and neurotensin) (Brown et al., 1980; Konishi et al., 1981; Stapelfeldt and Szurszewski, 1989; Benarroch, 1994).

The neurovascular junction is arranged so as to facilitate coordinated vascular constriction. The unmyelinated axons of sympathetic post-ganglionic neurons do not make defined synaptic junctions with vascular smooth muscle cells; instead, the axons of sympathetic post-ganglionic neurons branch extensively and form multiple (up to 20,000 for a single neuron) varicosities that appose multiple neighbouring vascular smooth muscle cells (Burnstock, 2004). The noradrenaline released from these varicosities (but also circulating noradrenaline originating from the adrenal medulla or ‘spill-over’ from other visceral tissues) induces vasoconstriction via activation of post-synaptic α_1 and, in some cutaneous beds, α_2 receptors (Polonia et al., 1985; Guimaraes and Moura, 2001). In turn, vascular smooth muscle cells are electrically coupled to one-another through low resistance gap junctions, thus enabling a spread of contraction along the vascular wall (Welsh and Segal, 1998).

Properties of sympathetic nerves

Cardiovascular sympathetic efferents (i.e., those that supply the vasculature, heart, kidneys and adrenal gland) fall into three categories: thermosensitive, glucosensitive or barosensitive (Guyenet, 2006). Thermosensitive efferents are comprised mostly of cutaneous vasoconstrictor neurons and are stimulated most prominently by hypothermia, but also hyperventilation and emotional stimuli (Janig and Habler, 2003). Glucosensitive efferents are responsible for the release of adrenaline from the adrenal medulla and are activated by hypoglycaemia and exercise (Morrison and Cao, 2000). The final group, barosensitive

efferents, are the most expansive group; they supply the non-cutaneous resistance arterioles, veins, kidneys, heart and adrenal chromaffin cells that secrete noradrenaline, and are characterised by a discharge pattern that varies with the arterial pulse and fluctuations in blood pressure (Pang, 2001; Guyenet, 2006). Barosensitive cardiovascular efferents are the most important group of cardiovascular efferents for the regulation of arterial pressure and therefore will be considered more closely beneath.

Barosensitive sympathetic nerves display a tonic level of activity that contributes to a significant degree of 'vasomotor tone'. This was the seminal finding of Claude-Bernard (1851) and Brown-Séquard (1852) who observed that sectioning of a sympathetic nerve in the neck of rabbits lead to an increase in ear artery diameter and local blood flow (cited by Coote (2007)). A level of tone is important because it allows the sympathetic nervous system to bidirectionally alter the distribution of cardiac output to different vascular beds. For example, sympathetic nerve outflow to the leg muscles decreases during arousal but increases after ingestion of a meal (Fagius and Berne, 1994; Donadio et al., 2002), reflecting the required level of blood flow to the legs during each respective condition. Generalised activation of sympathetic vasomotor tone is rare, except perhaps during acute physiological stress (Dampney et al., 2008) and certain disease states such as heart failure (Rundqvist et al., 1997).

Adrian et al. (1932) were the first to record from whole sympathetic nerves supplying vasomotor targets in mammals (rabbits and cats). Though these early recordings had very poor signal-to-noise, they nevertheless revealed the characteristic firing pattern of sympathetic vasomotor nerves: the discharge is "persistent" (i.e., tonic), with bursts that are entrained to the cardiac cycle, respiration and fluctuations in blood pressure. This characteristic pattern of activity has been observed in multiple vasomotor (and cardiac) sympathetic nerves and in every mammalian species examined (Ling et al., 1998; Miki et al., 2004; Ramchandra et al., 2013; Salman et al., 2015b; Kobuch et al., 2018).

Single-unit recordings of post-ganglionic sympathetic neurons supplying vasomotor targets show that they typically fire at a slower rate than the 'bursts' observed in multifibre recordings, although cardiac and respiratory rhythmicity is still observed when averaged over multiple cardiac or respiratory cycles (Macefield et al., 1994; Burke et al., 2016). The 'bursting' of whole sympathetic nerves therefore most likely reflects the summed synchronous firing of a large number of neurons whose axons are contained within the nerve

bundle. Consequently, a change in the pattern of discharge across the entire pool of individual units contained within a sympathetic nerve differentially affects the frequency and amplitude of bursts (Ramchandra et al., 2009; Burke et al., 2016), with the former representing the rate of discharge of active units whereas the later indicates changes in the number of fibres recruited (Malpas, 2010). Sympathetic nerve bursts are thought to enhance the efficiency of sympathetic transmission in two regards. Firstly, bursts in preganglionic nerves may increase the probability of action potentials in sympathetic ganglia through the summation of weak synaptic inputs (McAllen and Malpas, 1997). Secondly, bursts of discharge appear to enhance neurovascular transduction, with classic studies showing that field stimulation of isolated vessels evokes greater vasoconstrictor responses when the impulses are triggered off a recorded sympathetic nerve activity (SNA) waveform compared to being delivered at a constant frequency (Nilsson et al., 1985; Sjoblom-Widfeldt and Nilsson, 1990).

There is functional heterogeneity in individual neurons whose axons make up sympathetic nerve bundles. In the rat, the most commonly used species for cardiovascular investigations, multifibre SNA is generally recorded from branches of the renal, splanchnic, lumbar and adrenal nerves. The general functions and properties of these nerves are described in Table 1.1.

Table 1.1: General functions and properties of the major 'cardiovascular' sympathetic nerves in the rat.

Nerve	Pre-/post-ganglionic	Class of efferent	Functions	Reference
Renal	Post	Barosensitive	Renal blood flow, renin release and sodium reabsorption.	(Scislo et al., 1998; DiBona, 2005)
Splanchnic	Pre or post depending on recording site	Barosensitive	Blood flow in the splanchnic circulation and gastrointestinal and reproductive functions. Can contain some adrenal fibres.	(Morrison, 2001)
Lumbar	Mostly post	Barosensitive and thermosensitive	Blood flow to muscle and (to a lesser extent) cutaneous beds in the hind-limbs.	(Scislo et al., 1998; Kenney et al., 1999; Montano et al., 2009)
Adrenal	Mostly pre	Glucosensitive (adrenaline-regulating neurons) and barosensitive (noradrenaline-regulating neurons)	Pre-ganglionic fibres innervate chromaffin cells that secrete adrenaline (80% of fibres) and noradrenaline. Post-ganglionic fibres control adrenal blood flow and modulate cortical functions.	(Carlsson et al., 1992; Morrison, 2001)

The origins of sympathetic tone

Presympathetic neurons

Early experiments in cats showed that rather than being intrinsically active, the sympathetic nervous system requires input from the brain in order to generate its activity because: firstly, the action potentials of sympathetic preganglionic neurons are always preceded by an excitatory post-synaptic potential (Dembowsky et al., 1985); and secondly, separating the spinal cord from the brain (via cooling of the medulla) virtually eliminates SNA (Coote and Downman, 1966). A series of landmark anatomical studies led by Loewy described the distribution of neurons in the brain that supply multiple sympathetic targets by utilising a modified rabies virus that is transported across synapses in a retrograde direction at a mostly predictable rate (Strack et al., 1989; Schramm et al., 1993; Geerling et al., 2003). Their experiments indicated that 'presympathetic' neurons that supply the kidneys, splanchnic and hindlimb vasculature, adrenal gland and heart are located in six principle regions; the hypothalamic paraventricular nucleus (PVN), rostral ventrolateral medulla (RVLM), rostral ventromedial medulla, A5 catecholaminergic cell group, caudal raphe nuclei and lateral hypothalamic/perifornical area (Figure 1.1). Interestingly, these regions contain neurons that supply both the heart and adrenal gland simultaneously, presumably via collateral projections to sympathetic pre-ganglionic neurons in different spinal segments (Jansen et al., 1995; Geerling et al., 2003). This important finding indicates that these presympathetic regions contain so called 'command neurons' that are hard-wired to generate specific patterns of sympathetic outflow.

Functional data indicate that these six presympathetic regions are not equal in their capacity to drive barosensitive efferents. Studies using chemical activation of neurons in each region (e.g. with glutamate) suggest that the PVN (Porter, 1988; Kannan et al., 1989; Martin et al., 1991; Martin et al., 1997; Haselton and Vari, 1998; Deering and Coote, 2000; Kenney et al., 2001; Page et al., 2011; Mendonca et al., 2018), RVLM (Dampney et al., 1982; Ross et al., 1984; Morrison, 1999; Ramchandra et al., 2013), A5 cell group (Stanek et al., 1984; Huangfu et al., 1992; Maiorov et al., 1999) and lateral hypothalamus (Allen and Cechetto, 1993) have a strong capacity to modulate outflows to the resistance vasculature and kidneys, whereas the raphe and rostral ventromedial medulla preferentially drive thermoregulatory outflows (e.g., to the tail, brown adipose tissue and sweat glands) (Morrison, 1999; Rathner and McAllen, 1999; Nakamura et al., 2005; Cao and Morrison, 2006; Shafton and McAllen, 2013), with a

population of raphe neurons identified to drive cardiac sympathetic outflow as well (Zaretsky et al., 2003; Salo et al., 2009).

By extending the survival time of rabies-inoculated animals, trans-neuronal tracing has also been used to identify higher-order neurons antecedent to presympathetic neurons. These studies confirm that the vasculature, heart, kidneys and adrenal gland receive polysynaptic input from neurons located in several central structures strongly suggested to provide sensory input to barosensitive sympathetic efferents on the basis of prior functional data (Sly et al., 1999; Westerhaus and Loewy, 1999; Gao et al., 2014). These include the forebrain lamina terminalis, a region which contains sensory circumventricular organs that detect haemal stimuli such as blood osmolality and angiotensin II levels (McKinley et al., 2001), and the nucleus of the solitary tract (NTS), the so called 'viscerosensory gateway' of the CNS, where visceral cranial afferents terminate (Llewellyn-Smith and Verberne, 2011) (Figure 1.1).

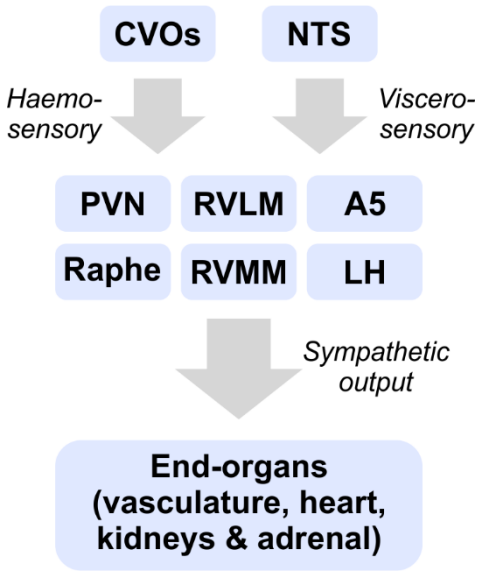

Figure 1.1. The hierarchical organisation of structures that have the capacity to modulate sympathetic outflow to the vasculature, heart, kidneys and adrenal as revealed by trans-synaptic viral tracing. CVOs, circumventricular organs; NTS, nucleus of the solitary tract; PVN, hypothalamic paraventricular nucleus; RVLM, rostral ventrolateral medulla; RVMM, rostral ventrolateral medial medulla; LH, lateral hypothalamus.

Role of the RVLM

The central origins of resting sympathetic vasomotor tone had been investigated long before the afferent connections of the sympathetic nervous system were mapped. The first attempts were made in the 19th century in Carl Ludwig's laboratories by examining how arterial

pressure changed in response to systematically positioned lesions in anaesthetised cats. These experiments indicated that the generation of vasomotor tone was localised to a broad area in the ventral medulla (cited by Seller (1996)). However, this finding remained ignored for almost 100 years until a seminal study by Guertzenstein and Silver (1974) found that focal application of the inhibitory amino-acid glycine bilaterally to the ventral brainstem between the hypoglossal nerve and caudal portion of the trapezoid body produced a profound (80 mmHg) and rapid reduction in arterial pressure in anaesthetised cats. Subsequent work mostly in the rat found that this region, which would later become known as the RVLM, contains spinally-projecting neurons which contact sympathetic preganglionic neurons (Ross et al., 1981; Zagon and Smith, 1993) and display a tonic discharge that is strongly barosensitive in-vivo (Brown and Guyenet, 1985; Koshiya et al., 1993; Moreira et al., 2006). In anaesthetised animals, activating RVLM neurons with glutamate was found to evoke substantial increases blood pressure (> 70 mmHg) and renal SNA (Dampney et al., 1982; Ross et al., 1984) whereas inhibiting them markedly lowered SNA (Stein et al., 1989; Schreihofer et al., 2000). Thus, these experiments unequivocally show that neurons within the RVLM region are responsible for the generation of sympathetic vasomotor tone in anaesthetised animals.

It is less clear whether RVLM neurons make an equally large contribution to vasomotor tone in conscious animals and also whether different neuronal populations are involved. The majority of spinally-projecting RVLM neurons are both glutamatergic and peptidergic, with ~50% also belonging to the C1 cell group that are marked by the expression of enzymes required to synthesise catecholamines (Phillips et al., 2001; Stornetta, 2009; Dempsey et al., 2017a). C1 neurons have additional projection targets throughout the brainstem (e.g. locus coeruleus, A5 cell group and dorsal motor nucleus of the vagus) and hypothalamus (e.g., PVN and lateral hypothalamus) (Ross et al., 1984; DePuy et al., 2013; Li et al., 2015a; Stornetta et al., 2016), and are implicated in a range of autonomic and humoral responses (Schreihofer and Guyenet, 2000; Madden and Sved, 2003; Madden et al., 2006; Abe et al., 2017; Wenker et al., 2017; Zhao et al., 2017). Several studies have directly examined the relative contribution of C1 neurons to the maintenance of vasomotor tone by utilising a catecholaminergic neurotoxin, anti-dopamine beta-hydroxylase (DBH)-saporin. Madden and Sved (2003) found that depletion (by ~80 %) of C1 neurons only reduced arterial pressure by ~10 mmHg in conscious animals whereas Schreihofer and Guyenet (2000) found that lesioning of spinally-projecting C1 neurons did not affect the magnitude of the depressor or

sympathoinhibitory response to acute pharmacological inhibition of the RVLM in anaesthetised rats. Together these studies suggest that C1 neurons contribute minimally to the maintenance of sympathetic vasomotor tone and that non-C1 RVLM neurons may be more important in this regard.

However, a more recent study by Wenker et al. (2017) suggests that this view is likely too simplistic. These authors used inhibitory optogenetics to tease apart the role RVLM C1 and non-C1 neurons in maintenance of arterial pressure in conscious rats. They found that photoinhibition of C1 neurons selectively or RVLM neurons non-selectively produced a negligible (< 5 mmHg) reduction in blood pressure, an effect that was exaggerated under anaesthesia. These data indicate that excitatory drive to preganglionic sympathetic neurons is far less dependent on RVLM neurons in conscious than in anaesthetised settings, and that the contributions made by C1 and non-C1 neurons is probably comparable. That being said, because of inherent limitations associated with viral transduction, it is possible that the total number of RVLM neurons inhibited in this study was less than that which can be achieved using pharmacological tools. Nevertheless, attempts to pharmacologically inhibit RVLM neurons in conscious animals have produced varied results, with one study observing that bilateral RVLM inhibition with the $GABA_A$ receptor agonist muscimol lowers arterial pressure by ~35 mmHg (Menezes and Fontes, 2007) while others have found that glycine (Araujo et al., 1999) or GABA (Lacerda et al., 2003) both slightly (15-20 mmHg) decrease arterial pressure but that the depressor response to GABA is twice as large under anaesthesia.

If RVLM neurons are less important for the maintenance of arterial pressure in conscious unchallenged animals, then the possibility arises that another presympathetic region makes a greater contribution under conscious conditions. Considering this possibility, either no change or a small reduction in baseline blood pressure is encountered in conscious animals when PVN neurons are acutely inactivated (Martins-Pinge et al., 2012; Ramchandra et al., 2013), A5 neurons are lesioned (Taxini et al., 2017) or inhibited (Maiorov et al., 2000) or upon depletion of orexin neurons (Kayaba et al., 2003), a dominant presympathetic cell cluster in the lateral hypothalamus (Geerling et al., 2003). These data do not support the notion that the PVN, A5 cell group or lateral hypothalamus serve as a sole ‘vasomotor centre’ in conscious conditions. An alternative and perhaps more likely possibility is that the tonic drive of sympathetic vasomotor efferents is more distributed than previously thought, potentially emerging from the coordinated activity of multiple groups of presympathetic neuron. This possibility remains to be directly examined but is indicated by the strong

interconnectivity between presympathetic regions (Swanson and Kuypers, 1980; Abbott et al., 2012; Dempsey et al., 2017a; Dergacheva et al., 2017) and observations from Fos studies that 'symapthoexcitatory' stimuli (e.g., hypoxia (Hirooka et al., 1997) and acute psychological stress (Furlong et al., 2014)) activate neurons in multiple presympathetic regions. This level of complexity cannot be revealed with conventional loss-of-function pharmacological or lesion experiments and will ultimately require technologies that permit the manipulation of specific neuronal populations according to their projection target.

Baroreceptor reflex

The arterial baroreceptor reflex (baroreflex) is the principle mechanism by which arterial pressure is controlled in the short-term. Arterial baroreceptors are afferent neurons with stretch-activated terminal endings located in the adventitia layer of the carotid sinus and aortic arch (Lau et al., 2016). The action potential frequency of baroreceptor afferents changes linearly with arterial pressure over a large physiological range and with a cardiac cycle resolution (Barrett and Bolter, 2006). Baroreceptor loading (i.e., an increase in firing produced by an increase in arterial pressure above a resting value) reflexively inhibits sympathetic outflow to the vasculature and heart while simultaneously exciting cardiac vagal efferents to collectively reduce total peripheral resistance and cardiac output and thus restore arterial pressure. Unloading of the baroreceptors following a decrease in arterial pressure produces the opposite effect on sympathetic and cardiac vagal outflows (Dampney, 2016).

Assessment of baroreflex function

The baroreflex therefore dictates the relationship between arterial pressure and sympathetic activity and heart rate, at least over relatively short timescales. As such, baroreflex function can be examined by plotting sympathetic activity or heart rate (the readily measurable component of cardiac output) against changes in arterial pressure that are autogenic or are induced with vasoactive substances (Kent et al., 1972; Hildreth et al., 2013a; Dampney, 2016). The former method can provide a relatively non-invasive assessment of cardiac baroreflex sensitivity and is used primarily in conscious animals and human subjects. The latter method of analysing baroreflex function with induced changes in blood pressure has the advantage of generating additional measures of baroreflex function, such as how much of heart rate and SNA is regulated by the baroreflex and over what range the baroreflex operates (Hildreth et al., 2013a). With this method, a logistic sigmoidal curve provides the best-fit to analyse the input-output function of the baroreflex (Figure 1.2) (Dampney, 2016). The main parameters that can be derived from this curve are: 1) the sensitivity of the reflex as indicated

by the maximal gain or slope of the curve; 2) the maximum and minimum output under the control of the baroreflex (upper and lower plateau); 3) the threshold and saturation points of the baroreflex; and 4) the operational range of the baroreflex (Kent et al., 1972; Dampney, 2016).

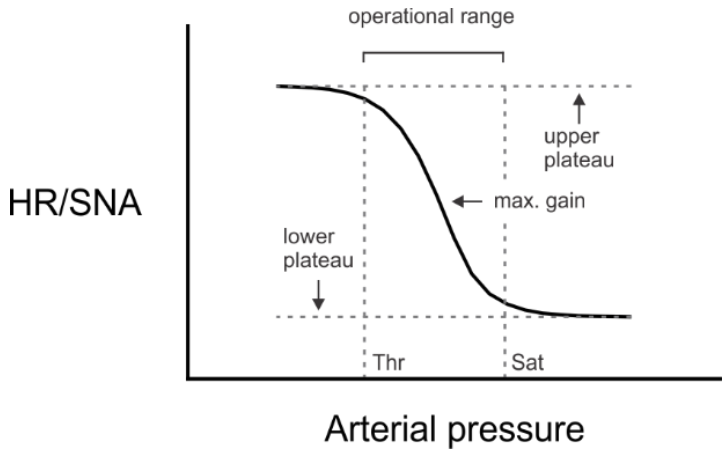

Figure 1.2. An illustration of the sigmoidal baroreflex function curve including the main parameters that can be derived from it. Adapted from (Dampney, 2016). HR, heart rate; Thr, threshold point, Sat, saturation point; Max., maximum.

Central baroreflex circuitry

A core network of neurons in the medulla oblongata are responsible for producing baroreflex-mediated changes in sympathetic and cardiac vagal outflow. Carotid and aortic baroreceptor afferents reach the brain via the carotid sinus (a branch of the glossopharyngeal) and aortic depressor (a branch of the vagus) nerves, respectively (Lau et al., 2016). Baroreceptor afferents terminate within the NTS of the dorsal medulla oblongata. This synapse is glutamatergic involving both NMDA and non-NMDA receptors (Guyenet et al., 1987; Leone and Gordon, 1989; Gordon and Leone, 1991; Lawrence and Jarrott, 1994; Ohta and Talman, 1994).

The NTS is the location where the baroreflex pathway diverges to control cardiac vagal and sympathetic outflows. Anatomical data support a monosynaptic glutamatergic projection from the NTS to the nucleus ambiguus (Loewy and Burton, 1978; Standish et al., 1994; Neff et al., 1998), where most cardiac vagal preganglionic neurons controlling heart rate are located (Nosaka et al., 1979; Stuesse, 1982). This monosynaptic pathway most likely underlies the baroreflex control of the cardiac vagus since baroreflex mediated bradycardia is virtually eliminated following administration of inotropic glutamate antagonists into the nucleus ambiguus (Guyenet et al., 1987; Hildreth and Goodchild, 2010).

Baroreflex control of SNA is mediated via the disinhibition of tonically generated sympathetic outflow. The anatomical substrate is an indirect projection from the NTS to the RVLM via a population of GABAergic interneurons in the caudal ventrolateral medulla (CVLM). This is supported by several key lines of evidence: firstly, both GABAergic RVLM-projecting CVLM neurons (Schreihofer and Guyenet, 2003) and spinally-projecting RVLM neurons (Brown and Guyenet, 1985) are strongly barosensitive; secondly, inhibitory post-synaptic potentials are evoked in spinally-projecting RVLM neurons upon stimulation of arterial baroreceptors (Lipski et al., 1996); and thirdly, baroreflex control of SNA is virtually eliminated following blockade of ionotropic glutamate receptors in the CVLM (Masuda et al., 1992) or $GABA_A$ receptors in the RVLM (Sun and Guyenet, 1985; Masuda et al., 1992).

Baroreflex resetting

The arterial baroreflex is capable of resetting to different levels of arterial pressure. There are two primary forms of baroreflex resetting: peripheral resetting of the baroreceptors following a sustained change in arterial pressure (e.g., induction of hypertension) (Sleight et al., 1977; Krieger, 1989); and central resetting as a feed-forward response when higher (e.g. exercise and acute physiological stress) or lower (e.g., sleep) levels of arterial pressure are desirable (Dampney, 2017). Peripheral resetting involves both an acute-phase, which arises within ~20 minutes and is attributed to a partial shift in the pressure threshold of the baroreceptors (Munch et al., 1983), and a chronic-phase, which occurs in hypertension due to structural changes of the vasculature and baroreceptors resulting in an irreversible shift in baroreceptor pressure threshold and sensitivity (Thrasher, 2005). Central resetting is less well understood but appears to arise from distinct mechanisms depending on the physiological context. For instance, resetting of the cardiac baroreflex during exercise involves modulation of baroreceptor afferent transmission at the level of the NTS by peptidergic inputs originating from the PVN (Michelini and Bonagamba, 1988; Dufloth et al., 1997) and GABAergic inputs that are stimulated by skeletal muscle afferents (Potts et al., 2003), whereas resetting of the sympathetic baroreflex during acute physiological stress has been suggested to involve activation of neurons in the dorsomedial hypothalamus and adjacent perifornical area (McDowall et al., 2006).

Humoral control of arterial pressure

There are various humoral factors that have the capacity to modulate arterial pressure by influencing extracellular fluid homeostasis or vascular tone. Here I will consider three dominant humoral mechanisms that originate from (i.e., vasopressin and HPA axis) or

interact with (i.e., the RAS) the CNS. These humoral systems contribute minimally to basal vasomotor tone but are nonetheless engaged during different (patho)physiological conditions to support cardiovascular function via direct effects on the vasculature or by modulating the gain of the sympathetic nervous system.

Vasopressin

The osmotic pressure of the extracellular fluid generated by effective solutes, mainly sodium, that do not readily permeate the plasma membrane (i.e., the tonicity) dictates the distribution of water in the intracellular and extracellular compartments (Verbalis, 2007). Mammals must tightly control their plasma osmolality (to ~280 mmol/kg in humans and ~295 mmol/kg in rats) in order to prevent fluctuations in cell volume which are traumatic for cells and tissues, particularly the brain as it is encased in a rigid structure (Pasantes-Morales and Tuz, 2006; Bourque et al., 2007). Vasopressin is chief among the humoral effectors that defend extracellular fluid homeostasis by minimising renal water loss (Bankir, 2001). Additionally, during the shock states of haemorrhage and early-sepsis, vasopressin levels surge to increase vasomotor tone in an attempt to prevent circulatory collapse (Schwartz and Reid, 1981; Matsuoka and Wisner, 1997; Sharshar et al., 2003).

Vasopressin is released into the peripheral circulation by hypothalamic magnocellular neurons where it binds to three receptors. The V_2 receptor is located almost exclusively in the kidneys, principally in the collecting tubule and duct. Activation of renal V_2 receptors enhances urine concentration mainly by increasing the permeability of the collecting duct lumen to water and secondly by increasing the osmotic gradient of the renal medulla by stimulating the transport of urea and sodium into the intersitium (Bankir, 2001). Within the periphery, V_{1A} receptors are found in vascular smooth muscle, where it produces vasoconstriction, and in liver hepatocytes, where it stimulates urea production and gluconeogenesis (Bankir, 2001). Vascular V_{1A} receptors are most abundantly expressed in the renal circulation, specifically the vasa recta which controls inner medullary blood flow (Phillips et al., 1990; Turner and Pallone, 1997). The V_{1b} receptor is expressed in the anterior pituitary and in the adrenal medulla where it facilitates adrenocorticotropic hormone (ACTH) and catecholamine release, respectively (Guillon et al., 1998; Aguilera, 2011). Activation of V_{1b} receptors in the anterior pituitary depends not only on the concentration of vasopressin in the blood but also on the terminal release of vasopressin from parvocellular PVN neurons, which is subject to distinct control mechanisms (Aguilera, 1994).

The circulating level of vasopressin required to exert anti-diuresis versus increase arterial pressure is vastly different. Normal euhydrated levels of vasopressin are remarkably low (2-4 pg/ml in rats, dogs and humans) yet exert a strong tonic anti-diuretic action such that V_2 receptor inhibition or genetic vasopressin deletion produces marked polyuria (Valtin, 1982; Casteleijn et al., 2017a). In contrast, circulating vasopressin typically does not contribute to resting blood pressure in healthy subjects (Bussien et al., 1984) and only does so when levels increase to values (~ 40 pg/ml) beyond that required to achieve maximal anti-diuresis (Mohring et al., 1981; Cowley and Barber, 1983; Cowley, 2000). Thus, the difference in the effective concentration for vasopressin's anti-diuretic and pressor actions allows for these functions to be differentially engaged during normal fluctuations in hydration versus abnormal shock states.

There are several possible reasons underlying the large difference in effective concentration for V_{1A} receptor pressor compared to V_2 receptor anti-diuretic actions. Firstly, differences in receptor sensitivity likely contribute, with binding assays showing that the affinity of the V_{1A} receptor for vasopressin is ~50% lower than the V_2 and V_{1b} receptor (Koshimizu et al., 2012). However, receptor sensitivity is unlikely to be the dominant factor because in-vitro organ bath experiments demonstrate that vasopressin constricts renal efferent and mesenteric arterioles at concentrations within the 'normal' physiological range (1-10 pg/ml) (Altura, 1975; Edwards et al., 1989). Furthermore, in-vivo doppler measurements show that small increases (< 5 pg/ml) in plasma vasopressin reduce renal medullary blood flow (Franchini and Cowley, 1996). Since these plasma concentrations of vasopressin do not influence arterial pressure, it is likely that any V_{1A} receptor-induced vasoconstriction in renal and potentially mesenteric arterioles is normally offset by dilation in other vascular beds and/or a reduction in cardiac output. Indeed, seminal work in dogs (Cowley et al., 1974; Montani et al., 1980), and later rabbits (Hasser and Bishop, 1990) and rats (Brizzee and Walker, 1990; Zhang et al., 1992), showed that circulating vasopressin powerfully enhances the gain of baroreflex bradycardia and sympathoinhibition via activation of central V_{1A} receptors located in the area postrema (Hasser and Bishop, 1990; Zhang et al., 1992). The enhancement of baroreflex gain by circulating vasopressin is likely very important in preventing arterial pressure from rising when vasopressin release is increased within the anti-diuretic range. This is exemplified by observations that much lower concentrations of vasopressin increase arterial pressure in experimental animals (Montani et al., 1980; Osborn et al., 1987) and humans (Mohring et al., 1980) without a functional baroreflex.

Pharmacological inhibition of V_{1A} receptors or vasopressin release modestly reduces (by < 20 mmHg) the arterial pressure of the spontaneous hypertensive rat (SHR) (Yamada et al., 1994), 5/6 nephrectomised rat (Bouby et al., 1990) and rats with salt-sensitivity that is either genetic (Dahl strain) (Crofton et al., 1993) or produced with deoxycorticosterone (DOCA) (Yamada et al., 1994). These observations suggest that vasopressin-dependent vasoconstriction may contribute to a component of some forms of hypertension. However, the plasma level of vasopressin (< 20 pg/ml) exhibited in these hypertensive models, though higher than their respective normotensive controls (Crofton et al., 1978; Matsuguchi et al., 1981; Bouby et al., 1990; Yamada et al., 1994), is lower than the previously stated 'pressor threshold' of vasopressin (Mohring et al., 1981; Cowley and Barber, 1983; Cowley, 2000). Therefore, smaller increases in circulating vasopressin appear to be capable of increasing arterial pressure in hypertension. It is possible that this is because of a background of autonomic dysfunction and consequently an impaired ability to buffer vasopressin-induced elevations in blood pressure. Observations in the SHR support this assertion; this model exhibits baroreflex dysfunction (Head and Adams, 1992) and markedly reduced bradycardic responses to boluses of vasopressin alongside a four-fold lower pressor threshold to vasopressin than normotensive controls (Mohring et al., 1981). Alternatively, in some forms of hypertension the lower pressure threshold for vasopressin could reflect an enhancement of vascular V_{1A} receptor sensitivity as this characteristic is present in SHR (Touyz et al., 1996), though not in rats with DOCA-salt hypertension (Bockman et al., 1992).

The renin-angiotensin system (RAS)

The RAS is a peptide cascade that has both endocrine and paracrine functions, many of which are directed at cardiovascular and extracellular fluid homeostasis. Briefly, in the endocrine RAS, angiotensinogen, the substrate of the system, is cleaved in the circulation by renin which is released from the renal juxtaglomerular apparatus. This interaction forms angiotensin I that in turn is converted to angiotensin II by angiotensin converting enzyme (ACE) (Paul et al., 2006). As the primary effector of the RAS, angiotensin II via its AT_1 receptor (AT1R) increases vasoconstriction, central sympathetic drive (predominately to non-renal vascular beds), vasopressin release, thirst and renal sodium reabsorption directly or indirectly by stimulating aldosterone release from the adrenal cortex (Tobey et al., 1983; Iovino and Steardo, 1984; Matsukawa et al., 1991; Mehta and Griendling, 2007). As will be described subsequently, the CNS actions of circulating angiotensin II are mediated by the sensory circumventricular organs that are capable of detecting plasma angiotensin II by virtue

of an incomplete blood-brain barrier (Ferguson and Bains, 1997; McKinley et al., 1998). Renin secretion is the rate limiting step of the endocrine RAS and is thus heavily regulated (von Lutterotti et al., 1994). The primary stimuli for renin release are renal hypoperfusion, sodium chloride concentration at the macula densa and catecholamines, and there is feedback inhibition from circulating angiotensin II (Leenen and Stricker, 1974; Friis et al., 2013).

The importance of the RAS in blood pressure control is exemplified by observations that pharmacological inhibitors of the RAS (i.e., ACE and renin inhibitors and AT1R antagonists) are effective at lowering blood pressure in some hypertensive patients (Brunner et al., 1979; Boger et al., 1990; Goa and Wagstaff, 1996). This is not controversial. What is controversial, however, is *how* these drugs lower blood pressure because they are often effective in patients with normal or even low plasma renin (Brunner et al., 1979; Boger et al., 1990). This implies that the paracrine actions of the RAS are likely to be important in setting the level of arterial pressure in certain forms of hypertension or alternatively that AT1R transduction is enhanced in some individuals. Considering the former, the enzymes required for angiotensin II synthesis are expressed in the kidneys (Bader and Ganten, 2008), brain (de Kloet et al., 2015), heart (Lindpaintner et al., 1988) and vasculature (Ganten et al., 1970). However, studies have shown that after bilateral nephrectomy angiotensin I/II levels in the heart and vasculature fall dramatically in line with plasma levels (Kato et al., 1993; von Lutterotti et al., 1994), but actually increase in the brain (Trolliet and Phillips, 1992). Also, unilateral renal artery clipping increases angiotensin I/II levels significantly more in the clipped versus non-clipped kidney (Guan et al., 1992). Thus, the kidneys and brain, but not heart or vasculature, contain a paracrine RAS that can be differentially regulated from the endocrine system. It nevertheless remains to be clarified how angiotensin II is synthesised in the brain as the components of the RAS are distributed among different fluid compartments and cell types (de Kloet et al., 2015).

Whether the paracrine actions of the RAS in the brain or kidneys contribute to human hypertension is difficult to examine directly and is therefore mostly unknown. What is known is that both of these locally-acting RAS have the capacity to increase arterial pressure in experimental rodent models. With respect to the intra-renal RAS, it has been observed that selective knockout of ACE in the kidneys (Gonzalez-Villalobos et al., 2013) or renin in the collecting ducts (Ramkumar et al., 2014) does not affect basal arterial pressure but attenuates the hypertension produced by chronic systemic administration of angiotensin II or a nitric oxide inhibitor. Whether the intra-renal RAS participates in other forms of experimental

hypertension thought to be more clinically-relevant (e.g., SHR or salt-sensitive) requires clarification (Yang and Xu, 2017). With respect to the brain RAS, pharmacological studies show that inhibiting the generation of angiotensin II in the brain with centrally administered ACE inhibitors is anti-hypertensive in SHR (Sattar et al., 1985; Heringer-Walther et al., 2001) and DOCA-salt hypertension (Itaya et al., 1986) but not in their respective normotensive controls. However, an important caveat to consider when interpreting these studies is that pharmacological inhibition of brain ACE can have non-specific effects because ACE degrades certain neuropeptides, such as substance P (Hooper and Turner, 1987). Nevertheless, similar results have been obtained in these hypertensive models following brain-specific genetic ablation of the (pro)renin receptor (Shan et al., 2010; Li et al., 2014), a receptor that markedly enhances catalytic activity of renin (Nguyen, 2007; Shan et al., 2008) and is necessary for the stimulatory action of the brain RAS on arterial pressure (Li et al., 2014). These data therefore support the possibility that the paracrine actions of the RAS in the kidneys and brain can elevate arterial pressure in rodents. Thus, it is possible that in hypertensive patients augmented paracrine RAS actions in the kidneys and/or brain may underlie the anti-hypertensive efficacy of RAS blockers, many of which cross the blood-brain barrier irrespective of hydrophobicity (Li et al., 1993; Tan et al., 2005; Ahmad et al., 2008).

The hypothalamic-pituitary-adrenal (HPA) axis

The HPA axis is an endocrine system that facilities stress-adaptation of several physiological systems, including the cardiovascular system (Sapolsky et al., 2000). The HPA axis begins with parvocellular neurons in the PVN that stimulate the synthesis and secretion of ACTH from the anterior pituitary via the release of corticotrophin-releasing hormone (CRH) and vasopressin in the median eminence. ACTH in turn stimulates the adrenal cortex to synthesise and release glucocorticoids into the circulation (Aguilera and Liu, 2012). Glucocorticoids (cortisol in human and corticosterone in rodents) are the prototypical effector of the HPA axis; however, recent data indicates that ACTH and other adrenal hormones that are stimulated by ACTH, particularly endogenous ouabain, have important physiological actions, including vasoconstriction and enhanced muscle endurance (Sapolsky et al., 2000; Blaustein, 2018).

Peripheral administration of ACTH significantly raises blood pressure when administered acutely (Sabban et al., 2009) and chronically (Lorenz et al., 2008) in rodents. Furthermore, direct activation of CRH neurons in mice using optogenetics produces an acute pressor response (de Kloet et al., 2017; Wang et al., 2018) alongside a rise in ACTH (de Kloet et al.,

2017); although, these neurons have central projections that also contribute to this pressor response. The mechanisms underlying the pressor action of ACTH are not well delineated but may involve stimulation of noradrenaline release from sympathetic ganglia (Costa and Majewski, 1988; Serova et al., 2008) or the adrenal gland (Valenta et al., 1986). These effects act quickly and would be reversed once ACTH is eliminated from the circulation, and are considered part of the 'first-wave' of the stress response which involves concomitant sympathetic activation and, in the case of haemorrhage, vasopressin release (Sapolsky et al., 2000).

The persistent elevation in arterial pressure produced by ACTH excess requires intact adrenal glands and presumably therefore adrenal hormones (Whitworth et al., 1983; Whitworth et al., 1990). Glucocorticoids can potentiate peripheral sympathetic activity by enhancing vascular responsiveness to noradrenaline (Handa et al., 1984; Ullian, 1999) and suppressing catecholamine reuptake (Sapolsky et al., 2000). While certainly chronic glucocorticoid administration recapitulates ACTH-induced hypertension in rat (Mangos et al., 2000), neither glucocorticoid or mineralocorticoid receptor antagonism alters the development of ACTH-induced hypertension (Li et al., 1999). Together these observations indicate that chronic elevations in ACTH elevate arterial pressure through a novel adrenal effector. One effector that has been suggested to underpin ACTH induced hypertension is endogenous ouabain which is liberated from the adrenal glands by ACTH (Ludens et al., 1992; Laredo et al., 1994; Sophocleous et al., 2003). Chronic systemic administration of ouabain produces hypertension in some, but not all, rodent strains via an interaction with Na^+-K^+-ATPase in vascular smooth muscle (Yuan et al., 1993; Blaustein, 2018). Furthermore, in mutant mice with 'ouabain-resistant' Na^+-K^+-ATPase, ACTH-induced hypertension is virtually eliminated despite normal increases in adrenal steroids (Lorenz et al., 2008).

The extent to which ACTH and/or adrenal cortical hormones participate in the regulation of arterial pressure during different physiological conditions is yet to be fully clarified. Nonetheless, one stressor that has been found to engage the adrenal cortex to facilitate increases in blood pressure is water deprivation. In rats, but not dogs (Brooks and Keil, 1992), water deprivation increases plasma corticosterone as well as arterial pressure by ~15 mmHg (Ulrich-Lai and Engeland, 2002; Veitenheimer et al., 2012). This increase in blood pressure is maintained by an increase in sympathetic vasomotor tone because pharmacological inhibition of ionotropic glutamate receptors in the PVN (Freeman and Brooks, 2007; Bardgett et al., 2014b), RVLM (Brooks et al., 2004) or spinal-cord

(Veitenheimer and Osborn, 2013) produces an exaggerated fall in SNA and/or arterial pressure in water-deprived animals. However, Veitenheimer et al. (2012) found that the increase in blood pressure during water deprivation was significantly attenuated by not only by chronic pharmacological sympathetic blockade with but also adrenalectomy (with corticosterone and aldosterone clamped), yet was unaffected by celiac ganglionectomy, adrenal demedullation, lumbar sympathectomy or renal denervation. These observations indicate that an unidentified adrenal cortical hormone makes an important contribution to the elevation in arterial pressure during dehydration and that the mechanism is likely to involve an enhancement of sympathetic nervous system gain but not of one specific nerve outflow.

Hypothalamic control of arterial pressure

Pioneering studies by Hess and Ranson in the early 20th century showed that electrical stimulation or lesions of distinct sites of the hypothalamus in cats produced a range of autonomic, respiratory, thermoregulatory and reproductive responses (Ranson, 1937; Hess, 1954). Notably, some experiments describe a "hypothalamic sympathetic centre" located in the anterior-medial hypothalamus, roughly corresponding to the PVN, which upon stimulation promptly raised blood pressure. Other important experiments noted that ventrally positioned lesions produce diabetes insipidus and atrophy of soma adjacent to the optic chiasm, thus revealing the neural connections from the hypothalamus to pituitary gland necessary for the release of the "anti-diuretic substance" (i.e., vasopressin) (Ranson, 1937). Although these rather crude manipulations almost certainly stimulated/destroyed axons of neurons residing outside of the hypothalamus, subsequent investigations involving more refined approaches to not only manipulate but also record the activity of discrete neuronal populations and map their neural connections have cemented the hypothalamus as the most important integrative centre in the brain for individual survival and reproductive success (e.g., (Krashes et al., 2011; Abbott et al., 2016; Clarkson et al., 2017; Mandelblat-Cerf et al., 2017; Zhao et al., 2017; Augustine et al., 2018; Nomura et al., 2019)).

The hypothalamus is by its very nature integrative and therefore cannot defend homeostasis in isolation from the peripheral tissues or rest of the brain. Instead the hypothalamus must receive extensive sensory inputs from the viscera, blood and higher-order centres (e.g., limbic regions and thalamus) so as to be able to compare those inputs to ideal 'set-points' (Saper and Lowell, 2014). Moreover, groups of hypothalamic neuronal populations subserving different functions must communicate with each other in order to coordinate responses that might otherwise be functionally opposed; for instance, local hypothalamic circuits guarantee that

rats consume less food during dehydration (Garcia-Luna et al., 2017), presumably to minimise osmole intake and urine production. However, even hypothalamic neuronal populations with closely related functions are coordinated, an good example of which is the paracrine signalling between magnocellular vasopressin neurons and presympathetic neurons supplying the kidneys (Son et al., 2013).

Another striking feature of hypothalamic neurons and circuits is their capacity to adapt to the prevailing environmental conditions in which the organism is positioned. Consider again the secretion of vasopressin; during long-term increases in extracellular fluid tonicity, which terrestrial mammals face when environmental access to water is low, vasopressin neurons display a greater basal discharge which is facilitated partly by a reduction in the clearance of extracellular glutamate by local astrocytes (Tweedle and Hatton, 1984; Fleming et al., 2011) and also by a reduction in the efficacy of post-synaptic GABAergic hyperpolarising currents (Choe et al., 2015b). While there are countless other examples of environmental adaptation in different neuroendocrine systems (Schneider, 2004; van Bodegom et al., 2017), it is becoming increasingly clear that plasticity is also a prominent feature of hypothalamic circuits influencing autonomic nervous system functions. Importantly, these adaptive changes are observed not only during physiological (e.g. exercise training (Braga et al., 2000; Jackson et al., 2005)) but also pathophysiological conditions (e.g., chronic heart failure (Pyner, 2014) and hypertension (Li and Pan, 2017)). This thesis examines whether adaptive changes in the hypothalamus contribute to cardiovascular dysfunction in polycystic kidney disease (PKD).

The following section will consider the regulation of PVN presympathetic neurons and hypothalamic vasopressin-releasing neurons in response to physiological stimuli, particularly body-fluid disturbances and angiotensin II, and during hypertension.

Organisation of hypothalamic circuits subserving cardiovascular functions

Structure of the hypothalamus

The hypothalamus is a bilateral structure that is bounded rostrally by the optic chiasm and caudally by the mammillary bodies. The third cerebral ventricle separates the left and right hypothalami at a variable degree throughout its rostrocaudal length. The hypothalamus can be split rostrocaudally into three portions: the preoptic area, a tuberal part and a mammillary part (Saper and Lowell, 2014). The preoptic area is situated dorsal to the optic chiasm and contains nuclei that are important for fluid-balance, thermoregulation, circadian rhythm generation and sexual behaviour (Paredes, 2003; Abbott et al., 2016; Hastings et al., 2018;

Tan and Knight, 2018). The tuberal hypothalamus is the middle portion and lies above the pituitary stalk; it contains areas involved in feeding and sexual behaviour and also structures with neuroendocrine and autonomic outputs, including the PVN and supraoptic nucleus (SON) (Saper and Lowell, 2014). The caudal portion of the hypothalamus comprises the mammillary bodies and the hypothalamic nuclei dorsal to them which together are important for wake-sleep regulation and memory (Vann and Nelson, 2015; Pedersen et al., 2017).

Organisation of PVN and SON efferents

Magnocellular neurosecretory neurons are responsible for the synthesis and release of vasopressin and oxytocin into the peripheral circulation via an axonal projection to the neural lobe of the (posterior) pituitary gland. There are approximately 100,000 magnocellular neurons in humans and 10,000 in rat distributed between the PVN and SON, but also other smaller structures, most notably the accessory magnocellular nucleus (Figure 1.3A) (Brown et al., 2013). While the majority of SON neurons are considered magnocellular neurons (Smith and Armstrong, 1990; Armstrong, 1995), intracellular labelling techniques indicate that the axons of some SON neurons branch in the lateral hypothalamus as they travel to the pituitary (Randle et al., 1986), while others have projections to the hippocampus (Zhang and Hernandez, 2013).

The PVN is far more functionally heterogeneous than the SON, containing additional neuronal populations collectively termed parvocellular neurons. Parvocellular neurons are sub-divided according to their projection targets. Parvocellular neurosecretory neurons project to the median eminence, where they secrete hypophysiotropic hormones (e.g. CRH, vasopressin and thyrotropin-releasing hormone [TRH]) that control the peripheral release of anterior pituitary hormones (Stern, 2015). Parvocellular pre-autonomic neurons are a group of ~1500 neurons in the rat that innervate regions of the medulla and spinal-cord associated with autonomic control, including the RVLM, caudal NTS, medulla pressor area, dorsal motor nucleus of the vagus, nucleus ambiguus and intermediolateral cell group of the spinal cord (Saper et al., 1976; Swanson and Kuypers, 1980; Geerling et al., 2010). As a group, pre-autonomic PVN neurons provide integrative control over cardiovascular and gastrointestinal function through the modulation of tonic and reflexive sympathetic and vagal outflows (Stern, 2015). An additional population of centrally-projecting PVN neurons innervate limbic, cortical and sub-cortical sites and are thought to participate in the modulation of motivated and fear behaviours (Knobloch et al., 2012; Zhang and Hernandez, 2013).

There is a rough compartmental organisation of neuroendocrine and pre-autonomic neurons in the rat PVN (Figure 1.3B and 1.3C). Most magnocellular neurons are packed into a medial (mostly oxytocin neurons) and a lateral (mostly vasopressin neurons) subdivision. Parvocellular neurosecretory neurons are principally found in the medial and anterior parvocellular portions. Finally, parvocellular pre-autonomic PVN neurons are found in their majority in the dorsomedial cap, ventral and lateral posterior portions (Armstrong et al., 1980; Swanson and Kuypers, 1980; Stern, 2015).

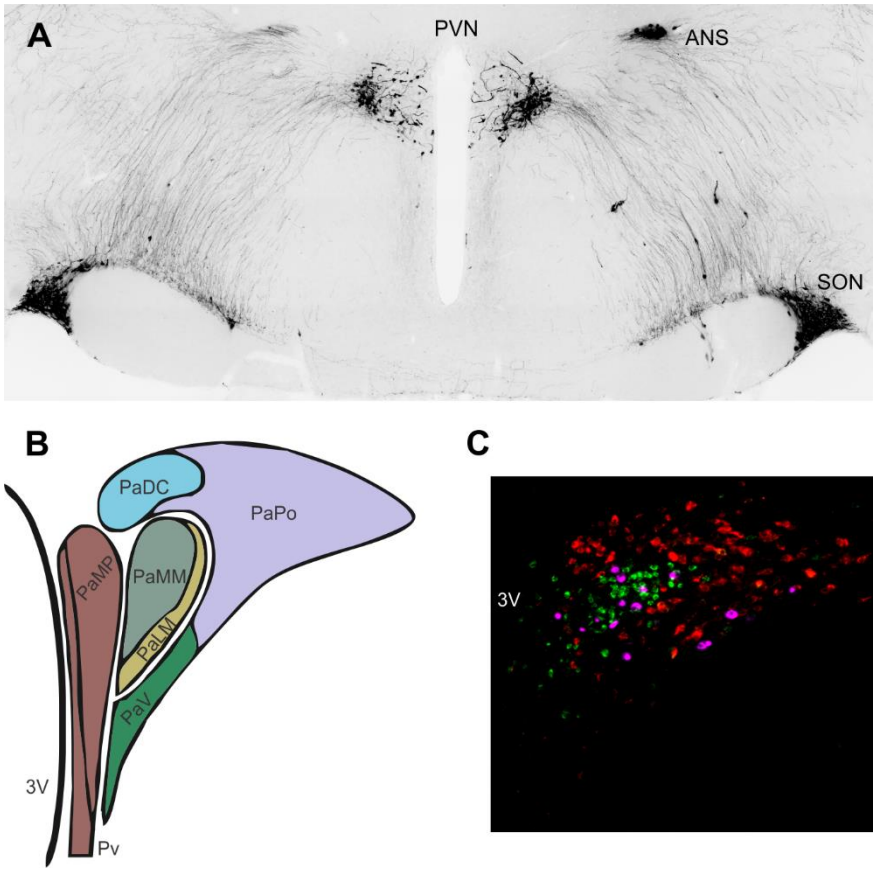

Figure 1.3. A: Photomicrograph showing the distribution of vasopressin neurons (black; detected with immunohistochemistry) in the rat PVN, SON and accessory magnocellular nucleus (ANS). **B**: An illustration of the cytoarchitectural sub-divisions of the PVN (adapted from (Feetham et al., 2018)). **C**: Photomicrograph of the posterior PVN showing the distribution of RVLM-projecting neurons (red), CRH neurons (green) and vasopressin neurons (magenta). RVLM-projecting neurons were retrogradely labelled with cholera toxin subunit b. CRH mRNA and vasopressin mRNA were detected with in-situ hybridisation (see Chapter 3 for details). 3V, third cerebral ventricle; PaMM, anterior-medial magnocellular division; PaLM, anterior-lateral magnocellular division; PaMP, medial parvocellular portion; PaAP, anterior parvocellular portions; PaDC, dorsomedial cap; PaV, ventral portion; PaPo, lateral posterior portion; PV, periventricular nucleus.

Lamina terminalis and its connections

Landmark experiments by Verney and colleagues showed that perfusing the arteries supplying the anterior hypothalamus with hypertonic saline produced potent anti-diuresis in dogs, providing the first evidence that the brain senses the ionic concentration of the blood directly (Verney, 1947; Jewell and Verney Ernest, 1957). Further studies showed that intracerebral hypertonic saline injections anterior to the third ventricle were dipsogenic in goats (Andersson, 1953) and that lesions in this region destroyed dipsogenic and anti-diuretic responses to hypertonic stimuli in rats and goats (Andersson et al., 1975; Buggy and Jonhson, 1977; Rundgren and Fyhrquist, 1978). Importantly, these lesions were also observed to eliminate dipsogenic responses to systemically administered angiotensin II, raising the possibility that the same structures that detect the ionic composition of the blood also detect circulating hormones (Andersson et al., 1975; Buggy and Jonhson, 1977). It is now established that a collection of interconnected structures contained within the forebrain lamina terminalis are responsible for detecting plasma solutes and hormones and in turn coordinating appropriate neurohumoral and behavioural responses (McKinley et al., 2001; Bourque, 2008; Toney and Stocker, 2010).

The lamina terminalis is a thin membrane (lamina) located in the medial anterior wall of the third ventricle at the rostral margin of the hypothalamus. It comprises two sensory circumventricular organs, the subfornical organ (SFO) and the organum vasculosum of the lamina terminalis (OVLT), at its dorsal and ventral edges, respectively, and the median preoptic nucleus (MnPO), a hypothalamic nucleus located between the SFO and OVLT in the dorsoventral plane that is interrupted midway through its rostrocaudal length by the anterior commissure (McKinley et al., 2001; McKinley et al., 2015). The SFO and OVLT are further sub-divided; the SFO contains a highly vascularised ventromedial core and a peripheral outer shell (Dellmann, 1998), whereas the OVLT contains a central capillary plexus, dorsal cap and lateral zone (Morita et al., 2016).

The SFO and OVLT lack a complete blood-brain barrier such that low, but not high, molecular weight tracers administered intravenously penetrate each region (Morita and Miyata, 2012; Langlet et al., 2013; Morita et al., 2016). In both regions this is achieved through capillary fenestration (Krisch et al., 1987; Sposito and Gross, 1987; Yamaguchi et al., 1993; Dellmann, 1998; Morita et al., 2016) and an absence of tight junctions between adjacent endothelial cells (Petrov et al., 1994; Morita et al., 2016). These anatomical features are most pronounced in the areas of richest vascularisation, which for the SFO is the

ventromedial core and for the OVLT is the central capillary plexus (Krisch et al., 1987; Sposito and Gross, 1987; Yamaguchi et al., 1993; Petrov et al., 1994; Dellmann, 1998; Morita et al., 2016). Nevertheless, intravenous tracers fill the entire SFO and OVLT (Morita and Miyata, 2012; Langlet et al., 2013; Morita et al., 2016), albeit more intensely in the areas of greatest vascularisation, indicating that all cells contained within the SFO and OVLT are in continual contact with haemal stimuli. Interestingly, while the SFO and OVLT have ventricular surfaces, neither are labelled by tracer injections into the lateral ventricles (Langlet et al., 2013), highlighting that the passage of substances between the ventricular system and their parenchyma is restricted. This cerebrospinal fluid-barrier is thought to be produced by tight junctions between adjacent ependymal cells that line the ventricular surface (Petrov et al., 1994; Langlet et al., 2013) and may serve to minimise circulating factors from entering into the ventricular system via the circumventricular organs (Petrov et al., 1994; Langlet et al., 2013).

Cells within the SFO and OVLT have the capacity to detect various haemal stimuli (Table 1.2). In-vitro recordings of OVLT and SFO neurons in synaptic isolation show that their discharge is positively related to the extracellular fluid osmolality and sodium concentration (Anderson et al., 2000; Anderson et al., 2001; Ciura and Bourque, 2006; Kinsman et al., 2017a; Matsuda et al., 2017). In addition, some SFO and OVLT neurons express the AT1R (Allen et al., 1988; Song et al., 1992; Lippoldt et al., 1993; Krause et al., 2018) and are activated by peripherally administered angiotensin II (Tanaka et al., 1987; Gutman et al., 1988; Oldfield et al., 1994). As summarised in Table 1.2, other circulating factors can act in the SFO and OVLT to alter cardiovascular function.

Table 1.2: Summary of circulating factors that produce cardiovascular and/or fluid-balance responses via detection by the SFO and OVLT.

Circulating factor	Receptor	Region	Function/s	References
Osmolality (indiscriminate of effective solute)	Trpv1, Trpv4 and possibly others	SFO and OVLT	Thirst and vasopressin release (Trpv1-independent).	(Anderson et al., 2000; Anderson et al., 2001; Ciura and Bourque, 2006; Kinsman et al., 2017a; Ciura et al., 2018)
Sodium	Na_x channel	SFO and OVLT	Modulation of SNA (OVLT) and sodium preference (SFO).	(Matsuda et al., 2017; Nomura et al., 2019)
Angiotensin II	AT1R	SFO and OVLT	Increased thirst (SFO only), vasopressin release and sympathetic vasomotor tone.	(Mangiapane and Simpson, 1980; Knepel et al., 1982; Thrasher and Keil, 1987; Krause et al., 2008; Vieira et al., 2010)
Pro-inflammatory cytokines (TNF-α and IL-1β)	TNF-α and IL-1β receptors	SFO and OVLT	Increased sympathetic vasomotor tone (SFO).	(Ott et al., 2010; Wei et al., 2013; Wei et al., 2015)
Relaxin	RXFP1	SFO	Increased thirst, vasopressin and sympathetic vasomotor tone.	(Sunn et al., 2002; Coldren et al., 2015)
Endothelin	ET-3 receptor	SFO	Suppressed salt intake.	(Hiyama et al., 2013)
Leptin	Leptin receptor	SFO	Increased sympathetic vasomotor tone.	(Smith et al., 2009; Young et al., 2013)

The SFO and OVLT are reciprocally connected with each other and the MnPO (Figure 1.4) (Moore, 1977; Lind et al., 1982; Lind, 1986; Duan et al., 2008; Gizowski et al., 2016). These projections are both glutamatergic and GABAergic as indicated by recent studies using viral tracing in cre-dependent transgenic mice (Abbott et al., 2016; Leib et al., 2017; Matsuda et al., 2017; Augustine et al., 2018). Importantly, the PVN and SON receive afferent inputs from the lamina terminalis, arising predominately from glutamatergic neurons, but also a much smaller population of GABAergic neurons originating from the MnPO and OVLT (Oldfield et al., 1991a; Roland and Sawchenko, 1993; Armstrong et al., 1996; Ulrich-Lai et al., 2011a; Abbott et al., 2016; Leib et al., 2017; Matsuda et al., 2017; Nomura et al., 2019). While the neurochemical phenotype of lamina terminalis neurons with PVN or SON projections is not well defined, anatomical and in-vivo electrophysiological evidence indicates that SFO efferents that supply these hypothalamic nuclei also utilise angiotensin II as a neurotransmitter (Lind et al., 1984; Imboden et al., 1987; Jhamandas et al., 1989; Li and Ferguson, 1993a). Studies using in-vivo single-unit recordings, Fos activation or more recently fibre photometry show that acute systemic hyperosmolality and angiotensin II activates OVLT and SFO neurons, including those with projections to the PVN, SON and MnPO (Ferguson and Renaud, 1986; Tanaka et al., 1986; Tanaka et al., 1987; Gutman et al., 1988; Honda et al., 1990; Oldfield et al., 1994; Larsen and Mikkelsen, 1995; Augustine et al., 2018). As will be discussed subsequently, these connections enable the lamina terminalis to modulate vasopressin release and cardiovascular function in response to haemal stimuli (McKinley et al., 2001; Bourque, 2008; Toney and Stocker, 2010). Current thought is that the extensive excitatory and inhibitory interconnections within the lamina terminalis participate in feed-forward (i.e., anticipatory) and feedback gating of sensory stimuli, at least within the circuits that drive behavioural thirst responses (Matsuda et al., 2017; Zimmerman et al., 2017; Augustine et al., 2018). This is likely to be reinforced by extensive axonal collateralisation within the circuits that contain efferents supplying the PVN and SON (Weiss and Hatton, 1990; Oldfield et al., 1991b, 1992; Duan et al., 2008). For example, SFO neurons projecting to the PVN also have collateral projections to the MnPO and SON (Weiss and Hatton, 1990; Duan et al., 2008).

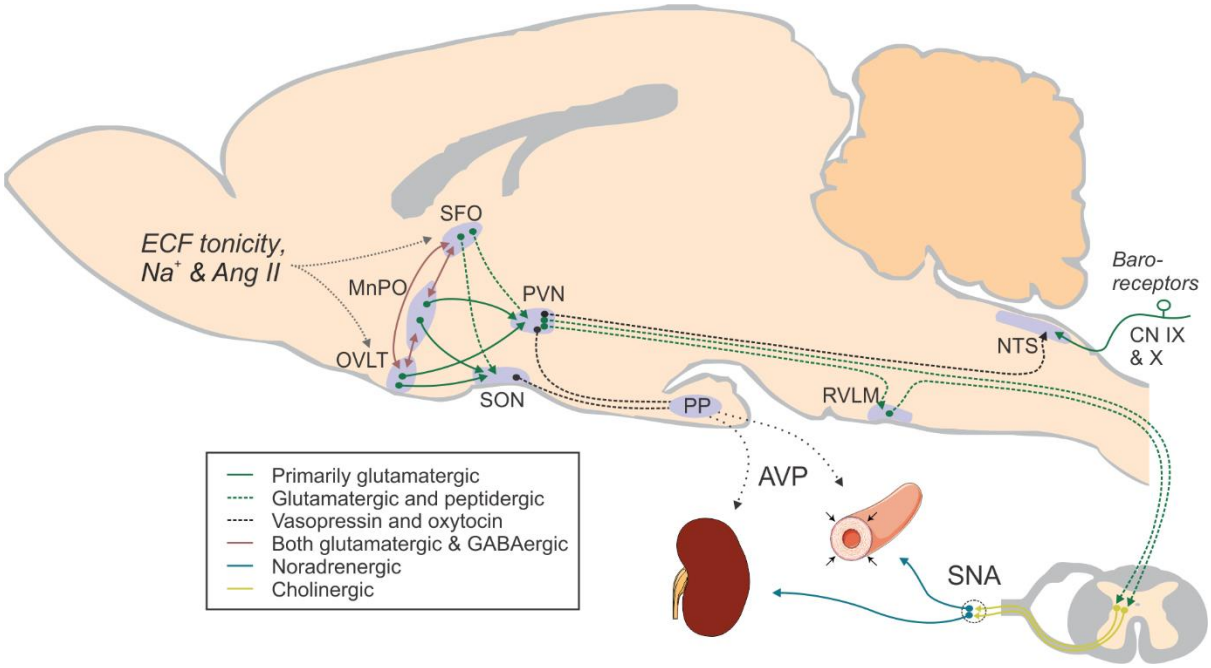

Figure 1.4. The organisation of important circuits in the lamina terminalis, hypothalamus and medulla that regulate SNA and vasopressin (AVP) release in response to changes in extracellular fluid (ECF) tonicity, sodium and angiotensin II (Ang II). Arrows indicate direction of the projection and circles indicate neuronal soma. OVLT, organum vasculosum of the lamina terminalis; SFO, subfornical organ; MnPO, median preoptic nucleus; PVN, hypothalamic paraventricular nucleus; SON, supraoptic nucleus; PP, posterior pituitary; RVLM, rostral ventrolateral medulla; NTS, nucleus of the solitary tract; CN, cranial nerve. Adapted from (Bichet, 2016) with graphics sourced from Laboratoires Servier (www.servier.com).

Lamina terminalis neurons project to numerous other structures in addition to the PVN and SON. The SFO and OVLT supply a common set of structures, including the bed nucleus of the stria terminalis, lateral hypothalamus, anterior periventricular preoptic nucleus, periaqueductal grey and dorsal raphé (Moore, 1977; Miselis, 1981; Lind et al., 1982; Lind, 1986; Gu and Simerly, 1997; Sunn et al., 2003; Uschakov et al., 2009; Kawano and Masuko, 2010). The additional efferent connections of the MnPO are also numerous, with the most prominent projection targets including the paraventricular nucleus of the thalamus, parvicellular hypothalamic nucleus lateral hypothalamus, dorsomedial hypothalamus, arcuate nucleus, parabrachial nucleus and ventrolateral periaqueductal grey (McKinley et al., 2015; Abbott et al., 2016; Leib et al., 2017).

Outside of the lamina terminalis, shared inputs to the SFO and OVLT include the locus coeruleus, dorsal and medial raphe, medial and ventral subdivisions of the PVN, septal nuclei and bed nucleus of the stria terminalis (Moore et al., 1978; Camacho and Phillips, 1981; Lind

et al., 1982; Larsen et al., 1991; Kawano and Masuko, 2001; Gizowski et al., 2016). In addition, the SFO has afferent connections with catecholaminergic and non-catecholaminergic cells in the ventrolateral medulla and NTS (Kawano and Masuko, 2001) and the OVLT with the suprachiasmic nucleus and midbrain central grey (Camacho and Phillips, 1981; Schwartz et al., 2011; Gizowski et al., 2016). The MnPO is extensively innervated by hypothalamic structures including the PVN, arcuate nucleus, ventral lateral preoptic area, anterior and preoptic periventricular nuclei (Saper and Levisohn, 1983). Extra-hypothalamic inputs to the MnPO include the parabrachial nucleus, Kolliker-Fuse nucleus, locus coeruleus and catcholaminergic and non-catecholaminergic neurons in the VLM and NTS (Saper and Levisohn, 1983; Saper et al., 1983).

Other afferent inputs to the PVN and SON

The PVN receives extensive afferent input from other diencephalic regions (e.g., bed nucleus of the stria terminalis, dorsomedial hypothalamus, posterior hypothalamus, arcuate nucleus, ventromedial hypothalamus, lateral hypothalamus and paraventricular thalamus) (Tribollet and Dreifuss, 1981; Sawchenko and Swanson, 1983; Ulrich-Lai et al., 2011a). Anterograde tracing experiments indicate that these inputs are preferentially directed at parvocellular subdivisions of the PVN, with the exception being the bed nucleus of the stria terminalis and dorsomedial hypothalamus that also innervate magnocellular portions (Sawchenko and Swanson, 1983). Studies that have combined tract-tracing with in-situ hybridisation for GABAergic or glutamatergic markers indicate that GABAergic inputs to the PVN are predominately restricted to the immediate vicinity of the PVN (i.e., the peri-PVN area) as well as the perifornical and anterior hypothalamic areas (Roland and Sawchenko, 1993), whereas glutamatergic inputs are more distributed in the forebrain (Ulrich-Lai et al., 2011a). However, diencephalic inputs to the PVN are no doubt more neurochemically diverse than glutamatergic or GABAergic, the full extent of which is starting to be appreciated with technologies that allow for cell-specific manipulations; for example, recent studies using optogenetics suggests that presympathetic PVN neurons receive inhibitory neuropeptide Y inputs originating from the arcuate nucleus (Shi et al., 2017) and excitatory orexinergic inputs from the lateral hypothalamus (Dergacheva et al., 2017).

The PVN receives additional afferent input from several brainstem sites; most notably, the ventrolateral medulla, NTS, periaqueductal grey, lateral parabrachial nucleus, raphe nuclei, laterodorsal tegmental area (Tribollet and Dreifuss, 1981; Ulrich-Lai et al., 2011a; Affleck et al., 2012). All of these regions supply glutamatergic projections (Ulrich-Lai et al., 2011a;

Affleck et al., 2012) while those in the ventrolateral medulla (A1/C1 cell groups) and NTS (A2/C2 cell groups) are also catecholaminergic (Sawchenko and Swanson, 1981, 1982; Cunningham and Sawchenko, 1988). More specifically, anterograde tracing experiments suggest that adrenergic innervation is most heavily concentrated in the parvocellular divisions and originates most prominently from the C1 neurons (Cunningham et al., 1990). In contrast, the A1 noradrenergic cell group primarily supplies the magnocellular portions of the PVN, while the A2 noradrenergic cell group innervates the medial parvocellular divisions where parvocellular neurosecretory neurons are located (Cunningham and Sawchenko, 1988). While this organisation could imply that different catecholaminergic cell groups preferentially control magnocellular versus parvocellular neurons, this is likely too simplistic because noradrenaline has been shown to activate local glutamate interneurons in the PVN whose soma and terminal fields may be contained within different cytoarchitectural domains (Daftary et al., 1998; Boudaba and Tasker, 2006).

In contrast to the extensive innervation of the parvocellular PVN, but similar to the magnocellular PVN, the SON receives few direct inputs from structures outside of the lamina terminalis. In the forebrain, inputs originate from a similar set of structures as the magnocellular PVN (i.e., the bed nucleus of the stria terminalis and dorsomedial hypothalamus) (Sawchenko and Swanson, 1983; Anderson et al., 1990). Inputs to the SON from the brainstem arise from parabrachial nucleus, caudal raphe, the A1 noradrenergic cell group and to a lesser extent medullary adrenergic cell groups (C1, C2 and C3) (Anderson et al., 1990; Cunningham et al., 1990). Also similar to the PVN, the SON is surrounded dorsally by a zone of GABAergic interneurons (Roland and Sawchenko, 1993; Armstrong and Stern, 1997). This ‘peri-nuclear zone’ is thought to relay other afferents to magnocellular SON neurons, such as those from the diagonal band of Broca which are important for conveying atrial-volume and arterial baroreceptor information (Sawchenko and Swanson, 1982; Cunningham and Sawchenko, 1988; Anderson et al., 1990; Grindstaff and Cunningham, 2001).

Regulation of sympathetic vasomotor tone by the PVN

Presympathetic efferents

Stimulation of the PVN *en masse* produces an abrupt increase in the discharge of multiple sympathetic nerves (renal, splanchnic, adrenal, lumbar and cardiac) and is associated with a concurrent rise in arterial pressure, heart rate, cardiac contractility, renin release, anti-natriuresis and central venous pressure (Porter, 1988; Kannan et al., 1989; Martin et al., 1991;

Martin et al., 1997; Haselton and Vari, 1998; Deering and Coote, 2000; Kenney et al., 2001; Page et al., 2011; Ramchandra et al., 2013; Mendonca et al., 2018). The PVN is therefore capable of generating sympathetic outflow to multiple targets to increase arterial pressure and modulate cardiac and renal function. Of note, in rabbits (Deering and Coote, 2000) and sheep (Ramchandra et al., 2013) chemical stimulation of the PVN produces renal sympathoinhibition. In rat, chemical stimulation of different regions of the PVN produces either an increase (most commonly) or decrease in renal SNA with no clear anatomical demarcation (Yang et al., 2002). It is therefore likely that the PVN contains local circuits that exert either a net inhibitory or excitatory influence on renal sympathetic outflow and the relative amount probably varies between different species.

The PVN is thought to control sympathetic outflow via three principle pathways: a direct pathway involving monosynaptic connections with sympathetic preganglionic neurons in the intermediolateral cell group (Hosoya et al., 1991; Ranson et al., 1998); an indirect pathway via projections to the RVLM (Pyner and Coote, 1999); and a collateral pathway involving neurons with projections to both the intermediolateral cell group and RVLM (Shafton et al., 1998; Pyner and Coote, 2000). In-vivo studies using either pharmacology or optogenetics confirm activation of PVN neurons with projections to either the spinal-cord or RVLM produces physiologically relevant increases in SNA and blood pressure (Malpas and Coote, 1994; Koba et al., 2018). The precise function of the collateral pathway is not known, but may represent an anatomical substrate that facilitates the coordinated output of different sympathetic outflows. It should also be noted that although the PVN is assumed to modulate sympathetic outflow through these three primary pathways, PVN neurons could conceivably influence sympathetic outflow through other indirect pathways, such as via projections to the A5 region (Byrum and Guyenet, 1987), lateral hypothalamus (Barone et al., 1981) or NTS (Singru et al., 2012).

Recent anatomical evidence suggests that PVN neurons likely innervate RVLM presympathetic neurons indirectly via local interneurons (Stornetta et al., 2016; Dempsey et al., 2017a). Specifically, two studies that mapped the monosynaptic inputs to spinally-projecting RVLM neurons in rat (Dempsey et al., 2017a) and C1 neurons in mouse (Stornetta et al., 2016) with a novel dual viral vector system observed that the majority of direct inputs arise from the immediate vicinity of the RVLM region and that the PVN contains far fewer monosynaptic inputs than that initially indicated with conventional retrograde tracers (Dampney et al., 1987; Van Bockstaele et al., 1989). Therefore, PVN neurons more than

likely innervate interneurons in the RVLM region that are antecedent to the spinally-projecting neurons. The physiological relevance of this pattern of connectivity is currently not known but would presumably permit a higher level of processing prior to the alteration of sympathetic outflow. However, an important consideration when interpreting these mapping studies is that both studies restricted their analysis to presympathetic RVLM neurons supplying T2-T3 spinal segments. Therefore, it is entirely possible that the hypothalamus provides greater monosynaptic input to RVLM neurons with projections to sympathetic preganglionic neurons located in lower spinal cord segments (i.e. those supplying the kidneys and hindlimb vasculature (Strack et al., 1989; Huang et al., 2002)).

PVN neurons with RVLM or spinal projections (henceforth collectively referred to as presympathetic neurons) have similar neurochemical phenotypes; both are glutamatergic (Stocker et al., 2006; Yang and Coote, 2007) and peptidergic, with oxytocin (Cechetto and Saper, 1988; Roy et al., 2018) and vasopressin (Gomez et al., 1993; Hallbeck and Blomqvist, 1999) most commonly expressed. Furthermore, the in-vivo discharge of PVN neurons with RVLM or spinal projections is largely comparable; approximately 30-50% are silent in anaesthetised normal animals, with most but not all of the remaining neurons firing in phase with the cardiac cycle, renal SNA and fluctuations in blood pressure (Chen and Toney, 2003; Chen and Toney, 2010; Xu et al., 2012). Therefore a significant portion of presympathetic PVN neurons are normally quiescent, at least in anaesthetised conditions, and those that are active likely supply either vasomotor or non-vasomotor targets, consistent with the pattern of innervation suggested by trans-neuronal tracing studies (Strack et al., 1989; Schramm et al., 1993; Kalsbeek et al., 2004).

As previously indicated, as a group PVN neurons likely contribute minimally if at all to resting sympathetic vasomotor tone in normal animals because pharmacological inactivation of the PVN does not change renal or lumbar SNA and reduces blood pressure only marginally (by at most 15 mmHg) in unchallenged anaesthetised rats irrespective of whether a $GABA_A$ receptor agonist, lidocaine or ionotropic glutamate antagonist is used (Stocker et al., 2004a; Stocker et al., 2005; Freeman and Brooks, 2007; Leite et al., 2012). Similarly in conscious conditions, inhibition of the PVN does not affect cardiac or renal SNA or blood pressure in sheep (Ramchandra et al., 2013), while blockade of ionotropic glutamatergic inputs produces a small depressor response in rats (Martins-Pinge et al., 2012). Nonetheless, loss-of-function studies indicate that PVN neurons are involved in: increasing lumbar and splanchnic SNA in response to acute plasma hypertonicity (Antunes et al., 2006; Holbein and

Toney, 2015); increasing lumbar and renal SNA during chronic water deprivation (Stocker et al., 2004b; Stocker et al., 2004a; Stocker et al., 2005; Freeman and Brooks, 2007); decreasing renal SNA in response to isotonic volume expansion (Haselton et al., 1994; Ramchandra et al., 2013); and increasing lumbar SNA during pregnancy (Shi et al., 2015b). Furthermore, a relatively small population of presympathetic PVN neurons express fos in response to hypotensive and non-hypotensive haemorrhage (Badoer et al., 1993; Badoer and Merolli, 1998) and conditioned fear (Carrive and Gorissen, 2008), indicating that PVN presympathetic neurons are also recruited during these conditions.

Glutamate-GABA interactions

Glutamate is the principle excitatory neurotransmitter in the brain (Fonnum et al., 1979; Meldrum, 2000). It is therefore not surprising that glutamate is the major source of excitatory drive to PVN neurons (Li and Pan, 2017). Anatomical studies show that the PVN is rich with glutamatergic terminals (van den Pol, 1991; Kaneko et al., 2002; Ziegler et al., 2005) and post-synaptic NMDA, AMPA and kainate ionotropic glutamate receptors (Herman et al., 2000; Ziegler et al., 2005). In addition, NMDA receptors are located pre-synaptically and can enhance glutamate release onto PVN presympathetic neurons (Ye et al., 2011). Physiological evidence demonstrates that ionotropic glutamate agonists increase the discharge of PVN neurons in-vitro (van den Pol et al., 1990) and in-vivo (Xu et al., 2012) and arterial pressure and renal and lumbar SNA when administered directly into the PVN in-vivo (Li et al., 2001; Ferreira-Neto et al., 2013; Stocker and Gordon, 2015). Thus, the activity of PVN neurons subserving cardiovascular functions is positively influenced by fast-acting glutamatergic transmission. However, consistent with the cardiovascular response elicited when PVN neurons are inhibited *en masse*, administration of ionotropic glutamate receptor antagonists into the PVN in normal rats does not affect resting SNA and reduces blood pressure only marginally or not at all (Li and Pan, 2007b; Bardgett et al., 2014b; Stocker and Gordon, 2015). Importantly, however, the same manipulation reduces blood pressure and SNA during dehydration (Bardgett et al., 2014b), hyperinsulinemia (Stocker and Gordon, 2015), heart failure (Li et al., 2003c) and hypertension (Li and Pan, 2007b). This strongly suggests that glutamatergic excitation of the PVN is augmented to increase SNA and blood pressure during certain conditions.

GABA is the principle inhibitory neurotransmitter in the PVN, with one-half of all synapses GABAergic (Decavel and Van den Pol, 1990). Unsurprisingly the PVN is rich with ionotropic $GABA_A$ receptors (Cullinan and Wolfe, 2000). Physiological evidence indicates

that fast-acting GABAergic transmission in the PVN has a very high level of physiological tone in normal conditions because pharmacological antagonism of $GABA_A$ receptors markedly increases neuronal discharge in-vitro (Li et al., 2003a; Li and Pan, 2005) and blood pressure, heart rate and SNA in anaesthetised and conscious animals (Martin et al., 1991; Chen et al., 2003; Li et al., 2006; Page et al., 2011; Ramchandra et al., 2013).

The ability of $GABA_A$ receptor antagonism to increase blood pressure and SNA above resting levels suggests that GABAergic inputs to cardiovascular PVN neurons tonically restrain a background level of excitatory drive. Since PVN neurons, including putative presympathetic neurons, do not display cell-autonomous firing (Bains and Ferguson, 1999; Chen and Toney, 2009), the tonic excitatory drive of PVN neurons must originate from synaptic input. This background level of excitatory synaptic drive appears to originate from glutamatergic inputs. This was first reported by Chen et al. (2003) who observed that the renal symapthoexcitatory and pressor response to PVN microinjection of the $GABA_A$ receptor antagonist bicuculline was eliminated if ionotropic glutamate receptors were antagonised with kynurenic acid. A subsequent investigation found that bicuculline dose-dependently increased the local concentration of glutamate when administered into the PVN in-vivo and increased glutamatergic synaptic events in RVLM-projecting PVN neurons in-vitro (Li et al., 2006). This indicates that GABAergic inputs to the PVN act through a pre-synaptic mechanism to restrain the quantile release of glutamate. The restraint of glutamatergic drive exerted by GABA more than likely underlies the quiescence observed in a substantial portion of presympathetic PVN neurons in-vivo (Chen and Toney, 2003; Chen and Toney, 2010; Xu et al., 2012). Interestingly, accumulating evidence indicates that other neurochemical inputs modulate PVN neuronal activity by altering the balance of glutamatergic excitation and GABAergic inhibition. For example, noradrenaline (Daftary et al., 1998; Daftary et al., 2000) and angiotensin II (Latchford and Ferguson, 2004; Stern et al., 2016) enhance glutamate transmission through presynaptic mechanisms, whereas nitric oxide (Li et al., 2004) and substance P (Womack et al., 2007) enhance GABA release. Thus, shifting the balance between excitatory-inhibitory ionotropic inputs determines the contribution of PVN neurons to the level of sympathetic outflow and arterial pressure.

Angiotensin II and its signalling mechanisms in the PVN

Angiotensin II is a well described modulator of different PVN neuronal populations. Early studies using in-vitro autoradiography revealed that the PVN is rich with angiotensin II binding sites (Mendelsohn et al., 1984; Millan et al., 1991; Song et al., 1992) due to a high

density of AT1R but few AT_2 receptors (Millan et al., 1991; Song et al., 1992; de Kloet et al., 2016a). These anatomical observations are supported by several electrophysiological recording studies showing that angiotensin II produces AT1R-dependent excitatory effects in different populations of PVN neurons in-vivo (Harding and Felix, 1987; Ambuhl et al., 1992) and in-vitro (Li and Ferguson, 1993b; Li et al., 2003a; Latchford and Ferguson, 2004; Cato and Toney, 2005; Latchford and Ferguson, 2005; Li and Pan, 2005). Exogenous administration of angiotensin II into the PVN promptly raises renal and lumbar SNA (Zheng et al., 2009; Buttler et al., 2016; Stern et al., 2016) and vasopressin release (Veltmar et al., 1992; Tsushima et al., 1994; Khanmoradi and Nasimi, 2016), concurrent with a surge in blood pressure that is reportedly mediated by both outputs (Khanmoradi and Nasimi, 2016). In contrast, blockade of AT1R in the PVN does not affect resting blood pressure or SNA acutely (Chen and Toney, 2001) or blood pressure chronically (Northcott et al., 2010; Chen et al., 2014) in normal unchallenged animals. Thus, ongoing AT1R activation in the PVN does not support arterial pressure in basal conditions. However, as will be discussed beneath, the activation of AT1R in the PVN appears to facilitate increases in SNA during plasma hypertonicity and hypertension.

There are several possible endogenous sources of angiotensin II in the PVN. Firstly, as previously indicated there is evidence that angiotensin II is released in the PVN from the terminals of SFO neurons (Lind et al., 1984; Jhamandas et al., 1989; Li and Ferguson, 1993a). Importantly, angiotensin II is not likely to have the same temporal and spatial constraints as amino-acid neurotransmitters because of its relatively prolonged half-life (16 sec versus 5 ms for amino-acid neurotransmitters) (Al-Merani et al., 1978; Ludwig and Leng, 2006). Thus, angiotensin II released from SFO efferents is likely capable of interacting with multiple neuronal and potentially non-neuronal cell populations distant from the site of exocytosis, a feature that is conducive with coordinating the activity of multiple groups of functionally heterogeneous PVN neuron. Secondly, angiotensin II might be synthesised locally, with evidence that all the components required to synthesise angiotensin II are found in the hypothalamus (Pickel et al., 1986; Paul et al., 1988; Stornetta et al., 1988; Intebi et al., 1990). Thirdly, a recent study suggests that SFO neurons might also secrete angiotensin II into the cerebrospinal fluid (Agassandian et al., 2017), noting however that this study was performed in transgenic mice that overexpress either renin or angiotensinogen and therefore remains to be substantiated. Finally, circulating angiotensin II has been show to access the

brain when the integrity of the blood-brain barrier is compromised, such as during hypertension (Biancardi et al., 2014a) and plasma hypertonicity (Yao and May, 2013).

Although the presence of angiotensin II binding sites in the PVN was first demonstrated over 30 years ago (Mendelsohn et al., 1984), the signalling mechanism by which angiotensin II acts within the PVN to increase SNA, vasopressin release and blood pressure is still contested. This has been contributed to by a reduced experimental capacity to evaluate the cellular and subcellular distribution of the AT1R protein due to concerns regarding the specificity of AT1R antibodies (Benicky et al., 2012). In-situ hybridisation and reporter strains have therefore been crucial to determine which cell populations transcribe AT1R in the PVN (Aguilera et al., 1995b; Lenkei et al., 1995; Gonzalez et al., 2012; Wang et al., 2016b; de Kloet et al., 2017); however, unfortunately these techniques cannot reveal the subcellular (e.g. pre- or post-synapse) localisation of the AT1R protein.

Neuroanatomical studies consistently show that the AT1R is expressed by parvocellular neurosecretory CRH and TRH neurons, but not vasopressin, oxytocin or pre-autonomic neurons or microglia in the PVN (Aguilera et al., 1995b; Lenkei et al., 1995; Oldfield et al., 2001; Gonzalez et al., 2012; Wang et al., 2016b; de Kloet et al., 2017). Whether the AT1R is expressed in astrocytes has been less consistently reported. A study using a transgenic reporter mouse found that a minor population of PVN astrocytes are AT1R-expressing (Gonzalez et al., 2012). In rat, though AT1R mRNA has been amplified from astrocytes isolated from broad hypothalamic tissue-punches (Stern et al., 2016), an early study using in-situ hybridisation did not observe AT1R mRNA in PVN astrocytes (Lenkei et al., 1995). Importantly, however, this early in-situ hybridisation study also failed to detect AT1R mRNA in the SON (Lenkei et al., 1995), a region with numerous AT1R-expressing cells when assessed with more sensitive in-situ hybridisation techniques (i.e., RNAscope technology) or a transgenic reporter (Sandgren et al., 2018). This thesis will use RNAscope in-situ hybridisation to determine whether the AT1R is expressed by astrocytes in the rat PVN.

The anatomical distribution of the AT1R in the PVN is supported by several in-vitro electrophysiological studies that show that after pharmacological synaptic isolation the excitatory actions of angiotensin II is eliminated in presympathetic and magnocellular PVN neurons (Li et al., 2003a; Latchford and Ferguson, 2004; Li and Pan, 2005; Stern et al., 2016), but persists in parvocellular neurosecretory neurons (Latchford and Ferguson, 2005). Conversely, Cato and Toney (2005) observed that angiotensin II continued to excite RVLM-

projecting PVN neurons during synaptic isolation in-vitro, indicating that angiotensin II acts directly on these neurons. However, this was not observed in other in-vitro recordings of RVLM-projecting PVN neurons (Li and Pan, 2005; Stern et al., 2016), nor is it consistent with anatomical data (Oldfield et al., 2001; de Kloet et al., 2017) or in-vivo experiments showing that ionotropic glutamate receptor activation is obligatory for the renal SNA response to PVN angiotensin II microinjection (Stern et al., 2016). Therefore, when considered together, anatomical and functional data suggest that angiotensin II stimulates presympathetic and magnocellular PVN neurons indirectly via a presynaptic mechanism yet activates parvocellular neurosecretory neurons directly.

Two mechanisms have been proposed to underlie the presynaptic action of angiotensin II on presympathetic PVN neurons; 1) presynaptic disinhibition and 2) inhibition of astrocyte glutamate transport. Support for the first putative mechanism came from studies by Li and Pan (Li et al., 2003a; Li and Pan, 2005). These authors measured the effect of angiotensin II on exogenously-evoked inhibitory and excitatory post-synaptic currents in PVN neurons with spinal (Li et al., 2003a) and RVLM (Li and Pan, 2005) projections in-vitro, and found that angiotensin II reduced inhibitory events, yet left the excitatory ones unaltered. Furthermore, in the presence of bicuculline, angiotensin II failed to increase presympathetic PVN neuronal discharge in-vitro (Li et al., 2003a; Li and Pan, 2005). These experiments indicate that angiotensin II acts at presynaptic GABAergic terminals to reduce the probability of GABA release. Nevertheless, this putative mechanism requires validation in-vivo, particularly because it is does not appear that neurons in the peri-PVN area, a dominant source of GABAergic input to the PVN (Roland and Sawchenko, 1993), express the AT1R (de Kloet et al., 2016a; de Kloet et al., 2016b).

The second mechanism thought to underlie the presynaptic action of angiotensin II on presympathetic PVN neurons – inhibition of astrocyte glutamate transporters – was proposed more recently by Stern et al. (2016). Astrocytes are responsible for clearing more than 90% of extracellular glutamate in the brain via the continual activity of their glutamate transporter-1 (GLT-1; also referred to as excitatory amino-acid transporter-2) (Rose et al., 2017). The ongoing efficacy of astrocyte glutamate clearance sets a “tonic” glutamate current in magnocellular and presympathetic PVN neurons due to the activation of extra-synaptic NMDA receptors, a phenomena that is differentially regulated from synaptic glutamatergic transmission (Fleming et al., 2011; Stern et al., 2016). Using in-vitro patch-clamp electrophysiology, Stern et al. (2016) found that angiotensin II inhibited glutamate transporter

activity in PVN astrocytes and produced a GLT-1-dependent “tonic” NMDA current in RVLM-projecting PVN neurons. These authors further showed that PVN microinjection of either a GLT-1 or NMDA receptor antagonist eliminated the sympathoexcitatory and pressor response to angiotensin II in anaesthetised animals. Together this work strongly suggests that the primary mechanism by which angiotensin II stimulates sympathetic outflow from the PVN is through an inhibition of astrocyte GLT-1 function and subsequent glutamatergic activation of presympathetic neurons. This mechanism is not necessarily opposed to the aforementioned model of presynaptic disinhibition proposed by Li and Pan; for example, it is conceivably an increase in ambient glutamate levels induced by angiotensin II might inhibit GABA release via activation of presynaptic group II metabotropic glutamate receptors (Chen and Bonham, 2005; Ye et al., 2013).

There are nevertheless unanswered questions concerning the signalling mechanism of angiotensin II in PVN astrocytes. Most notably, because of the aforementioned uncertainty concerning the expression of the AT1R in PVN astrocytes, it is unclear whether angiotensin II interacts with astrocytes directly or via an intermediary paracrine signal. It also awaits to be determined whether angiotensin II activates magnocellular PVN neurons via a similar mechanism, though this is suggested by the observation that glial toxins administered intracerebroventricularly impair angiotensin II-evoked vasopressin release (Flor et al., 2018).

How do PVN neurons adjust SNA during osmotic stress?

Terrestrial mammals encounter osmotic stress due to dehydration (water deprivation) or excessive sodium intake. While both of these conditions increase extracellular fluid sodium and osmolality, dehydration is a more complex challenge that is also associated with hypovolaemia and high circulating angiotensin II (Barney et al., 1983; Scrogin et al., 1999; Toney and Stocker, 2010). Importantly, both challenges are associated with an increase in sympathetic vasomotor tone in rat (Scrogin et al., 1999; Antunes et al., 2006; Toney and Stocker, 2010; Colombari et al., 2011; Holbein and Toney, 2015), which is dependent on the detection of the ionic concentration of the extracellular fluid by the brain because intracarotid infusion of hypotonic fluid rapidly and reversibly decreases lumbar SNA and arterial pressure in water deprived rats (Brooks et al., 2005), while transection of the lamina terminalis eliminates the lumbar sympathoexcitatory response to acute peripheral and central hypertonic saline (Antunes et al., 2006; Stocker et al., 2015).

Both acute sodium-loading and chronic dehydration increase lumbar and splanchnic SNA via the activation of ionotropic glutamate receptors in the PVN (Antunes et al., 2006; Bardgett et al., 2014b; Holbein et al., 2014; Holbein and Toney, 2015). During acute sodium-loading, both spinally- and RVLM-projecting PVN neurons are recruited as the evoked lumbar sympathoexcitation is attenuated with either intrathecal V_{1A} receptor antagonism (Antunes et al., 2006) or RVLM ionotropic glutamate receptor blockade (Stocker et al., 2015). In contrast, dehydration appears to preferentially activate glutamatergic RVLM-projecting PVN neurons as opposed to those with spinal projections as indicated by Fos expression (Stocker et al., 2004b; Stocker et al., 2006) and observations that water deprived animals display an exaggerated depressor response to RVLM ionotropic glutamate receptor blockade (Brooks et al., 2004) but not intrathecal V_{1A} receptor antagonism (Veitenheimer and Osborn, 2011). PVN neurons are also required to modulate renal SNA during both challenges, although in different directions; specifically, PVN neurons decrease renal SNA in response to acute hypertonic saline (Badoer et al., 2003; Frithiof et al., 2014) but positively contribute to resting renal SNA in water deprived rats (Stocker et al., 2004a; Stocker et al., 2005). However, the positive influence that the PVN exerts on resting renal SNA during dehydration is likely driven by a non-osmotic stimulus, such as hypovolaemia, because plasma hyperosmolality does not directly contribute to resting renal SNA in water deprived rats (Scrogin et al., 2002).

How is blood osmolality conveyed to presympathetic PVN neurons?

Stocker and colleagues first demonstrated that OVLT neurons are responsible for generating sympathoexcitation in response to an acute sodium-load. They showed that hypertonic saline injections into the lateral ventricle or OVLT produce concentration-dependent increases in lumbar and adrenal SNA and arterial pressure, which were eliminated if OVLT neurons were inactivated with muscimol (Kinsman et al., 2017b). In-vitro extracellular recordings performed by the same group indicate that there are two major populations of OVLT neuron that vary with respect to osmolyte detection: the largest population discharge in response to hypertonic solutions indiscriminately of the solute, while another population discharge specifically in response to hypertonic saline but not other effective osmoles (sorbitol and mannitol) (Kinsman et al., 2017a). This study suggests that a population of ‘NaCl-sensitive’ OVLT neurons are likely hard-wired to regulate SNA because hyperosmotic solutions containing effective osmoles other than NaCl (sorbitol and mannitol) did not produce sympathoexcitation when administered into the OVLT (Kinsman et al., 2017a).

Recently, Nomura et al. (2019) demonstrated that NaCl-sensitive OVLT neurons responsible for regulating SNA sense extracellular sodium concentration via a specialised sodium sensor, the Na_x channel. These authors observed that genetic deletion of the Na_x channel in mice eliminated the sympathetic, but not the vasopressin, component of the pressor response to intracerebroventricular hypertonic saline and the development of salt-induced hypertension. This phenotype is likely due to the removal of Na_x channel actions in the OVLT because OVLT neurons with PVN-projections were found to be NaCl-sensitive in-vitro and optogenetic inactivation of the glutamatergic population blunted the pressor response to central hypertonic saline (Nomura et al., 2019). These neurons do not sense sodium directly, however, because in the mouse OVLT (and SFO) the Nax channel is not present in neurons, only ependymal cells and astrocytes (Watanabe et al., 2006; Nehme et al., 2012). Indeed, Nomura et al. (2019) showed that NaCl-sensitive OVLT neurons produce sympathetically-mediated increases in blood pressure via a lactate/H^+ signal originating from local glia.

This body of work therefore demonstrates that PVN-projecting OVLT neurons are fundamental for producing increases in SNA during sodium-loading. There are nonetheless several important points that require clarification. *Firstly, is glutamate the only neurochemical involved in this pathway?* In that regard, it has been reported that antagonising the AT1R in the PVN moderately attenuates the sympathoexcitatory and pressor response to intracarotid hypertonic saline (Chen and Toney, 2001). Noting that AT1R activation enhances glutamatergic transmission in the PVN (Stern et al., 2016), one possibility is that angiotensin II potentiates the glutamatergic drive of PVN presympathetic neurons by OVLT neurons which has been described in SON vasopressin neurons (Stachniak et al., 2014). *Next, what role, if any, do SFO neurons play in the cardiovascular response to extracellular fluid hypertonicity?* Tiruneh et al. (2013) observed that hypertonic saline infused into the SFO produced a gradual increase in arterial pressure in rat and that lesioning the SFO reduced the pressor response to central hypertonic saline by ~50%. While SNA was not directly assessed in this study, it likely that this SFO-dependent pressor response to hypertonic saline was mediated via a humoral outflow, such as vasopressin, since the work by Kinsman et al. (2017b) and Nomura et al. (2019) discussed above demonstrates that the sympathetic response is almost certainly driven by the OVLT. Indeed, the pressor response to prolonged (several minutes) central hypertonic saline infusion has a vasopressin-component in mouse (Nomura et al., 2019) and an OVLT-independent component in rat (Kinsman et al., 2017b). It is possible that SFO neurons facilitate sympathetically- and/or humorally-mediated increases

in arterial pressure during dehydration when other stimuli are present (Scrogin et al., 1999; Veitenheimer et al., 2012). While this possibility has not been directly tested, there is some evidence that the SFO may be better suited to respond to chronic dehydration than the OVLT; specifically, some PVN-projecting SFO neurons discharge in response to both intracarotid hypertonic saline and angiotensin II in-vivo (Gutman et al., 1988), while the OVLT neurons that are activated by intravenous hypertonic saline or angiotensin II occupy distinct non-overlapping regions (Oldfield et al., 1991c; McKinley et al., 1992). *Finally, what role do MnPO neurons play?* In-vivo single-unit recordings show that populations of MnPO neurons with PVN projections increase their discharge in response to intracarotid hypertonic saline and/or angiotensin II (Stocker and Toney, 2005, 2007). Importantly, some of these neurons are also barosensitive (Stocker and Toney, 2005) or responsive to vagal afferents (Stocker and Toney, 2007). Thus, haemal and cardiovascular inputs converge on MnPO neurons, possibly representing an anatomical substrate by which the lamina terminalis can coordinate cardiovascular and behavioural responses to complex challenges such as dehydration (McKinley et al., 2015).

Important role of the PVN in hypertension

Soon after the discovery of PVN neurons with efferent connections to the spinal-cord by Saper et al. (1976), several researchers independently reported that large lesions of the PVN region substantially attenuated hypertension development in rat models of salt-sensitive hypertension (Azar et al., 1981; Goto et al., 1981; Nakata et al., 1989), primary hypertension (i.e., SHR) (Ciriello et al., 1984; Takeda et al., 1991) and renovascular hypertension (Earle and Pittman, 1995). These studies therefore indicated that the PVN is an important structure in the aetiology of multiple forms of hypertension. This conclusion is supported by more recent observations that acute pharmacological inactivation of PVN neurons or removal of excitatory glutamatergic drive produces an exaggerated depressor response in various hypertensive rat models under conscious (Martin and Haywood, 1998; da Silva et al., 2011; Gabor and Leenen, 2012, 2013) and anaesthetised (Li and Pan, 2007b; Xiong et al., 2012; Bardgett et al., 2014a) conditions, and is in keeping with chronic studies showing that hypertension development is mitigated following PVN over-expression of neuronal nitric oxide synthase in renovascular hypertensive rats (Rossi et al., 2010) and a hyperpolarising ion channel in SHR (Geraldes et al., 2014). Most interestingly, PVN neuronal inhibition is associated with an exaggerated reduction in lumbar (Allen, 2002), but not renal (Akine et al., 2003), SNA in SHR, yet produces an enhanced decrease in both renal and splanchnic SNA in

rats with angiotensin II-salt induced hypertension (Bardgett et al., 2014a). Thus, although PVN neuronal activity contributes to many forms of hypertension in the rat, the peripheral effectors recruited may vary according to the condition. A primary aim of this thesis is to examine whether PVN neurons contribute to hypertension in PKD and through what peripheral effector.

Several in-vivo pharmacological studies indicate that the balance between excitatory-to-inhibitory inputs to the PVN is shifted towards excitation in hypertension (Martin and Haywood, 1998; Li and Pan, 2007b; Gabor and Leenen, 2012, 2013). In SHR, this shift is reflected by both a suppression of GABAergic inputs and an enhancement of glutamatergic inputs. Specifically, Li and Pan (2007b) showed that, in SHR, the lumbar sympathoexcitatory and pressor response to PVN microinjection of the $GABA_A$ antagonist gabazine was blunted, whereas the reverse response evoked by PVN microinjection of kynurenic acid was greatly enhanced. In-vitro patch-clamp recordings suggest that suppressed GABAergic input to presympathetic PVN neurons in the SHR is due to a diminished post-synaptic $GABA_A$ receptor function (Li and Pan, 2006; Ye et al., 2012b), likely because of a combination of reduced $GABA_A$ receptor density (Kunkler and Hwang, 1995) and disrupted transmembrane chloride gradient (Ye et al., 2012b). In contrast, enhanced glutamatergic input to presympathetic PVN neurons in the SHR is due to both pre- and post-synaptic mechanisms as strongly suggested by several in-vitro electrophysiological studies showing that glutamatergic excitatory post-synaptic currents (EPSCs) in presympathetic PVN neurons occur at a higher basal frequency and have a higher amplitude when evoked with puffs of NMDA or an electrical stimulus (Ye et al., 2011; Li and Pan, 2017; Qiao et al., 2017). The enhanced terminal release of glutamate in SHR is attributed to activation of pre-synaptic NMDA receptors because the basal frequency of EPSCs in presympathetic PVN neurons is reduced in SHR but not controls animals with an NMDA receptor antagonist administered in the bath solution (i.e., extracellularly) despite being unaffected by an NMDA channel blocker included in the recording solution (i.e., administered intracellularly) in either strain (Ye et al., 2011). Therefore, enhanced activation of NMDA receptors at the pre- and post-synapse underlie the greater basal discharge of presympathetic PVN neurons observed in-vitro in the SHR (Ye et al., 2011; Li et al., 2015b; Li et al., 2017; Qiao et al., 2017). A gamut of different protein kinases have been reported to contribute to the enhanced pre- and post-synaptic NMDA receptor activity in the PVN and sympathetic outflow in SHR (Ye et al., 2011; Li et al., 2015b; Li et al., 2017; Qiao et al., 2017). However, surprisingly no study has determined

whether the augmented level of glutamatergic tone in the PVN in SHR is contributed to by an increased afferent drive originating in a separate brain region.

In the SHR, the augmentation of glutamatergic input to RVLM-projecting PVN neurons is reflected by an increase in excitatory drive to the RVLM. Specifically, while bilateral blockade of ionotropic glutamate receptors in the RVLM with kynurenic acid does not affect resting blood pressure in control animals, it lowers it by ~40 mmHg in SHR (Ito et al., 2000), a qualitatively similar effect to that observed when PVN excitation is blocked (Li and Pan, 2007b). The exaggerated reduction in blood pressure to RVLM kynurenic acid is also observed in other hypertensive models which exhibit increased PVN excitation, namely the Dahl salt-sensitive rat (Ito et al., 2001) (Gabor and Leenen, 2012) and renovascular hypertensive rat (Bergamaschi et al., 1995; Earle and Pittman, 1995). These findings are consistent with the notion that an excessive level of glutamatergic drive to RVLM-projecting PVN neurons contributes to the maintenance of heightened sympathetic vasomotor tone in hypertension. However, this schema is no doubt more complicated because other factors are also strongly implicated in excessive drive of presympathetic RVLM neurons in SHR, including peripheral chemoreceptor overactivity (Abdala et al., 2012; McBryde et al., 2013) and local brainstem ischemia (Marina et al., 2015). Whether these factors are independent or related to PVN excitation deserves clarification.

Angiotensin II is considered central to many forms of hypertension (Boger et al., 1990; Chapman et al., 2010; Santos et al., 2012; Yiannikouris et al., 2012). Acute pharmacological inhibition of the AT1R in the PVN reverses the hypertension produced by two-week continuous subcutaneous administration of angiotensin II in the rat (Gabor and Leenen, 2013). Moreover, in the rat, viral AT1R-knockdown in the PVN substantially reduces the acute pressor response to intravenous angiotensin II (Northcott et al., 2010) and prevents the rise in blood pressure produced by chronic subcutaneous angiotensin II administration (Chen et al., 2014). Clearly then the PVN region is critical for experimental angiotensin II-induced hypertension. Conceivably, circulating angiotensin II could activate AT1R in the PVN directly, by passing across the blood-brain barrier (Biancardi et al., 2014b), or indirectly, by activating AT1R on SFO neurons that themselves release angiotensin II in the PVN (Lind et al., 1984; Imboden et al., 1987; Jhamandas et al., 1989; Li and Ferguson, 1993a). The latter possibility is supported by observations that SFO lesion (Hendel and Collister, 2005) or knockdown of AT1R (Wang et al., 2016a) also largely prevents angiotensin II-induced hypertension in rats. However, there is debate as to whether SFO efferents release

angiotensin II into the PVN because although the pressor response to SFO electrical stimulation is dependent on PVN AT1R (Bains et al., 1992), the pressor response to SFO microinjection of bicuculline is not (Llewellyn et al., 2012). Furthermore, the mechanisms underlying angiotensin II-induced hypertension are no doubt more complex because kidney-specific AT1R knockout also impairs angiotensin II-induced hypertension (Crowley et al., 2006).

Several lines of evidence indicate that AT1R activation in the PVN in angiotensin II-induced hypertension involves an enhancement of local glutamatergic transmission. Gabor and Leenen (2013) showed that PVN microinjection of kynurenic acid lowers blood pressure to normal levels in rats with angiotensin II-induced hypertension. However, this manipulation did not lower blood pressure further if PVN AT1R were antagonised, suggesting that enhanced glutamatergic transmission is dependent on local AT1R activation. Subsequent work in mice indicated that NMDA receptor plasticity may be responsible. Specifically, subcutaneous angiotensin II infusion was found to increase the amplitude of post-synaptic NMDA currents in presympathetic PVN neurons (Wang et al., 2013). This enhancement of post-synaptic NMDA function is potentially driven by an increase in the trafficking of the GluN1 NMDA receptor subunit to the plasma membrane of PVN dendrites, which is supported by ultra-structural analysis and functional data showing that viral knockdown of GluN1 in the PVN significantly attenuates the development of angiotensin II-induced hypertension in mice (Glass et al., 2015). It remains to be determined whether enhanced NMDA receptor trafficking at the post-synapse arises secondary to the acute actions of angiotensin II in the PVN (i.e., through astrocyte glutamate handling) or potentially through a separate paracrine signal. Irrespective, when considered together these findings highlight that persistently heightened local ‘angiotensin tone’ strengthens glutamate transmission in the PVN, a conceivable consequence of which might be to enhance the cardiovascular response to subsequent stimuli that recruit glutamatergic inputs to the PVN (e.g., dehydration or angiotensin II itself) (Bardgett et al., 2014b).

Modulation of baroreflex function by PVN neurons

The PVN has extensive projections to all brainstem regions that constitute the primary baroreflex circuit (Geerling et al., 2010) and is therefore anatomically primed to modulate baroreflex function. Disinhibiting the PVN through antagonism of $GABA_A$ receptors (Page et al., 2011) or neuropeptide Y, a tonically active inhibitory input (Cassaglia et al., 2014; Shi et al., 2017), enhances the gain and upper range of the lumbar sympathetic baroreflex in

anaesthetised rats. There are several possible interpretations of these findings. One interpretation is that since these experimental manipulations significantly raise resting SNA and therefore likely augment the basal discharge of presympathetic RVLM neurons, the degree of 'presympathetic tone' capable of being inhibited by the baroreflex is greater which in and of itself would enhance baroreflex-mediated sympathoinhibitory responses. Alternatively, these findings could suggest that the PVN contains a population of neurons that restrain baroreflex regulation of lumbar SNA and that their activity is tonically inhibited by GABAergic and neuropeptide Y inputs. However, this neuronal population would appear to be maximally suppressed under normal conditions because *en masse* inactivation of PVN neurons with muscimol does not affect baroreflex regulation of lumbar SNA (Cassaglia et al., 2011; Shi et al., 2015a). The neural pathway downstream of the PVN that mediates sympathetic baroreflex modulation has not been characterised, but as noted by Dampney (2017) could theoretically result from a suppression of baroreceptor transmission at the level of the NTS or a facilitation of GABAergic CVLM neurons.

There is also evidence that PVN neurons are capable of modulating baroreflex control of heart rate. This was initially suggested by pharmacological studies which observed that PVN microinjection of glutamate agonists (Jin and Rockhold, 1989; Chen et al., 1996) or noradrenaline (Hwang et al., 1998) blunted the magnitude of baroreflex-evoked bradycardia. Unfortunately, these studies did not generate logistic-sigmoid baroreflex function curves and therefore it is not clear whether this attenuated baroreflex response reflects a depression of reflex sensitivity or a resetting to a higher level of arterial pressure. These studies nonetheless show that PVN neurons can influence cardiac baroreflex function and that the direction is inhibitory when stimulated *en masse*. In normal unchallenged animals, control of cardiac baroreflex function by the PVN is probably minimal because neither acutely silencing (Cassaglia et al., 2011; Shi et al., 2015a) nor chronically ablating (Rockhold et al., 1990) PVN neurons affects baroreflex regulation of heart rate.

Subsequent work uncovered that there are at least two PVN neuronal populations that exert opposing influences on the cardiac baroreflex and together facilitate adaptive responses to exercise (Michelini and Bonagamba, 1988; Dufloth et al., 1997; Braga et al., 2000; Higa et al., 2002). Vigorous exercise demands greater perfusion pressures concurrent with tachycardia (Raven et al., 2002). Thus, the baroreflex is reset to higher levels of arterial pressure and heart rate (i.e., an upwards and rightwards shift of the baroreflex function curve) (Dampney, 2017). Studies by Michelini and colleagues indicate that baroreflex resetting

during exercise is mediated at least in part by vasopressinergic PVN efferents that innervate the NTS (Buijs, 1978; White et al., 1984). They showed that administering vasopressin directly into the NTS region in conscious rats displaced the baroreflex to higher pressure and heart rate values, while antagonising NTS V_{1A} receptors in exercising animals moderately attenuated exercise tachycardia (Michelini and Bonagamba, 1988; Dufloth et al., 1997). Further investigations revealed that oxytocinergic PVN efferents terminating in the NTS region exert an opposing role, serving to enhance the lower range and sensitivity of the heart rate baroreflex (Higa et al., 2002). Oxytocinergic efferents supplying the NTS appear to be recruited in exercise trained animals to facilitate a lower level of tachycardia during exercise (Braga et al., 2000). The divergent influence of different NTS-projecting PVN efferents on cardiac baroreflex function is consistent with several in-vitro recording studies in brainstem slices which show that vasopressin inhibits (Bailey et al., 2006), whereas oxytocin enhances (Peters et al., 2008), glutamatergic EPSCs in NTS neurons that receive monosynaptic input from cranial viscerosensory afferents. Thus, vasopressin and oxytocin most likely affect cardiac baroreflex function via a presynaptic action in the NTS, involving the differential modulation of glutamate release from baroreceptor afferent terminals.

Magnocellular vasopressin neurons

Vasopressin is synthesised in the soma of magnocellular neurons and is packaged into neurosecretory vesicles as a prohormone before being transported along the axon to the posterior pituitary gland via the hypothalamo-neurohypophysial tract (Morgenthaler et al., 2008). Magnocellular neurons project a single axon to the pituitary gland, with each axon arborising extensively to give rise to thousands of terminals, each containing many neurosecretory vesicles (Leng et al., 1999).

The half-life of vasopressin is extremely short, less than 5 minutes in rat (Forsling et al., 1973; Morgenthaler et al., 2006). Magnocellular neurons are therefore required to continually release vasopressin into the peripheral circulation in order to concentrate urine. The exocytosis of vasopressin is chiefly governed by action potentials originating at the soma of magnocellular neurons (Brown et al., 2013). In-vitro studies using isolated rat neural lobes show that vasopressin release increases with the frequency of continuous stimulation up to ~13 Hz, but plateaus or even decreases thereafter (Bicknell, 1988). However, the pattern of discharge is also important. Magnocellular vasopressin neurons display a range of firing properties in-vivo: silence, continuous, irregular or phasic (Scott et al., 2009a). Stimulation studies in isolated neural lobes highlight that phasic discharge is the most effective pattern for

vasopressin release (Dutton and Dyball, 1979). Importantly, magnocellular vasopressin neurons are intrinsically silent and require glutamatergic afferents to generate their tonic activity (Brown et al., 2004). As will be discussed beneath, a major source of afferent glutamatergic drive originates from the forebrain lamina terminalis. However, vasopressin release is capable of being modulated at the level of the pituitary gland too, specifically by purines (Knott et al., 2007) and local ionic gradients (Marrero and Lemos, 2010).

Osmotic control of vasopressin release

Changes in the effective osmotic pressure of the extracellular fluid is a strong stimulus for vasopressin release (Verney, 1947; McKinley et al., 1978). The stimulus-response relationship between plasma osmolality and vasopressin concentration is linear and extremely sensitive; for instance, an increase in plasma osmolality of just 2% results in a 2- to 3-fold elevation in circulating vasopressin concentration (Dunn et al., 1973). In response to sodium-loading or water deprivation, magnocellular neurons in the SON and PVN robustly express Fos initially and other immediate-early genes, namely Fra-1 and Fra-2, after prolonged stimulus exposure (Sharp et al., 1991; Miyata et al., 2001; Pirnik et al., 2004). Furthermore, the strong relationship between plasma osmolality and vasopressin release is clearly reflected in the discharge of PVN and SON magnocellular vasopressin neurons in-vivo (Leng et al., 2001; Joe et al., 2014; Hernández et al., 2015).

The lamina terminalis is fundamental for the osmotic stimulation of vasopressin release. Lesions of the OVLT and MnPO, together or separately, impair but often do not eliminate hyperosmotic stimulation of vasopressin release in rat, sheep and dog (Thrasher et al., 1982; Mangiapane et al., 1983; McKinley et al., 1984; Leng et al., 1989). Lesions of the SFO have also been reported to reduce vasopressin release in response to hypertonic saline in rat (Mangiapane et al., 1984). However, because of the close proximity of these nuclei, particularly the OVLT and MNPO, it is unlikely lesions positioned in one region completely spared the neuronal processes of other regions. A study by McKinley et al. (2004) found that hypertonic saline-induced vasopressin release in sheep was partly inhibited by lesions of the SFO, OVLT or MnPO separately. Although these authors observed that combined SFO and OVLT lesion had an additive effect, complete removal of the lamina terminalis was required to eliminate the response altogether. Thus, the SFO, OVLT and MnPO likely all contribute to the osmotic stimulation of vasopressin release.

Ionotropic glutamate transmission is responsible for driving the activity of SON magnocellular vasopressin neurons during plasma hypertonicity in-vivo (Brown et al., 2004). In a classic experiment, Richard and Bourque (1995) recorded from magnocellular neurons in hypothalamic explants orientated such that osmotic stimuli could be selectively applied to the OVLT. With this preparation the authors clearly demonstrate a non-NMDA receptor-dependent increase in magnocellular neuron EPSP and action potential frequency with increasing osmolality of the OVLT perfusion solution. However, intravenous infusion of hypertonic saline in-vivo not only increases the concentration of glutamate in the SON, but also GABA (Leng et al., 2001). Furthermore, exogenous stimulation of the OVLT and MnPO evokes inhibitory $GABA_A$ receptor-dependent responses in magnocellular neurons in-vivo (Nissen and Renaud, 1994; Leng et al., 2001). Modelling suggests that this GABAergic input to SON magnocellular neurons enables magnocellular neurons to respond linearly over the physiological range of plasma osmolality (Leng et al., 2001).

Unlike OVLT neurons that regulate SNA via sodium- but not osmolality-based sensing, the circumventricular organ neurons regulating vasopressin release appear to use both sodium- and osmolality-based detection because hypertonic solutions of NaCl or sugar produce anti-diuresis when administered peripherally or centrally, but the response is greater with equi-osmotic NaCl solutions (McKinley et al., 1978). However, the precise mechanism by which the circumventricular organ neurons regulating vasopressin release detect sodium and osmolality is still not known. Initial work of Bourque and colleagues indicated that a mechanosensitive variant of the Trpv1 channel is required for the detection of osmolality in OVLT neurons via the transduction of cell volume (Ciura and Bourque, 2006; Ciura et al., 2011). However, subsequent studies showed that the Trpv1 receptor is dispensable for osmoregulation of vasopressin release because Trpv1 knockout mice and rats display normal vasopressin responses to hyperosmotic stimuli (Kinsman et al., 2014; Tucker and Stocker, 2016). Furthermore, hyperosmolality-induced Fos activation in the OVLT, PVN and SON is unaffected in Trpv1 knockout mice (Taylor et al., 2008; Kinsman et al., 2014). This could be due to the presence of redundant osmoregulatory mechanisms involving peripheral osmoreceptors (see below) or other molecular mechanisms of central osmoreception, such as sodium-sensing. Nonetheless the Na_x channel does not appear to be involved in vasopressin release because mice lacking this channel show normal osmoregulatory vasopressin responses (Nagakura et al., 2010). Another candidate sodium-sensor that has been proposed is the epithelial sodium channel that is expressed in SFO and OVLT neurons and glia (Amin

et al., 2005; Miller and Loewy, 2013; Miller et al., 2013). There is some evidence that this channel is involved in central osmoregulation of vasopressin release because intracerebroventricular administration of benzamil, an epithelial sodium channel inhibitor, virtually eliminates hyperosmotic saline evoked vasopressin release (Nishimura et al., 1998). Nonetheless, more detailed investigations involving specific manipulations of the epithelial sodium channel in OVLT and SFO cells will be required to further examine the role of this channel in the osmoregulation of vasopressin release.

Both water and salt consumption produce changes in plasma vasopressin levels before detectable changes in plasma osmolality (Geelen et al., 1984; Spinelli et al., 1987; Huang et al., 2000). This suggests that tonicity is detected or anticipated in the periphery before or immediately following fluid absorption in the gut. Classic studies indicated that the act of drinking itself, via oropharyngeal receptors, is sufficient to inhibit vasopressin release in dogs (Appelgren et al., 1991) and sheep (Blair-West et al., 1987). In contrast, in mice, SON vasopressin neurons are regulated by oropharyngeal and gastrointestinal afferents but also via a feed-forward anticipatory mechanism (Mandelblat-Cerf et al., 2017; Zimmerman et al., 2019).

Finally, it should be noted that magnocellular vasopressin neurons display cell-autonomous osmosensitivity in-vitro (Oliet and Bourque, 1992), such that SON administration of hyperosmotic solutions evoke vasopressin release in-vivo (Ludwig et al., 1994). The physiological relevance of this intrinsic osmosensitivity is uncertain because magnocellular neurons require glutamatergic input to discharge, including under hyperosmotic conditions (Brown et al., 2004). One possibility is that cell-autonomous osmosensitivity may serve to modulate the sensitivity of magnocellular neurons to synaptic input and therefore facilitate the overall control of vasopressin release.

Blood volume and pressure influence on vasopressin release

Hypotension and hypovolaemia are other potent stimuli for vasopressin release. In contrast to the linear relationship between plasma osmolality and vasopressin concentration, the relationship between blood volume and vasopressin release is exponential, with circulating vasopressin level showing little to no increase in response to depletions in blood volume of <10% but incrementally larger increases in response to further volume depletion (Dunn et al., 1973). The differing sensitivities of vasopressin to blood osmolality versus volume presumably provides another means to gate the hormones anti-diuretic and pressor actions.

While certainly there are physiological conditions when changes in blood osmolality and volume occur independently (i.e. mild dehydration and haemorrhage), water deprivation simultaneously elevates plasma osmolality and contracts blood volume. In this context, the increase in vasopressin produced by hyperosmolality and hypovolaemia is additive and, in some cases, synergistic (Dunn et al., 1973; Stricker and Verbalis, 1986; Kondo et al., 2004).

Arterial blood pressure is transduced by arterial baroreceptors whereas blood volume is transduced by low-pressure volume receptors located in the atria and great veins (Harris, 1979; Hainsworth, 2014). In-vivo recordings and Fos studies show that magnocellular vasopressin neuron activity is enhanced by decreases in blood volume and pressure and vice versa is suppressed by increases in blood volume and pressure (Roberts et al., 1993; Schiltz et al., 1997; Grindstaff et al., 2000b). It should be noted, however, that acute experimental changes in blood volume (i.e. haemorrhage and volume expansion) are often (but not always) associated with reciprocal changes in arterial pressure, making it difficult to tease apart the relative contributions of volume receptors and arterial baroreceptors to the regulation of vasopressin release in conditions such as haemorrhage or volume expansion. Furthermore, experimental increases in arterial pressure with phenylephrine, an α_1 receptor agonist, reduces venous return and may therefore also activate atrial volume receptors (Cannesson et al., 2012). Nevertheless it is clear that direct activation of arterial baroreceptor afferents and of atrial volume receptors (via balloon inflation near the atrial-vena cava junction) both effectively silence the phasic discharge of magnocellular vasopressin neurons (Yamashita and Koizumi, 1979; Grindstaff et al., 2000b).

The modulation of vasopressin release by volume receptors and arterial baroreceptors is mediated via a polysynaptic pathway involving brainstem catecholaminergic neurons. An impairment in magnocellular neuron barosensitivity is observed following neurotoxic lesion of A6, and to a lesser extent A1, cell groups (Banks and Harris, 1984; Grindstaff et al., 2000a). Importantly, however, retrograde lesion of noradrenergic inputs to the SON and perinuclear region does not affect the barosensitivity of magnocellular vasopressin neuron discharge (Day and Renaud, 1984), demonstrating that A6 and A1 noradrenergic neurons must relay baroreceptor information through another brain region. Subsequent loss-of-function studies combined with anatomical data suggested that noradrenergic neurons likely mediate baroreflex inhibition of SON magnocellular neurons through a projection to the forebrain diagonal band of Broca which in turn inhibits SON magnocellular vasopressin neurons via efferent connections with GABAergic interneurons in the perinuclear zone (Jones

and Moore, 1977; Cunningham et al., 1992b, a; Nissen et al., 1993; Voisin et al., 1994; Grindstaff et al., 2000a). Less clear is the central pathway through which the atrial volume receptors inhibit magnocellular vasopressin neurons, although a synapse in the perinuclear zone also appears to be obligatory (Grindstaff and Cunningham, 2001).

Angiotensin II modulation of vasopressin release

Systemic administration of angiotensin II reliably increases circulating vasopressin in all species examined (Bonjour and Malvin, 1970; Knepel et al., 1982; Brooks et al., 1986; Shi et al., 2003; Matsukawa and Miyamoto, 2011). The SFO and OVLT are activated by circulating angiotensin II via the AT1R (Allen et al., 1988; McKinley et al., 1992; Song et al., 1992; Li and Ferguson, 1993b; Oldfield et al., 1994). Oldfield et al. (1994) analysed Fos expression in SFO, OVLT and MnPO neurons with SON projections and found that the percentage of Fos expressing neurons with SON projections was twice as high in the SFO compared to the OVLT and MnPO. Lesion studies further support an important role of the SFO in mediating the stimulatory effect of circulating angiotensin II on magnocellular neuron discharge and vasopressin release in rat (Knepel et al., 1982; Ferguson and Renaud, 1986). Nevertheless, lesions of the OVLT do reduce systemic angiotensin II-evoked vasopressin release in dog (Thrasher and Keil, 1987). Of course the limitation of these studies is that lesions will likely destroy not only monosynaptic connections with the magnocellular neurons but also the interconnectivity between the circumventricular organs and MnPO. Therefore most likely circulating angiotensin II effects vasopressin release via the detection by both forebrain sensory circumventricular organs.

In addition to its circulating actions, angiotensin II also influences vasopressin release via local actions within the SON and PVN. Direct administration of angiotensin II into the SON or PVN stimulates peripheral vasopressin release (Shoji et al., 1989; Veltmar et al., 1992; Tsushima et al., 1994; Khanmoradi and Nasimi, 2016). RNA analysis indicates that the AT1R is present in magnocellular vasopressin neurons in the mouse SON contrasting the PVN where it is absent (Sandgren et al., 2018). Recent work by Sandgren et al. (2018) suggests that AT1R in SON magnocellular vasopressin neurons are involved in the osmotic regulation of vasopressin release. These authors showed that genetic deletion of AT1R from SON vasopressin neurons in mice elevated basal plasma osmolality and severely impaired urinary concentrating ability in response to an osmotic load. A possible mechanism underlying this effect is suggested by the work of Bourque and colleagues which shows that local angiotensin II enhances the osmosensitivity gain of SON magnocellular neurons

(Chakfe and Bourque, 2000; Sharif Naeini et al., 2006; Stachniak et al., 2014). Since the SON, like the PVN, receives angiotensinergic input from the SFO (Lind et al., 1984; Imboden et al., 1987; Jhamandas et al., 1989; Li and Ferguson, 1993a), it is possible that angiotensin II is co-released with glutamate from SFO terminals to potentiate the osmotic release of vasopressin. Another intriguing possibility is that this mechanism may enable elevated levels of circulating angiotensin II, such those observed during hypovolaemia as a consequence of water deprivation (Barney et al., 1983), to enhance the sensitivity of vasopressin release through direct actions in the SON. Angiotensin II may also enhance osmotically-evoked vasopressin release via actions in the PVN because blockade of the AT1R in this nucleus reportedly reduces vasopressin release following central hypertonic saline administration (Qadri et al., 1998). Although as previously noted, precisely how angiotensin II interacts with PVN magnocellular vasopressin neurons is unclear, but a mechanism involving astrocyte glutamate transport is suggested (Stern et al., 2016; Flor et al., 2018).

Recent evidence shows that AT_2 receptors have the capacity to suppress vasopressin release. Systemic administration of compound 21, an AT_2 receptor agonist that crosses the blood-brain barrier, decreases pituitary and plasma vasopressin concentration and enhances the vasopressin release evoked by intracerebroventricular angiotensin II (Hohle et al., 1995; Macova et al., 2009; de Kloet et al., 2016a). Determining the distribution of the AT_2 receptor in the brain has historically been problematic because antibodies are non-specific and autoradiography suffers from poor resolution. A recent study by de Kloet et al. (2016b) overcame these limitations using recent advances in in-situ hybridisation combined with a transgenic mouse that expresses a fluorescent reporter under the control of the AT_2 promoter. They found that although the PVN and SON did not contain AT_2 receptor-expressing cell bodies, PVN vasopressin neurons were enwrapped by fibres/terminals of AT_2 receptor-expressing neurons, of which were found to originate in part from GABAergic interneurons in the peri-PVN area. Slice electrophysiology showed that compound 21 activated AT_2 receptor-expressing peri-PVN neurons and produced GABAergic IPSCs in PVN vasopressin neurons (de Kloet et al., 2016a). Thus, one pathway by which activation of AT_2 receptor inhibits vasopressin release is via GABAergic interneurons in the PVN. The overall relevance of the AT_2 receptor to the control of vasopressin release by angiotensin II remains to be determined.

Plasticity of magnocellular vasopressin neurons in hypertension

There is strong evidence that the elevation of vasopressin release in chronically salt-loaded rats is facilitated by changes in the $GABA_A$ receptor chloride channel function in magnocellular vasopressin neurons. In a seminal study, Kim et al. (2011) measured the reversal potential for GABA (i.e., the membrane potential at which there is no net ion flow across the cell membrane) in PVN and SON magnocellular neurons of salt-loaded rats with in-vitro intracellular recordings. They found that GABAergic post-synaptic potentials (PSPs) were inhibitory in control animals, reflecting a GABA reversal potential close to resting membrane potential. In contrast, in rats chronically administered a high-salt diet, GABAergic PSPs were excitatory, reflecting a GABA reversal potential that was far more positive than resting membrane potentials and greater than the threshold for firing. Consequently, in salt-loaded rats, muscimol, a $GABA_A$ receptor agonist, increased the discharge of magnocellular neurons, whereas bicuculline, a $GABA_A$ receptor antagonist, lowered circulating vasopressin when given intravenously, effects not seen in control animals. This polarity-switch in $GABA_A$ receptor function in salt-loaded rats was shown to be due to an upregulation of NKCC1, a chloride importer, the consequence of which was presumably a greater intracellular chloride concentration.

Subsequent studies have demonstrated the relevance of the shift in $GABA_A$ receptor function in magnocellular neurons in rats with hypertension induced by high-salt intake (Choe et al., 2015b), DOCA-salt (Kim et al., 2013) and renin overexpression (Korpal et al., 2017). In all of these models, systemic V_{1A} receptor antagonism was found to moderately attenuate the hypertension observed (Kim et al., 2013; Choe et al., 2015b; Korpal et al., 2017). Both the DOCA-salt and renin-overexpressing model exhibited $GABA_A$ receptor excitation. In the DOCA-salt model this was reflect by an incremental decrease in the reversal potential for GABA over time as plasma osmolality and vasopressin increased and hypertension became more severe and also an abnormal pressor response to muscimol microinjection into the SON (Kim et al., 2013). In the renin-overexpressing model, $GABA_A$ receptor excitation was reflected by an increase in magnocellular neuron discharge in-vivo following local administration of muscimol and a lack of stimulation following bicuculline (Korpal et al., 2017). In the high-salt intake model, the reversal potential for GABA was more positive than controls but did not exceed the threshold for firing, suggesting that $GABA_A$ receptor inhibition was weakened but not converted to excitation (Choe et al., 2015b). Importantly, all three studies showed that baroreceptor activation failed to inhibit magnocellular neurons in-vivo in hypertensive animals. Thus, this pathological change in $GABA_A$ receptor function

may allow the facilitation of high vasopressin levels despite a chronic elevation of arterial pressure.

Hypertension and neurohumoral dysfunction in PKD

In the previous section the fundamental neurohumoral regulatory systems involved in the regulation of arterial pressure were reviewed in the context of physiological and pathophysiological states, particularly hypertension. Hypertension and neurohumoral dysfunction arise secondary to a number of chronic kidney diseases (CKD) (Ridao et al., 2001; Klein et al., 2003b), including PKD (Klein et al., 2001; Zittema et al., 2014). The following section will review the renal and cardiovascular manifestations of PKD, highlighting emerging evidence that implicates the CNS in the aetiology of both.

Overview of PKD and its manifestations

PKD is a group of monogenetic disorders characterised by the acquisition of multiple bilateral renal cysts that originate from the dilation or "ballooning" of the renal tubule epithelium (Nigro et al., 2015). PKD can be broadly classified into three types that occur with different incidences: autosomal dominant PKD (ADPKD; 1:2000 individuals), autosomal recessive PKD (ARPKD; 1:20,000) and nephronophthisis (1:50,000-100,000) (Al-Bhalal and Akhtar, 2008; Telega et al., 2013; Willey et al., 2017). All forms of PKD share a common set of key clinical features; namely, renal insufficiency, impaired urine concentrating ability and high rates of hypertension (Rall and Odel, 1949; Hansson et al., 1974; Waldherr et al., 1982; Gabow et al., 1984; Kaariainen et al., 1988; Kaplan et al., 1989; Hildebrandt et al., 1992; Fick et al., 1994; Zerres et al., 1996; Ridao et al., 2001; Guay-Woodford and Desmond, 2003; Schrier et al., 2003; Ho et al., 2012; Zittema et al., 2012; Sweeney et al., 2014). Nevertheless, the clinical onset and severity of renal insufficiency varies according to the type of PKD; ADPKD affects mostly adults and is the least aggressive in its presentation, whereas ARPKD and nephronophthisis affects mostly children and commonly requires renal replacement therapy (i.e. dialysis or renal transplant) or is lethal before adolescence (Hildebrandt and Zhou, 2007; Sweeney et al., 2014).

PKD arises from a mutation in one of several genes. Importantly, the proteins encoded by these genes are all are localised to the same organelle of the renal epithelial cells – the primary cilium – where they form a network of interacting proteins to influence a set of common signalling pathways (Hanaoka et al., 2000; Wang et al., 2007). This network-like interaction underpins the strong phenotypic convergence observed among different PKD genotypes.

Phenotypic convergence has proved extremely useful for preclinical investigations. A substantial fraction of PKD research has been directed at improving the management of ADPKD because it is not only the most prevalent form of PKD but also has the slowest disease progression. ADPKD is thus most likely to benefit from therapeutic interventions that slow cyst growth. However, until recently rodent models with orthologous ADPKD mutations have been largely inadequate for research because of the phenotype produced is either too mild or conversely too severe (Wu et al., 2000; Boulter et al., 2001). Fortunately rodent models with orthologous ARPKD or nephronophthisis mutations recapitulate many of the features of human ADPKD, and have been successful in testing of anti-cyst and anti-hypertensive therapies (Keith et al., 1994; Gattone et al., 2003; Torres et al., 2004; Wang et al., 2005; Zafar et al., 2007; Zittema et al., 2016). Therefore, despite notable differences among PKD types with respect to clinical onset and severity, the pathogenic mechanisms underlying cyst growth and hypertension are likely similar.

PKD genes and proteins

The majority of ADPKD cases arise from a mutation in either PKD1 (78%) or PKD2 (15%) genes that encode polycystin-1 and -2, respectively (Cornec-Le Gall et al., 2018). Polycystin-1 is an integral membrane protein whereas polycystin-2 is a transient receptor potential-like non-selective cation channel (Lemos and Ehrlich, 2018). Together polycystin-1 and -2 form a complex which is involved in intracellular Ca^{2+} signalling (Hanaoka et al., 2000; Nauli et al., 2003; Lemos and Ehrlich, 2018). It is likely that other rarer genes are yet to be discovered as shown by the recent identification of a third gene, GANAB, that causes a rare (0.3% of cases) mild form of ADPKD in association with severe cystic liver disease (Porath et al., 2016). Though ADPKD shows an autosomal dominant pattern of inheritance, heterogeneous mutations in a single PKD1 or PKD2 allele alone is not sufficient for cyst formation (Reeders, 1992). Instead cyst formation only initiates once the function of the non-mutant allele is lost in a progenitor epithelial cell due to either a somatic mutation or insufficient expression (Reeders, 1992; Eccles and Stayner, 2014).

Both ARPKD and nephronophthisis show an autosomal recessive pattern of inheritance. ARPKD is caused by a mutation in the PKHD1 gene which encodes fibrocystin (Al-Bhalal and Akhtar, 2008). Although the precise function of fibrocystin is not clear, it appears to interact with and support the function of polycystin-2 in the primary cilium (Kim et al., 2008). Nephronophthisis is produced by a mutation in one of nine genes (NPHP1-9). Again,

all proteins are localised to the primary cilia and some have been found to directly interact with the polycystin proteins (Hildebrandt et al., 2009; Omran, 2010).

Renal manifestations

Mechanism of cystogenesis

The primary cilium is a non-motile, microtubule-based protrusion on the cellular surface and in renal epithelial cells is involved in sensing urine-flow and paracrine factors (Yoder et al., 2002; Otto et al., 2003; McCooke et al., 2012; Praetorius and Leipziger, 2013). Precisely how disruption of PKD proteins within the renal primary cilia produces cysts is poorly understood. In normal physiological conditions, polycystin-dependent calcium signalling in the primary cilium regulates cell proliferation and apoptosis (Lemos and Ehrlich, 2018). In PKD, the initiating event for cyst production appears to be abnormal cell proliferation (Hanaoka and Guggino, 2000; Belibi et al., 2004; Chen et al., 2011). Uncontrollable and disorientated cell proliferation is thought to subsequently produce microscopic focal dilations of the renal tubule (Nigro et al., 2015). With further proliferation these dilations may form “blister like” structures that eventually detach from the renal tubule to form isolated cysts. Over time, continual cell proliferation and secretion of fluid into the cystic lumen progressively enlarges the cysts and ultimately the kidneys themselves (Grantham et al., 2006b; Gustavo and Wallace, 2013). Importantly, both cell proliferation and fluid secretion are intimately dependent on intracellular cyclic adenosine monophosphate (cAMP) concentration (Mangoo-Karim et al., 1989; Belibi et al., 2004; Chen et al., 2011), which has proven to be an extremely useful therapeutic target (Torres and Harris, 2009).

Renal function

As cysts grow, they encroach on the normal renal parenchyma to progressively impinge on renal function. In 1957, Dalgaard showed that renal enlargement preceded uraemia in PKD patients (Dalgaard, 1957). This seminal observation was subsequently confirmed by studies using modern imaging techniques (King et al., 2000; Fick-Brosnahan et al., 2002; Grantham et al., 2006b). Of note is the work of Grantham et al. (2006b) who demonstrated, using magnetic resonance imaging, a strong relationship between kidney volume (an index of cyst abundance) and the annual decline in glomerular filtration rate (GFR) in patients. However, GFR is not the only aspect of renal function that is compromised in PKD. The majority of PKD patients show a marked impairment in the ability to concentrate urine (Waldherr et al., 1982; Kaariainen et al., 1988; Hildebrandt et al., 1992; Fick et al., 1994; Ho et al., 2012; Zittema et al., 2012), presenting clinically as polyuria and polydipsia (Waldherr et al., 1982;

Omran et al., 2000; Simms et al., 2009; Sweeney et al., 2014; Jeewandara et al., 2015). In addition, approximately 20% of PKD patients also show moderate proteinuria (Chapman et al., 1994; Dell et al., 2016). Therefore, renal cysts impair the handling of uraemic solutes and plasma proteins and compromise the preservation of extracellular fluid homeostasis.

The age at which GFR declines in PKD varies according to the underlying pathology. For ADPKD, though a small number of cysts are commonly observed in children (Sweeney et al., 2014), most patients maintain a normal GFR until about the 5th decade of life, after which patients typically progress to renal failure in 10 years (Chapman, 2008; Harris and Rossetti, 2010). In contrast, ARPKD and nephronophthisis predominately affect children and adolescents and are a major cause of renal failure in this age bracket (Hildebrandt and Zhou, 2007; Sweeney et al., 2014).

There is also substantial variability in the onset of renal insufficiency *within* each type of PKD. This variation is the widest for ADPKD where renal failure presents in childhood in some individuals and not at all in others (Sedman et al., 1987; Grantham et al., 2006a). In ARPKD and nephronophthisis, the phenotypic spectrum ranges from severe cases that are lethal in the neonatal period to less severe cases in which individuals may not require renal replacement therapy till adolescence (Hildebrandt and Zhou, 2007; Al-Bhalal and Akhtar, 2008). The large phenotypic variation within each type of PKD is in part due the causative gene mutation (i.e. certain gene mutations produce more severe forms of ADPKD and nephronophthisis), but likely also due to other genetic and environmental modifiers (Hateboer et al., 1999; Harris and Rossetti, 2010).

Role of vasopressin in PKD progression

Vasopressin accelerates cyst growth

The rate of renal cyst growth intimately depends on intracellular cAMP concentration in renal tubular epithelial cells. Circulating vasopressin tonically stimulates cAMP production in the renal tubules via its V_2 receptor (Belibi et al., 2004; Torres, 2008b). In rodent PKD models, chronic pharmacological V_2 receptor antagonism or suppression of vasopressin release (through genetic vasopressin deletion or high-water consumption) substantially slows cyst growth (Gattone et al., 2003; Torres et al., 2004; Wang et al., 2005; Nagao et al., 2006; Wang et al., 2008; Hopp et al., 2015; Zittema et al., 2016). Consistent with these animal studies are large randomised controlled trials that have demonstrated that a V_2 receptor antagonist, tolvaptan, slows renal cyst growth and GFR decline in ADPKD patients with preserved

(Torres et al., 2012; Torres et al., 2018) or impaired renal function (Torres et al., 2017). Thus, circulating vasopressin makes an important contribution to the progression of PKD. Accordingly, several countries have recently approved tolvaptan as the first treatment for ADPKD (Mustafa and Yu, 2018). Whether vasopressin participates in any of the extrarenal manifestations of PKD, particularly hypertension, has never been tested.

Peripheral vasopressin release is elevated in PKD

Greater levels of circulating vasopressin are associated with faster PKD progression in rodents (Wang et al., 2008) and humans (Boertien et al., 2013; Nakajima et al., 2015). Most studies report plasma vasopressin and/or copeptin (a stable fragment of the vasopressin pre-prohormone which acts a reliable surrogate for vasopressin release (Szinnai et al., 2007; Balanescu et al., 2011; Meijer et al., 2011) as being elevated in ADPKD, particularly in hypertensive patients (Danielsen et al., 1986b; Danielsen et al., 1986a; Michalski and Grzeszczak, 1996; Zittema et al., 2014), implying that they are potentially related through causality or share a common origin. The largest (n = 256) study was performed by Zittema et al. (2014), who observed that plasma copeptin in ADPKD patients was twice that of healthy controls. Two small studies reported that plasma copeptin and/or vasopressin were not significantly higher in ADPKD patients (Ho et al., 2012; Zittema et al., 2012). However, in both of these studies, plasma samples were extracted from participants immediately prior to an overnight fluid deprivation test. It is therefore quite likely that participants were adaquately hydrated in anticipation of this challenge, which is entirely consistent with the postulated mechanism underlying high vasopressin release in PKD (see below).

Remarkably only a single study has assessed circulating vasopressin levels in an animal model of PKD. Fonseca et al. (2014) measured plasma vasopressin in mice with mosaic Pkd1 deletion and found seven-fold greater levels compared to controls, although this did not quite reach significance (P=0.13). Their measurements were however hugely variable, probably because they collected blood via a retro-orbital bleed, a method known to evoke vasopressin release in small animals (Bankir, 2001). The notable absence of data relating to plasma vasopressin levels in preclinical PKD models highlights a common thread of basic research in this field; that is, the majority of studies focus on the molecular mechanisms of cystogenesis and rarely investigate the integrative aspects of this systemic disease.

Impaired urine concentrating ability stimulates vasopressin release

The ability of the kidneys to produce concentrated urine is necessary to conserve water and therefore maintain body fluid homeostasis. Maximal urine concentrating ability is universally reduced in all forms of PKD (Waldherr et al., 1982; Kaariainen et al., 1988; Hildebrandt et al., 1992) most likely due to the anatomical distortion of the renal medulla and consequent disruption of local osmotic gradients (Bankir and Bichet, 2012; van Gastel and Torres, 2017). A reduced ability to concentrate urine likely predisposes PKD patients to recurrent periods of plasma hyperosmolality. This assertion is supported by experimental evidence that consistently demonstrates that overnight water restriction produces an exaggerated increase in plasma osmolality in ADPKD patients compared to healthy individuals (Ho et al., 2012; Zittema et al., 2012), and is in keeping with cross-sectional data highlighting that ADPKD patients with a more severe urinary concentrating defect tend to have a higher basal plasma osmolality (Casteleijn et al., 2015). This assertion is furthermore reinforced by observations in rodent PKD models which show reduced urine concentrating ability concurrent with a higher plasma osmolality during resting conditions (Fonseca et al., 2014; Zittema et al., 2016; Sagar et al., 2019) which is suppressed with high-water consumption (Sagar et al., 2019).

Impaired urinary concentrating capacity is the most likely determinant of the high vasopressin levels typically observed in PKD. This is supported by studies showing that high-water intake suppresses plasma vasopressin levels in ADPKD patients and urinary vasopressin excretion in PKD models (Nagao et al., 2006; Hopp et al., 2015; Amro et al., 2016). Moreover, Zittema et al. (2012) found that overnight water restriction in ADPKD patients was associated with an exaggerated elevation of plasma vasopressin and copeptin, reaching values that were more than two-fold higher than healthy controls. However in a similar study, Ho et al. (2012) observed that overnight water restriction and a concordant increase in plasma osmolality failed to increase plasma vasopressin in another small cohort of ADPKD patients, leading them to reach the conclusion that the osmoregulation of vasopressin secretion might actually be impaired in ADPKD. The reason for the inconsistent finding of Ho et al. (2012) is unclear, but could reflect the use of an inappropriate statistical method (i.e., unpaired t-test for within-subject comparisons) and/or variability in their vasopressin measurements since the relationship between vasopressin and plasma osmolality was extremely weak in controls subjects (R^2 values were not reported). Furthermore, this study is not supported by the other work previously mentioned, nor is it consistent with a study that acutely raised plasma osmolality with an intravenous infusion of hypertonic saline

and found that this effectively increased plasma vasopressin in ADPKD patients, and that this effect was in fact slightly exaggerated compared to healthy controls (Graffe et al., 2012). Thus, taken together these studies indicate that a reduced ability to conserve body water most likely drives a greater osmotic stimulation of vasopressin secretion in PKD. Circulating vasopressin and cyst growth are therefore likely to be linked through a vicious cycle in PKD (Figure 1.5).

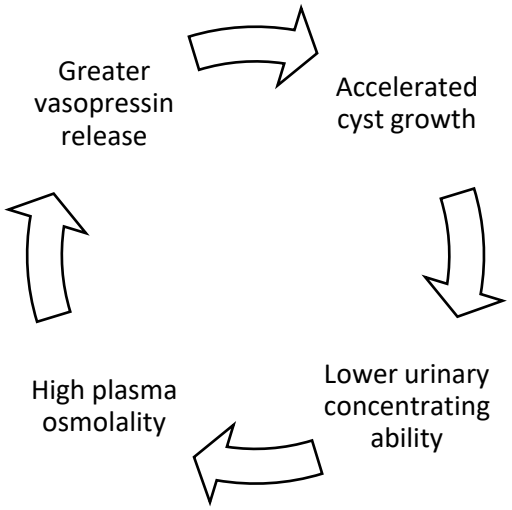

Figure 1.5. Vasopressin and renal cyst growth are likely linked through a vicious cycle in PKD.

Cardiovascular manifestations

Cardiovascular disease is the most common cause of death in ADPKD patients with and without end-stage renal disease (Fick et al., 1995; Rahman et al., 2009). Though the specific causes of cardiovascular mortality are numerous, the most common conditions are ischemic heart disease, congestive heart failure, sudden cardiac death and vascular haemorrhage (Rahman et al., 2009). Hypertension increases cardiovascular disease risk significantly in ADPKD (Chapman et al., 2010) and is likewise a risk factor for the development of cardiovascular disease in ARPKD and nephronophthisis (Kaplan et al., 1989; Zerres et al., 1996; Saito et al., 2017; Stokman et al., 2018); although the relatively acute and aggressive nature of these conditions means that renal failure and respiratory distress are a more common cause of death (Roy et al., 1997; Simpson et al., 2009; Dempsey et al., 2017b).

Cardiovascular remodelling

Several structural and functional changes to the heart and vasculature likely increase cardiovascular disease risk in ADPKD. As noted in Table 1.3, many of these cardiovascular

pathologies likely arise independently of hypertension, however others, particular left-ventricular hypertrophy, are exacerbated by hypertension.

Table 1.3: Cardiovascular abnormalities commonly observed in ADPKD patients.

Characteristic	Prevalence	Clinical relevance	Pathogenesis	Reference
Intracranial aneurysms	8%	Rupture poses a significant risk to morbidity and mortality.	Vascular expression of mutant genes.	(Pirson et al., 2002)
Left-ventricular hypertrophy	41%	Progressive cardiac dysfunction and greater morbidity and mortality.	Hypertension exacerbates it but is not obligatory for its development.	(Koren et al., 1991; Chapman et al., 1997)
Cardiac valvular abnormalities	18%	Associated with mitral and aorta regurgitation.	Unknown.	(Leier et al., 1984; Chapman et al., 1997; Lumiaho et al., 2001)
Endothelial dysfunction	Unknown	May impair autoregulation and promote atherosclerosis.	Unknown. Angiotensin II may contribute.	(Wang et al., 2003; Nowak et al., 2017; Quek et al., 2018)
Arterial stiffness	Unknown	Inflates arterial pulse pressure and may promote target organ damage.	Vascular expression of mutant genes and/or uraemia.	(Qian et al., 2003; Briet and Burns, 2012; Bulley et al., 2018)

Baroreflex dysfunction

Cardiac baroreflex dysfunction impairs the heart's ability to buffer fluctuations in blood pressure and is strongly associated with adverse cardiac arrhythmias in the general population (Farrell et al., 1991; Hohnloser et al., 1994). Cardiac baroreflex function can be assessed by analysing spontaneous changes in blood pressure and heart rate in time or frequency domains, both of which predominantly reflect the ability of the cardiac vagus to be modulated by fluctuations in blood pressure (Swenne, 2013). While no study has examined spontaneous cardiac baroreflex sensitivity in PKD patients specifically, several studies show that it is reduced in patients with CKD of various underlying conditions, including PKD (Johansson et al., 2005; Lal et al., 2017). In these patients, decreased spontaneous cardiac baroreflex sensitivity is predictive of sudden cardiac and all-cause mortality (Johansson et al., 2007; John et al., 2008). In addition to analysing spontaneous oscillations of blood pressure, baroreflex function has been assessed in CKD patients by measuring heart rate responses to pharmacologically evoked changes blood pressure. These studies agree that cardiac baroreflex function is impaired in CKD patients relative to healthy controls (Tomiyama et al., 1980; Agarwal et al., 1991). Furthermore, cross-sectional studies indicate that cardiac baroreflex dysfunction is worse in CKD patients with lower levels of GFR (Bavanandan et al., 2005; Lacy et al., 2006), suggesting that this abnormality is directly related to renal insufficiency. Therefore, it is likely that cardiac baroreflex function declines as renal function deteriorates in PKD in line with other forms of CKD. There are currently no treatments to protect against cardiac baroreflex dysfunction in renal disease patients, ultimately reflecting a poor understanding of its pathogenesis.

Baroreflex control of muscle SNA has also been assessed in CKD patients through pharmacological evoked changes in blood pressure. Due to technical constraints, the only barosensitive sympathetic nerve that can be measured non-invasively in humans supplies the muscle vasculature, which is far less barosensitive than other vasomotor sympathetic nerves innervating visceral targets, at least in rat (Scislo et al., 1998). Studies assessing baroreflex control of muscle SNA in CKD patients report the gain as either reduced (Tinucci et al., 2001) or alternatively normal (Ligtenberg et al., 1999). The only study of exclusively ADPKD patients was performed by Klein et al. (2001) who found that baroreflex gain of muscle SNA was normal irrespective of GFR status. These inconsistent findings may reflect a subtle difference in how each study analysed their data, with Tinucci et al. (2001) expressing muscle SNA as a percentage of baseline, to corrected for large differences in resting SNA

levels, whereas this correction was not performed in the studies by Ligtenberg et al. (1999) or Klein et al. (2001). Therefore, whether baroreflex control of SNA (supplying muscle or other vascular beds) is dysfunctional in patients with PKD or CKD more generally is unresolved.

Hypertension in PKD

Prevalence and unique features of hypertension in PKD

The first associations between PKD and hypertension were made at the beginning of the 20th century (Vollard and Fahr, 1914; Braasch, 1916). Schacht (1931) found that hypertension (>145 mmHg systolic) was present in 75% of PKD patients compared to only 26% of control subjects matched for age and sex. Indeed more recent studies of ARPKD and ADPKD patients typically report a hypertension prevalence of 65-75% (Rall and Odel, 1949; Hansson et al., 1974; Gabow et al., 1984; Kaplan et al., 1989; Zerres et al., 1996; Ridao et al., 2001; Guay-Woodford and Desmond, 2003; Schrier et al., 2003), with case-controlled studies indicating that the prevalence of hypertension in ADPKD is ~3-fold greater than unaffected family members (Gabow et al., 1984) and the general population (Kelleher et al., 2004). Early investigations performed before the introduction of modern anti-hypertensives show that hypertension is often severe in PKD, with systolic values greater than 160 mmHg observed in over 40% of patients (Schacht, 1931; Dalgaard, 1957). Yet even in the modern era only a half of hypertensive ADPKD patients have their blood pressure controlled below 140/90 mmHg (Kelleher et al., 2004). Therefore, hypertension is very common in PKD but poorly controlled.

Hypertension occurs in other forms of CKD with an overall prevalence roughly comparable with PKD (Ridao et al., 2001). However, the development of hypertension in PKD is uniquely different from other forms of CKD in that it occurs early, before GFR declines in most cases (Nash and Jr, 1977; Kaplan et al., 1989; Gabow et al., 1990; Zerres et al., 1996; Ecder and Schrier, 2001; Schrier et al., 2003; Saito et al., 2017; Stokman et al., 2018). In fact, hypertension is most commonly diagnosed at least a decade earlier than when GFR first declines in ADPKD (Nash and Jr, 1977; Gabow et al., 1990; Ecder and Schrier, 2001; Schrier et al., 2003), including in childhood in 20% of cases (Sedman et al., 1987; Fick et al., 1994; Ivy et al., 1995). The temporal profile of hypertension development in ARPKD and nephronophthisis is less well described, however affected individuals are commonly hypertensive before any evident impairment in GFR (Kaplan et al., 1989; Zerres et al., 1996; Saito et al., 2017; Stokman et al., 2018). The early occurrence of hypertension in PKD strongly suggests that its origin are probably unrelated to CKD *per se* and more likely related

to unique aspects of the pathology that present before any decline in GFR. Nevertheless, a mechanistic understanding of the origins of hypertension in PKD is lacking.

Renin-angiotensin system (RAS) activation

ACE inhibitors and AT1R antagonists typically reduce blood pressure in PKD patients (Chapman et al., 1990; Watson et al., 1992; Schrier et al., 2014) and animal models (Keith et al., 1994; Zafar et al., 2007), suggesting a causative role for the RAS in the maintenance of hypertension observed in PKD. Less clear is how the RAS serves to elevate blood pressure in PKD. In PKD patients and animal models, systemic RAS activation, as assessed through measurements of plasma renin concentration or enzymatic activity, is inconsistently reported as normal, high or even low (Nash and Jr, 1977; Ramunni et al., 2004; Doulton et al., 2006; Phillips et al., 2007; Salih et al., 2017). In contrast, urinary angiotensinogen and renin, thought to reflect the activity of the intra-renal RAS, are elevated in PKD patients (Kocyigit et al., 2013; Salih et al., 2017). Intra-renal RAS activation may be localised to the cysts themselves because all components of the RAS have been detected in the cystic fluid and epithelium of patients and animal models (Torres et al., 1992; Loghman-Adham et al., 2004; Loghman-Adham et al., 2005; Saigusa et al., 2015).

While it is theoretically possible that upregulation of the intra-renal RAS could contribute to hypertension in PKD (Kobori et al., 2007), causation has not been demonstrated. In fact, urinary angiotensinogen is higher independent of whether an ADPKD patient is hypertensive or not (Kocyigit et al., 2013), indicating that intrarenal RAS activation alone is not sufficient to produce hypertension. Furthermore, work in animal models of PKD highlight that the temporal relationship between intrarenal RAS activation and hypertension is discordant with a cause-effect relationship (Goto et al., 2010; Fonseca et al., 2014). Specifically, mice with mosaic Pkd1 deletion are hypertensive before renal expression of components of the RAS are elevated (Fonseca et al., 2014), whereas the PCK rat model shows an increase in renal RAS expression at a young age despite resting blood pressure being normal and unaffected by systemic RAS inhibition (Goto et al., 2010). Therefore, taken together these data do not support the view that activation of an intra-renal RAS is a primary driver of hypertension in PKD.

There are certainly other possibilities that could explain why RAS inhibitors lower blood pressure in PKD despite systemic RAS not being elevated. In that regard, it is possible that actions of circulating angiotensin II are sensitised (e.g. due to increased AT1R density or

transduction efficacy) at the vasculature or forebrain sensory circumventricular organs, both of which are observed in the SHR (Jackson et al., 1999; Miyakubo et al., 2002; Bhatt et al., 2014). Another possibility is that the anti-hypertensive benefit of RAS inhibitors in PKD reflects an inhibition of central angiotensin II actions since: firstly, angiotensin II levels in the hypothalamus are regulated independently of the peripheral RAS (Trolliet and Phillips, 1992); and secondly, many RAS inhibitors cross the blood-brain barrier normally (Li et al., 1993; Sink et al., 2009) and this is probably enhanced in the context of hypertension (Biancardi et al., 2014b). These possibilities remain to be tested.

Sympathetic vasomotor tone

Measurements of sympathetic tone

Several studies have measured plasma catecholamine concentrations in ADPKD patients as an index of resting sympathetic tone. Two studies found that plasma noradrenaline and adrenaline were 30-50% higher in hypertensive ADPKD patients with normal renal function compared to individuals with primary hypertension (Cerasola et al., 1998; Martinez-Vea et al., 2000). Cerasola et al. (1998) observed a modest correlation between 24-hour ambulatory blood pressure and plasma noradrenaline as measured with high-performance liquid chromatography, however no such association was identified in other studies using radioimmunoassay (Bell et al., 1988; Martinez-Vea et al., 2000), possibly reflecting differences in the sensitivity of each measurement technique (Peaston and Weinkove, 2004). Therefore, whether an elevation in plasma noradrenaline is related to hypertension in ADPKD patients remains uncertain. However, it should be noted that plasma noradrenaline level is a rather poor index of sympathetic activity since it reflects not only the net effect of noradrenaline release from all sympathetic terminals and the adrenal gland, but also the effectiveness of catecholamine clearance (Peaston and Weinkove, 2004).

More specific assessments of sympathetic outflow have been obtained using microneurography. In the study by Klein et al. (2001) described before, muscle SNA was measured in patients with ADPKD and healthy individuals after RAS blockers and sympatholytics were discontinued. They found that compared to healthy controls, muscle SNA was greater in hypertensive but not normotensive ADPKD patients with preserved GFR. This difference was also observed in hypertensive ADPKD patients with reduced GFR. Interestingly there was a moderate positive correlation between blood pressure and muscle SNA in entire PKD cohort, such that muscle SNA accounted for 40% of the variability in mean arterial pressure. Two other studies measured muscle SNA in mixed cohorts of CKD

patients, of which ADPKD was the underlying pathology in 50-60% of patients (Klein et al., 2003b; Klein et al., 2003a). Both studies found that muscle SNA was likewise elevated in these CKD cohorts compared with healthy controls. Therefore, sympathetic outflow to muscle vascular beds is likely increased in PKD before GFR declines and appears to be associated with hypertension.

Origins of high sympathetic tone

The origins of high sympathetic drive in PKD are poorly understood. Some have suggested that a contributing factor could be renal afferent nerve activation (Klein et al., 2001), which exerts a sympathoexcitatory action in some forms of hypertension (Banek et al., 2018). This is an attractive hypothesis because intrarenal ischemia, a stimulus for renal afferent nerve activation (Banek et al., 2018), is a prominent feature of PKD (Torres et al., 1991; Ow et al., 2014). There is however limited evidence to support this hypothesis. Firstly, while no study has directly measured sympathetic tone in PKD following renal denervation (i.e., combined afferent and efferent nerve transection), there are studies that have performed this procedure in hypertensive PKD models and patients and measured blood pressure as a proxy for sympathetic tone. Gattone et al. (2008) performed renal denervation in the Han:SPRD-Cy/+ rat model of PKD and observed that the intervention normalised blood pressure in this model. These data are far from conclusive however because blood pressure was measured through tail-cuff plethysmography at a single timepoint 2 months following the procedure. In PKD patients, renal denervation has produced far from consistent results. Several case reports have observed that renal denervation lowers blood pressure in some but not all ADPKD patients (Casteleijn et al., 2014; Riccio et al., 2014; Pietila-Effati et al., 2018). In addition, two small uncontrolled studies have performed unilateral or bilateral renal denervation in ADPKD patients with the primary purpose of reducing kidney pain (Casteleijn et al., 2017b; de Jager et al., 2017). These studies found that renal denervation improved pain management and produced a variable effect on blood pressure and the requirement for antihypertensive medications (Casteleijn et al., 2017b; de Jager et al., 2017). Thus, further experimental studies are required to elucidate what role if any the renal nerves play in the development and/or maintenance of high sympathetic drive and hypertension in PKD.

Two clinical studies raise the possibility that the RAS may be involved in generating higher levels of sympathetic drive in PKD (Ligtenberg et al., 1999; Klein et al., 2003a). These studies found that 6-week treatment with either an ACE inhibitor or AT1R antagonist reduced muscle SNA by 20% in mixed CKD cohorts containing a high percentage of

ADPKD patients. Unfortunately, these studies only report on the pooled analysis of all patients. Whether this sympatholytic action of systemic RAS inhibition is due to an inhibition of angiotensin II actions in the CNS is unknown.

The CNS sites underlying high sympathetic drive in PKD have never been investigated. Nevertheless there are studies that have examined the contribution of neurons in the RVLM and PVN to sympathetic regulation in rodent models of CKD induced by surgical reductions in renal mass (i.e., 5/6 nephrectomy). Dugaich et al. (2011) found that acute inhibition of the RVLM with a bilateral microinjection of GABA produced an exaggerated fall in blood pressure in anaesthetised 5/6 nephrectomised rats, indicating that the discharge of RVLM neurons may contribute to an additional level of blood pressure in this model, presumably via the regulation of sympathetic outflow. A separate study observed that the pressor and lumbar sympathoexcitatory response to inhibition of $GABA_A$ receptors in the PVN was significantly blunted in 5/6 nephrectomised mice (Nishihara et al., 2017). These authors concluded that diminished GABAergic tone in the PVN contributes to chronic sympathoexcitation in 5/6 nephrectomised mice. However, this conclusion is somewhat premature since the authors did not assess tonic excitatory inputs to the PVN nor did they examine the contribution of PVN neuronal 'tone' to resting sympathetic discharge. Nevertheless, taken together these data indicate that rodents with CKD exhibit functional changes in the RVLM and PVN that contribute to altered sympathetic regulation. Whether similar CNS changes are present in PKD is uncertain however, particularly because GFR impairment does not appear to be obligatory for the development of sympathetic overactivity or hypertension in PKD patients or rodent models (Klein et al., 2001; Phillips et al., 2007; Goto et al., 2010; Salman et al., 2014).

Relationship between hypertension and osmoregulation in PKD

Plasma vasopressin appears to be higher in hypertensive versus normotensive ADPKD patients (Danielsen et al., 1986b; Danielsen et al., 1986a). In fact, Danielsen et al. (1986a) observed that plasma vasopressin concentration accounted for 60% of the variability in office blood pressure in their cohort. These data have led some to suggest that vasopressin via its V_{1A} receptor contributes to hypertension in PKD (Torres, 2008a; Sans-Atxer et al., 2013). However, currently this suggestion is unsubstantiated because no study has examined the effect of vasopressin V_{1A} receptor antagonism on hypertension in PKD patients or animal models.

There is nevertheless some data to suggest that osmotic stimuli may be linked with the development of hypertension in PKD. Reduced urinary concentrating capacity is one of the earliest features of PKD, commonly preceding hypertension development in ADPKD (Figure 1.6), and predisposes PKD patients to recurrent elevations in plasma osmolality (Fick et al., 1994; Ho et al., 2012; Zittema et al., 2012). Seeman et al. (2004) found that the presence of hypertension was seven-times more common in ADPKD children and adolescents that had impaired urinary concentrating ability, and that there was a significant inverse relationship between urine osmolality and ambulatory blood pressure across the cohort. This relationship has also been observed in ADPKD adults. In a retrospective analysis of a large study of ADPKD patients, lower urine osmolality at baseline predicted the presence of hypertension (Devuyst et al., 2017). This relationship may explain why hypertension consistently associates with indices of cyst abundance (Gabow et al., 1990; Cadnapaphornchai et al., 2008), the most probable determinant of the urine concentrating impairment (Fonseca et al., 2014).

It is currently unknown whether the association between urine concentrating impairment and hypertension in PKD is because these features share a common origin or alternatively because of a cause-effect relationship. One possibility is that in PKD, frequent elevations in plasma hyperosmolality stimulate and/or sensitise central osmoregulatory circuits to initiate or exacerbate hypertension development via generating a greater level of vasomotor sympathetic outflow and vasopressin release. This hypothesis is examined in the present thesis.

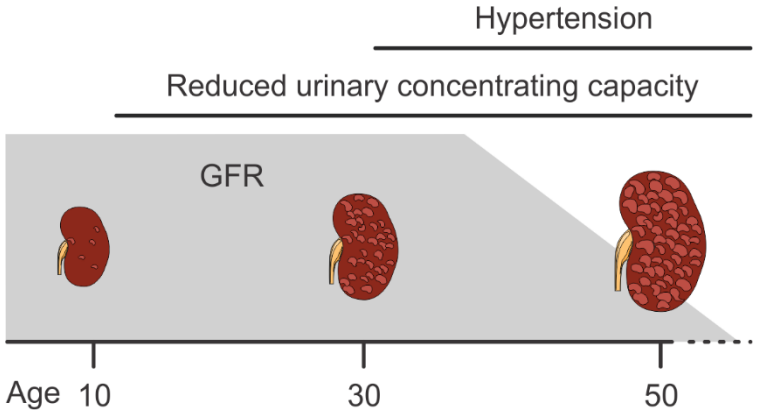

Figure 1.6. Timeline illustrating the presentation of key clinical features of ADPKD in relation to age (years). Note that the reduction in urinary concentrating capacity precedes the development of hypertension and the decline in glomerular filtration rate (GFR; grey area). Data were acquired from (Chapman, 2008; Ho et al., 2012). Images sourced from Laboratoires Servier (www.servier.com).

Consequences of hypertension in PKD

Renal

Higher levels of blood pressure (Gabow et al., 1992; Choukroun et al., 1995) and muscle SNA (Siddiqi et al., 2010) predict a faster annual GFR decline in ADPKD patients. Furthermore, in a survival analysis, Johnson and Gabow (1997) found that ADPKD individuals diagnosed with hypertension younger than 35 years reached end-stage renal disease 14 years earlier than those who are diagnosed with hypertension after 35 years (Johnson and Gabow, 1997). Thus, hypertension and sympathetic overactivity are directly related to disease progression in ADPKD.

In a large randomised control trial of patients with early-stage ADPKD, the HALT-PKD trial, aggressively lowering blood pressure with RAS inhibitors was associated with a slightly lower annual increase in total renal volume (an index of cyst growth) compared to patients with standard therapy (Schrier et al., 2014). The HALT-PKD trial and other longitudinal studies identified that anti-hypertensive treatment also protects against proteinuria in ADPKD patients, particularly those with higher baseline levels (Jafar et al., 2005; Zeltner et al., 2008; Schrier et al., 2014). Post-hoc analyses of these studies suggest that the beneficial effect of anti-hypertensive treatment on proteinuria is directly related to the haemodynamic effect of lowering blood pressure and not the amount or type of anti-hypertensive medication used (Zeltner et al., 2008; Brosnahan et al., 2018). These studies therefore show that hypertension is a modifiable factor affecting renal disease progression in PKD.

Cardiovascular

Left-ventricular hypertrophy is greater in ADPKD patients that are hypertensive (Ivy et al., 1995; Chapman et al., 1997; Valero et al., 1999; Cadnapaphornchai et al., 2008) and is improved with aggressive lowering of blood pressure with RAS inhibitors (Schrier et al., 2002; Schrier et al., 2014). This benefit is likely due to haemodynamic changes rather than direct cardiac actions of the drug because another study found that left ventricular hypertrophy improved in ADPKD patients with rigorously controlled blood pressure irrespective of whether an ACE inhibitor or a β-blocker was used (Zeltner et al., 2008). Hypertension may also increase the risk of intracranial aneurysm rupture in PKD patients, with evidence that normalisation of blood pressure powerfully mitigates aneurysm rupture in hypertensive mice (Tada et al., 2014).

In summary, hypertension and excessive sympathetic activity confer poor cardiovascular and renal outcomes in PKD. As such therapies that alleviate hypertension and sympathetic overactivity would no doubt be beneficial to the clinical management of PKD. A better understanding of the origins of hypertension and sympathetic overactivity in PKD is therefore paramount.

Rodent models of PKD

Rodent models of PKD have proved extremely useful for the study of pathogenic mechanisms and for preclinical testing. This is most clearly demonstrated by the recent clinical success of V_2 receptor antagonism which was first tested in rodent PKD models 15 years ago (Gattone et al., 2003). Although there is an ever-growing list of rodent models, blood pressure has only been measured in a select few. Table 1.4 describes the characteristics of PKD models in which hypertension has been noted. Among these PKD models, the Lewis Polycystic Kidney (LPK) rat is by far the most extensively characterised for the study of hypertension and neurohumoral dysfunction. The LPK rat model will be examined more closely in the proceeding section.

Table 1.4: Overview of hypertensive rodent models of PKD.

Rodent model	Genotype	Renal progression	Temporal profile of hypertension	Sympathetic activity	Circulating vasopressin	References
$Pkd1^{cond/cond}$: $Nestin^{cre}$ mouse	Conditional, mosaic Pkd1 inactivation (ADPKD orthologue)	Progressive cyst growth preceding a moderate reduction in renal function.	Moderate hypertension before GFR reductions.	Unknown.	Trends high.	(Bankir, 2001; Shillingford et al., 2010)
PCK rat	Pkhd1 mutation (ARPKD orthologue)	Very slow progression, mimicking human ADPKD.	Moderate hypertension before GFR is significantly impaired.	Unknown.	Unknown.	(Lager et al., 2001; Goto et al., 2010; O'Meara et al., 2012; Yoshihara et al., 2013)
Han:SPRD Cy rat	Anks6 mutation (non-orthologous)	Rapid progression in homozygotes and slow ADPKD-like progression in heterozygotes.	Blood pressure is inconsistently reported as mildly elevated (at most 15 mmHg) or normal.	Unknown.	Unknown.	(Schafer et al., 1994; Kennefick et al., 1999; Wang et al., 1999; Al-Nimri et al., 2003; Zafar et al., 2007; Ibrahim et al., 2016)
Lewis Polycystic Kidney rat	Nek8 (nephronophthisis orthologue)	ARPKD-like renal phenotype with progressive renal function decline culminating in renal failure by 24 weeks.	Hypertension before GFR declines. Becomes more severe with age.	High.	High.	(Phillips et al., 2007; McCooke et al., 2012) (Hildreth et al., 2013b; Underwood et al., 2017)

The LPK rat

Genotype

The Lewis rat is an inbred strain derived from Wistar rats that are normotensive and have a normal GFR (Hildreth et al., 2013b; Carrara et al., 2016). The LPK rat arose spontaneously from the Lewis rat due to a single point mutation in the never in mitosis gene A-related kinase 8 (Nek8) gene (Phillips et al., 2007; McCooke et al., 2012). The Nek8 protein is localised to the primary cilia (McCooke et al., 2012) where it regulates the expression and localisation of the polycystin proteins (Sohara et al., 2008). Mutation in the human homologue (NPHP9) produces nephronophthisis (Otto et al., 2008).

Renal phenotype

LPK animals exhibit progressive cyst growth and renal enlargement with age. Cysts arise from dilations of the collecting tubules, mimicking human ARPKD. A few small focal dilations are observed in the outer renal medulla at 1 week of age, but cysts are largely absent (Phillips et al., 2007). Between 1 and 3 weeks of age, cyst growth increases 6-fold, but then slows dramatically, such that the total cyst area at 12 weeks of age is just 70% greater than at 3 weeks of age (Schwensen et al., 2011).

LPK rats show a progressive decline in glomerular filtration with age that initiates after the bulk of cyst growth has occurred. While GFR has not been directly measured, it is probably within normal limits at 6 weeks of age because although plasma urea is mildly elevated, plasma creatinine is normal (Phillips et al., 2007). However by 12 weeks of age, GFR is more than likely significantly reduced in these animals because both plasma urea and creatinine are markedly elevated and creatinine clearance rate is reduced by 60% (Phillips et al., 2007; Jeewandara et al., 2015). Plasma urea and creatinine continue to rise thereafter, reaching levels indicative of renal failure around 24 weeks of age (Phillips et al., 2007). LPK rats can therefore be studied before and after the development of renal insufficiency.

The renal phenotype of LPK rats reflects other features of human PKD. Notably, proteinuria arises early, typically around 6 to 8 weeks, and increases incrementally with age (Phillips et al., 2007; Quek et al., 2016; Yimin et al., 2017). In addition, polyuria and polydipsia are present from 7 weeks of age and increase with age concurrent with a reduction in urine concentration (Phillips et al., 2007; Ding et al., 2012; Sagar et al., 2019). Possibly as a consequence of this urinary concentrating defect, basal plasma osmolality is significantly

elevated (Sagar et al., 2019) and plasma vasopressin is approximately two-fold higher (Underwood et al., 2017).

Hypertension development

Blood pressure has been measured in conscious LPK rats with tail-cuff plethysmography and radiotelemetry. These measurements show that hypertension is present by 5 weeks of age and becomes progressively severe with age, reaching systolic values in excess of 200 mmHg at 10 weeks of age (Phillips et al., 2007; Ng et al., 2011a; Hildreth et al., 2013b; Jeewandara et al., 2015; Ameer et al., 2016; Quek et al., 2018).

Several chronic interventions have been reported to attenuate, but not prevent, hypertension in LPK rats. Ng et al. (2011a) found that LPK rats treated with the ACE inhibitor perindopril from 6 weeks of age showed no further increase in tail-cuff systolic blood pressure from 7 to 11 weeks. Likewise, LPK rats treated with the AT1R antagonist valsartan from 4 weeks of age show significantly lower tail-cuff pressures during the treatment duration (Ameer et al., 2016; Quek et al., 2018). A mineralocorticoid receptor antagonist has been reported to moderately attenuate hypertension development in female but not male LPK rats (Jeewandara et al., 2015). However, while chronic inhibition of angiotensin II or aldosterone are both anti-hypertensive in LPK animals, plasma renin activity and angiotensin II levels are lower, whereas plasma aldosterone is normal, in this PKD model, at least at age 10-12 weeks (Phillips et al., 2007). This indicates that in LPK rats either the density and/or signalling of the AT_1 and mineralocorticoid receptors are greater or that more angiotensin II and aldosterone is synthesised locally in certain tissues.

A very recent article by Sagar et al. (2019) found that high-water intake treatment reduced tail-cuff systolic blood pressure ~20 mmHg in LPK rats concurrent with a normalisation in plasma osmolality and an improvement in renal function. While this finding awaits to be confirmed with radiotelemetry, it is nevertheless consistent with our hypothesis that chronic osmotic stress participates in the development of hypertension PKD. It remains to be determined whether lowering of plasma osmolality through high-water intake protects against other cardiovascular manifestations of PKD.

Cardiac and vascular changes

Cardiac structural changes are observed in LPK rats. Left-ventricular mass is greater in LPK rats by 12 weeks of age and the left-ventricular free wall shows significant perivascular fibrosis (Ameer et al., 2015; Jeewandara et al., 2015). Left-ventricular hypertrophy shows

variable responses to AT1R antagonism (Ameer et al., 2015; Quek et al., 2018) while perivascular fibrosis is ameliorated by mineralocorticoid receptor antagonism (Jeewandara et al., 2015).

LPK animals also exhibit vascular structural and functional changes. The thoracic aorta is hypertrophied, calcified and stiff as indicated by lower elastin/collagen ratio and higher pulse-wave velocity measured in-vivo (Ng et al., 2011a; Ng et al., 2011b). The mesenteric arteries are structurally different in LPK rats also in that they are hypertrophied with a narrower lumen and lower elastin/collagen ratio (Quek et al., 2016). Ex-vivo organ bath experiments show that endothelium-dependent relaxation is impaired from 18 weeks in the thoracic aorta and 12 weeks in the mesenteric arteries, and that at 18 weeks of age (but not earlier) both vessels are more sensitive to α-adrenergic stimulation (Ameer et al., 2015; Quek et al., 2016). Most of these structural and functional vascular abnormalities are improved by chronic ACE inhibition or AT1R antagonism, suggesting that their development involves direct or indirect actions of angiotensin II (Ng et al., 2011a; Ng et al., 2011b; Ameer et al., 2015; Ameer et al., 2016; Quek et al., 2016; Quek et al., 2018).

Higher vasomotor sympathetic tone

Several lines of evidence suggest that LPK animals display a greater level of sympathetic vasomotor tone. Firstly, in-vivo electrophysiological studies have reported that LPK rats show a greater voltage level of SNA in the splanchnic, renal and lumbar nerves (Salman et al., 2014; Salman et al., 2015b; Yao et al., 2015). Next, conscious LPK animals show higher low-frequency systolic blood pressure variability (Hildreth et al., 2013b), a measure that has been demonstrated to represent sympathetic vasomotor tone in this model (Ameer et al., 2014). The final weight of evidence that the sympathetic control of resting blood pressure is heightened in LPK rats is data showing that ganglionic blockade with hexamethonium produces an exaggerated fall in blood pressure in conscious and anaesthetised animals (Ameer et al., 2014; Salman et al., 2015b). The central origins of high sympathetic vasomotor tone observed in LPK animals remain to be determined.

Baroreflex dysfunction

Baroreflex function is overtly compromised in LPK rats. Hildreth et al. (2013b) studied cardiac baroreflex sensitivity by analysing spontaneous changes in blood pressure in conscious animals with radiotelemetry and found that while cardiac baroreflex sensitivity was normal in young LPK animals, it was reduced by ~35% as the renal disease progressed.

Experiments in anaesthetised animals analysing evoked changes in blood pressure demonstrate that LPK rats show an age-related reduction in both the range and gain of the cardiac baroreflex (Harrison et al., 2010; Salman et al., 2014; Salman et al., 2015a).

LPK rodents also exhibit impaired sympathetic baroreflex function. Salman and colleagues found that anaesthetised LPK rats display an early impairment in the gain of the renal sympathetic baroreflex, with a reduction in range of the reflex also present as the disease progresses (Salman et al., 2014; Salman et al., 2015a). Sympathetic baroreflex dysfunction observed in LPK rats appears to be specific to the renal and possibly splanchnic nerves (Harrison et al., 2010; Yao et al., 2015), with baroreflex control of lumbar SNA apparently unaffected (Yao et al., 2015). Since the lumbar nerve supplies muscle vasculature in rat, this regional selectivity in sympathetic baroreflex dysfunction may explain why muscle SNA baroreflex gain is reportedly normal in PKD patients (Klein et al., 2001).

A CNS origin of baroreflex dysfunction in LPK

Experiments in the LPK rat model have provided insight into where in the baroreflex neuroaxis dysfunction arises in PKD. A study by Salman et al. (2014) found that early in the disease course in young male LPK animals the ability of the aortic depressor nerve to transduce arterial pressure is impaired, despite cardiac baroreflex function being largely maintained at this age. This baroreceptor afferent deficit in male LPK rats persisted with the onset of overt cardiac and sympathetic baroreflex dysfunction later in the disease progression, and most likely arose secondary to vascular remodelling and was compensated for by a central enhancement. Importantly, however, loss of baroreflex function in older LPK rats was found to involve a CNS impairment because the ability of direct aortic depressor nerve stimulation to reflexively decrease heart rate and renal SNA was significantly reduced relative to control Lewis and younger LPK rats (Salman et al., 2014). This therefore suggests that baroreflex dysfunction in male LPK arises from deficits in both the afferent and central components of the reflex arc. While the former presents early in the disease course before baroreflex function is overtly compromised, development of the latter appears to be necessary for the full expression of baroreflex dysfunction.

The critical importance of a central alteration in the development of baroreflex dysfunction in LPK rats is further emphasised by work in female LPK animals. Salman et al. (2015a) showed that similar to males, female LPK rats exhibit a reduction in heart rate and renal sympathetic baroreflex function with age, coinciding with an impaired bradycardic and

sympathoinhibitory responses to aortic depressor nerve stimulation. However, unlike male LPK rats, baroreflex dysfunction in female LPK rats does not occur alongside an impairment of baroreceptor afferent transduction. Furthermore, bradycardic responses to efferent vagal stimulation were normal in female LPK animals, indicating that efferent vagal transduction is not affected (Salman et al., 2015a). Thus, in both male and female LPK animals, altered central processing is the primary determinant of baroreflex dysfunction. The basis of this central impairment is current unknown. However, since both the heart rate and sympathetic baroreflexes are affected, it is plausible that this abnormality may involve a central site that is common to both reflex pathways, such as the NTS via descending hypothalamic modulation.

A study by Yao et al. (2015) examined the hypothesis that acute AT1R activation contributes to sympathetic baroreflex dysfunction in the LPK model of PKD. They observed that intravenous losartan shifted sympathetic baroreflex curves to lower levels of blood pressure, consistent with the reduction in baseline blood pressure. Interestingly, losartan selectively increased the gain of the splanchnic, but not renal or lumbar, sympathetic baroreflex in LPK but not Lewis control animals. Therefore, tonic AT1R activation may be involved in maintaining the impaired baroreflex control of splanchnic SNA in this PKD model.

Other CNS alterations in LPK

As well as baroreflex dysfunction and high sympathetic drive, the LPK animals display other central autonomic changes. A study by Salman et al. (2017) raises the possibility that other vagal reflexes may be dysregulated in LPK rats. High frequency (16 Hz) stimulation of the central end of a cut cervical vagus nerve produces a biphasic response characterised by acute sympathoinhibition immediately followed by sympathoexcitation, reflecting the engagement of both unmyelinated C-fibres and myelinated A-fibres. In LPK animals, the sympathoinhibitory component of this response is smaller while the sympathoexcitatory component has a longer duration but a similar magnitude. Furthermore, the vagally-mediated bradycardia associated with vagal afferent stimulation is impaired in female animals, but not male, LPK animals. These abnormalities are not present in young LPK rats and only develop as renal function declines (Salman et al., 2017).

Other studies show that LPK rats have a blunted capacity to drive increases in SNA in response to certain stimuli. Under conscious and anaesthetised conditions, activation of peripheral chemoreceptors with hypoxia or activation of central chemoreceptors with hypercapnia increases SNA in control Lewis animals, yet this sympathoexcitatory response is

completely eliminated in LPK animals (Salman et al., 2015b; Yao et al., 2015). In addition, LPK animals also show a blunted ability to drive SNA during acute psychological stress, with evidence that open-field stress produces a ~50% smaller increase in renal SNA and blood pressure in LPK compared to Lewis animals (Salman et al., 2015b).

These studies, combined with observations that LPK animals display higher resting levels of SNA (Salman et al., 2014; Salman et al., 2015b; Yao et al., 2015) and circulating vasopressin (Underwood et al., 2017), demonstrate that the central neurohumoral control of the circulation is fundamentally altered in LPK rodents. Changes within the CNS may therefore contribute to the pathological dysregulation of the cardiovascular system and hypertension observed in PKD (and potentially also CKD more generally). It is intriguing to note that in LPK rats at least some of the cardiovascular abnormalities are either absent (e.g., central baroreflex dysfunction) or are less severe (e.g., hypertension) in young animals, indicating that certain central alterations are not innately present in LPK animals and are therefore likely to develop in response to disease-specific stimuli. Currently, these stimuli are elusive, as are the CNS pathways that they engage. Nevertheless, considering that both autonomic and vasopressin pathways are affected in LPK rats, and that the stimuli suspected to be involved in producing the hypertension in PKD are angiotensin II and increased osmotic drive, the hypothalamus and adjunct sensory structures of the lamina terminalis must be considered as prime candidates for mediating hypertension and neurohumoral dysfunction the LPK model of PKD.

Thesis Aims

Cardiovascular disease poses the single biggest threat to the life expectancy of PKD patients (Fick et al., 1995; Rahman et al., 2009), and is likely attributed to by uncontrolled hypertension (Kelleher et al., 2004) and baroreflex dysfunction (Johansson et al., 2005; Johansson et al., 2007; John et al., 2008; Lal et al., 2017). The central hypothesis examined in this thesis is that hypertension and baroreflex dysfunction is contributed to by altered signalling within the hypothalamus and elevations in extracellular fluid osmolality in the LPK rat model of PKD. The main experimental aims are as follows:

- Chapter 2: To determine whether ongoing PVN neuronal activity contributes to the maintenance of hypertension and baroreflex dysfunction in juvenile (6 weeks old) and adult (13-14 weeks old) LPK rats.
- Chapter 3: To examine whether enhanced angiotensin II signalling in the PVN contributes to hypertension in LPK rats.
- Chapter 4: To determine whether the pro-hypertensive action of PVN excitation in LPK rats is driven by the forebrain lamina terminalis and is reduced by chronic suppression of plasma angiotensin II or osmolality.
- Chapter 5: To assess whether high-water intake improves baroreflex dysfunction in LPK rats.

2.

Increased Excitatory Regulation of the Hypothalamic Paraventricular Nucleus and Circulating Vasopressin Results in the Hypertension Observed in Polycystic Kidney Disease

Underwood C.F., Boyd, R., Phillips J.K. & Hildreth C.M.

This chapter is a modified version of the following paper published in The Journal of Hypertension: Underwood CF, Boyd R, Phillips JK & Hildreth CM (2018). Increased excitatory regulation of the hypothalamic paraventricular nucleus and circulating vasopressin results in the hypertension observed in polycystic kidney disease. *Journal of Hypertension.* ***37****(1), 109-115.*

For Figure 2.6A, 80% of the experiments were performed during the candidates Masters of Research project.

Abstract

Hypertension and baroreflex dysfunction confer poorer outcomes in patients with polycystic kidney disease (PKD). We examined whether hypothalamic paraventricular nucleus (PVN) activation or circulating vasopressin contribute to hypertension and baroreflex dysfunction in Lewis polycystic kidney (LPK) rats. Bilateral PVN inhibition with muscimol reduced systolic blood pressure (SBP) further in urethane-anaesthetised adult LPK than control Lewis rats (-43±4 vs. -18±3 mmHg; $P<0.0001$, $n=14$), but was not associated with a greater reduction in sympathetic nerve activity (SNA) or improvement in HR or SNA baroreflex function. Blockade of ionotropic glutamatergic input to the PVN with kynurenic acid also reduced SBP ($P<0.001$), but not SNA, more in both adult and juvenile LPK rats. No differences in AMPA or NMDA receptor mRNA expression were noted. Systemic V_{1A} receptor antagonism using OPC-21268 reduced SBP in adult LPK rats only ($P<0.001$) and had no effect on the depressor response to PVN inhibition ($P=0.394$). Combined peripheral V_{1A} receptor antagonism and PVN inhibition, however, normalised SBP in the adult LPK rats (122±11 vs. 115±6 mmHg; LPK vs. Lewis, $P>0.05$, $n=10$). Our data show that in the LPK model of PKD, hypertension is contributed to by increased PVN neuronal activity and, through an independent mechanism, systemic V_{1A} receptor activation. Treatments that reduce PVN neuronal activity and/or inhibit peripheral V_{1A} receptors may provide novel treatment strategies to ameliorate hypertension in individuals with PKD and limit overall disease progression.

Introduction

Patients with chronic kidney disease, including polycystic kidney disease (PKD), commonly exhibit both hypertension and impaired baroreflex function (Klein et al., 2001; Lacy et al., 2006; Chapman et al., 2010). Contrasting other forms of chronic kidney disease, the majority of PKD patients are hypertensive before renal function becomes impaired (Chapman et al., 2010). While the pathogenesis of hypertension in PKD is not well understood, both PKD patients and animal models, including those with normal renal function, display elevated levels of sympathetic nerve activity (SNA), in association with hypertension (Klein et al., 2001; Salman et al., 2014; Salman et al., 2015b) and circulating vasopressin (Zittema et al., 2012) another critical regulator of blood pressure. Nevertheless, whether vasopressin is a causative factor has not been directly tested. Further, the central origins of this high sympathetic drive and vasopressin release in PKD are unknown.

The hypothalamic paraventricular nucleus (PVN) comprises a functionally heterogeneous neuronal population that participates in the regulation of autonomic function and hormone release, and collectively, therefore, blood pressure. The PVN is one of two hypothalamic sites responsible for the production and secretion of vasopressin and also regulates sympathetic outflow to several target organs, including the kidneys, via direct and indirect projections to the spinal cord (Stern, 2015). In animal models of chronic heart failure and genetic hypertension, presympathetic PVN neurons display a greater tonic activity, as a result of enhanced glutamatergic drive (Li et al., 2008; Xu et al., 2012) which contributes to the elevated SNA commonly observed in these conditions (Li and Pan, 2007a; Carillo et al., 2012). Furthermore, another population of PVN neurons modulate cardiac baroreflex function (Michelini and Stern, 2009).

The Lewis polycystic kidney (LPK) rat is a rodent model of nephronophthisis, an autosomal recessive form of PKD, arising from a mutation in the never-in-mitosis A (Nek 8) gene (McCooke et al., 2012). These animals present with renal cysts by 3-weeks-old and a significant decline in renal function by 12-weeks-old (Phillips et al., 2007), exhibit hypertension and autonomic dysfunction under both conscious and anaesthetised conditions (Salman et al., 2014; Salman et al., 2015b), and have elevated vasopressin levels (Underwood et al., 2017). Additionally, PVN neuronal activity, assessed by Fos/Fra immunoreactivity, is increased in the LPK (Ang et al., 2007) suggestive of a critical role of the PVN in the autonomic dysfunction and elevated circulating vasopressin levels exhibited in this animal model.

The present study was therefore undertaken to examine the hypothesis that increased excitatory drive to the PVN contributes to the development and maintenance of hypertension, elevated SNA and baroreflex dysfunction observed in the LPK. The following questions were addressed: 1) does increased PVN neuronal activity maintain hypertension, resting SNA and baroreflex dysfunction in LPK rats? 2) is altered glutamatergic PVN tone in LPK rats a contributing factor and does this develop as renal function worsens? and finally 3) does PVN-dependent secretion of vasopressin contribute to the maintenance of hypertension in LPK rats?

Methods

Animals

Male juvenile (6-weeks-old) and adult (13-14-weeks-old) Lewis Polycystic Kidney (LPK) and Lewis rats were obtained from the Animal Resource Centre, Murdoch, Western Australia, Australia. Animals were group-housed in standard living conditions with a 12-hour light/dark cycle and access to standard rodent chow and tap water *ad libitum*. Experiments were performed in accordance with The Australian Code of Practice for the Care and Use of Animals (8th Edition, 2013) and approved by the Macquarie University Animal Ethics Committee.

Anaesthesia and surgical procedures

Animals were anaesthetised with ethyl carbamate (urethane, 1.2-1.3 g/kg i.p., Sigma Aldrich, Australia). Depth of anaesthesia was assessed by the absence of noxious tactile and corneal reflexes, and when required maintenance doses of urethane (65-130 mg/kg i.p. or i.v.) were administered. Body temperature was assessed with a digital rectal thermometer (Harvard Apparatus, MA, USA) and maintained at 37±0.5 ºC with a thermostatically controlled heating mat and manually operated infrared heating source. A tracheostomy was performed, and the animal was artificially ventilated with oxygen-enriched room air adjusted to maintain arterial pH at 7.4±0.5 and pCO_2 40±5 mmHg. Animals were paralysed with either cisatracurium (6 mg/kg/hr i.v.) or pancuronium (2 mg/kg/hr i.v.).

The right femoral artery was cannulated and connected to a pressure transducer to record arterial blood pressure, which was acquired with a CED 1401 plus (Cambridge Electronic Designs Ltd., Cambridge, UK). Heart rate (HR) was extracted from the arterial pressure signal in real-time. The right jugular vein and both femoral veins were cannulated for the administration of fluids (Hartmann's solution, 5 ml/kg/hr) and drugs, respectively.

For sympathetic nerve recordings, a dorsal flank incision was made to access the retroperitoneal cavity and expose the left kidney. One or more of the following nerves were isolated: the left greater splanchnic sympathetic nerve, a branch of the left renal sympathetic nerve or a branch of the left lumbar sympathetic nerve. Nerves were cut at their distal end and mounted onto a silver bipolar electrode. The SNA signals were band-pass filtered (30-1000 Hz), amplified with a bio-amplifier (2000x; CWE Inc., Ardmore, PA, USA) and sampled at 5 kHz with a CED 1401 plus and Spike2 software (Cambridge Electronic Designs Ltd.). The validity of the SNA recordings was verified by the presence of both pulse modulation and a

reduction in activity in response to a pressor dose of phenylephrine (50 µg/ml i.v. Sigma Aldrich).

Animals were placed in a stereotaxic apparatus with the head in the flat-scull position. A burr-hole was made in the midline of the scull immediately caudal to bregma. The PVN was functionally identified by assessing depressor responses to unilateral microinjections of GABA (50 nl, 50 mM; MP Biomedicals, OH, USA) with the pipette tip centred at 0.8-1.6 mm caudal to bregma, 0.3-0.5 mm lateral to the midline and 7.7-8.3 mm ventral to the surface of the dura. GABA microinjections that evoked a decrease in mean arterial pressure (MAP) of ~10 mmHg were considered within the PVN pressor region, as described previously (Li and Pan, 2007a). On average 3-4 GABA microinjections were performed per side.

Experimental protocols

Following a stabilisation period of at least 20 minutes, animals were then used as part of one of the following studies:

Study 1

Baseline measurements of baroreflex function, dP/dT_{max}, systolic blood pressure variability (SBPV) and resting systolic blood pressure (SBP), HR, renal SNA (rSNA), splanchnic SNA (sSNA) were acquired in adult Lewis (n = 7) and LPK (n = 6). Thereafter the $GABA_A$ agonist muscimol (10 mM) was microinjected bilaterally into the PVN and after a period of 30 minutes baroreflex function was reassessed. In n = 1 Lewis, sSNA could not be validated and the SNA data was therefore not analysed. In n = 1 Lewis and n = 1 LPK, rSNA could not be validated and the SNA data was therefore not analysed. All other parameters recorded from these animals were included in the final dataset so as to avoid possible experimental bias.

Baroreflex control of HR and SNA was assessed by first decreasing SBP to 50-80 mmHg over a period of 20-40 second with sodium nitroprusside (50 µg/ml in 0.9% saline; 5-20 µg/min i.v.), allowing for HR and SNA responses to plateau. Then SBP was increased by terminating the infusion of sodium nitroprusside and infusing phenylephrine at increasing rates (50 µg/ml in 0.9% saline; 5-20 µg/min i.v.) into the other femoral vein as previously described (Cassaglia et al., 2014).

Study 2

Initial experiments were performed in pancuronium (2 mg/kg i.v.) paralysed adult animals to ascertain whether glutamatergic neurotransmission is perturbed in the PVN of LPK rats. Rats

received a bilateral PVN microinjection of kynurenic acid (100 mM; *n* = 7 LPK and 6 Lewis), a non-selective ionotropic glutamate receptor antagonist and arterial blood pressure and lumbar SNA were recorded continuously.

In a different set of adult Lewis (*n* = 8) and LPK (*n* = 7) and juvenile Lewis (*n* = 8) and LPK (*n* = 7), baseline levels of baroreflex function, SBP, HR, rSNA were measured and then kynurenic acid (100 mM) was microinjected bilaterally into the PVN. After 30 minutes, baroreflex function was reassessed. In *n* = 1 adult Lewis, *n* = 2 juvenile Lewis and *n* = 2 juvenile LPK from this cohort, rSNA could not be validated and was therefore not analysed.

Study 3

In adult LPK (*n* = 4) resting levels of blood pressure and HR were measured before and 5 minutes after sequential administration of atenolol (1 mg/kg i.v.) and methylatropine (2 mg/kg i.v.) and then again 30 minutes after bilateral PVN microinjection of muscimol (10 mM). To confirm cardiac autonomic blockade, the bradycardic response to phenylephrine (25 µg/kg i.v.) was assessed before atenolol/methylatropine administration and 30-minuites following PVN microinjection. Atenolol/methylatropine administration eliminated the phenylephrine induced bradycardia in all animals ($P > 0.05$, data not shown).

Study 4

Preliminary studies using cumulative doses of OPC-21268 (Otsuka Pharmaceutical Co., Japan), a selective vasopressin V_{1A} receptor antagonist, were undertaken in an initial cohort of Lewis animals to determine the effective dose (Figure 2.1, *n* = 4). Animals were administered vasopressin (100 ng/kg in 0.9% saline; i.v.) then 20-minutes later, once arterial pressure had returned to baseline, OPC-21268 (0.3 mg/kg i.v.) was administered followed 5 minutes later by another bolus of vasopressin. The protocol was then repeated for 1 mg/kg and 3 mg/kg doses of OPC-21268. Then, the vehicle solution for OPC-21268 (20% dimethyl sulfoxide in saline; 0.75 ml/kg i.v.) was administered, which did not affect SBP ($P > 0.05$).

In adult Lewis (*n* = 7) and LPK (*n* = 6), baroreflex function and resting levels of blood pressure, HR, sSNA and rSNA was assessed before and 5 minutes after intravenous administration of OPC-21268 (3 mg/kg). In a subset of these animals (*n* = 5 per strain), muscimol (10 mM) was microinjected bilaterally into the PVN. In *n* = 2 Lewis and *n* = 4 LPK, the efficacy of OPC-21268 to block V_{1A} receptor was validated by administering a bolus dose of vasopressin (100 ng/kg i.v.) before and following OPC21268 and then again following muscimol. For the animals that received muscimol microinjection after OPC-21268

pre-treatment, sSNA could not be validated and was therefore not analysed in *n* = 1 Lewis and rSNA could not be validated in *n* = 1 LPK.

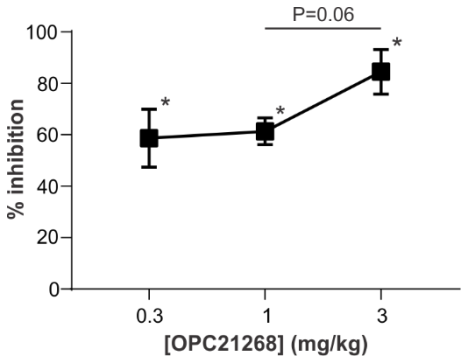

Figure 2.1. Cumulative dose response curve for OPC-21268 showing the percentage of inhibition of the systolic blood pressure response to vasopressin (100 ng/kg i.v.). * indicates $P < 0.05$ vs. baseline. *n* = 4 Lewis.

At the end of all experiments, animals were euthanised with an overdose of sodium pentobarbital (65 mg in 0.9% saline) and a death level of SNA recorded. Microinjection sites were marked with either pontamine sky blue dye after euthanasia or with FluroMax microbeads (1/10,000; Thermo Fisher Scientific, CA, USA) that were included in the drug solution. Brains were fixed in 10% formalin or 4% paraformaldehyde for at least 12 hours and cut at 100 µm with a vibrating microtome to identify the anatomical location of the injections sites relative to a rat brain atlas (Paxinos and Watson, 2013). A summary of microinjection sites is shown in Figure 2.2.

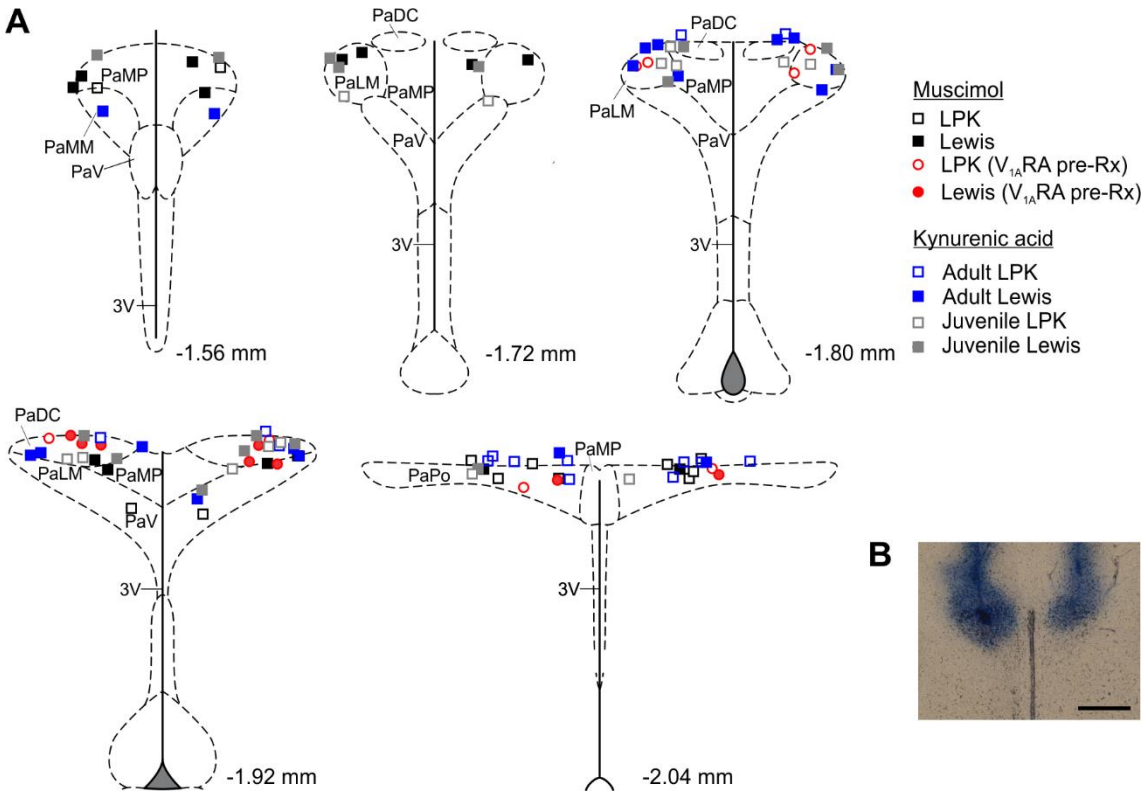

Figure 2.2. **A**: Summary of PVN microinjection sites for LPK and Lewis rats. Distance from Bregma is indicated in bottom left hand of corner. **B:** Representative injection site marked with dye. Scale bar = 500µm. V_{1A}RA pre-Rx indicates V_{1A} receptor antagonism pre-treatment; PaMP indicates paraventricular nucleus medial parvocellular; PaV indicates paraventricular nucleus ventral part; 3V indicates third ventricle; PaLM indicates paraventricular nucleus lateral magnocellular; PaDC indicates paraventricular nucleus dorsal cap; PaPo indicates paraventricular nucleus posterior part.

Microinjection drug solutions

Microinjection drugs were dissolved in phosphate-buffered saline (PBS). Kynurenic acid was adjusted to pH 7.3-7.4 with 1 M NaOH. The microinjection volume was 100 nl in all experiments. In a subset of adult Lewis that received kynurenic acid microinjection, PVN microinjection of PBS (n = 4) alone or PBS with FluroMax microbeads (n = 4) at the beginning of the experiment did not affect SBP, HR or SNA (all $P > 0.05$, data not shown).

Assessment of renal function

Animals were placed in a metabolic cage for 3-5 hours to collect urine 24-72 hours prior to undergoing the terminal experimental protocol to collect urine. Arterial blood (0.2-0.3 ml) was collected into heparinised tubes via the femoral artery during the surgical preparation and centrifuged to separate serum. Urine and serum samples were stored at -80 ºC until analysis. A VetTest chemistry analyser (IDEXX laboratories Pty Ltd., New South Wales, Australia)

was used to measure urinary concentrations of protein and creatinine and serum concentrations of urea. Urinary protein was normalised to urinary creatinine to account for hydration status (Methven et al., 2010).

Data analysis

All data was analysed offline using Spike2 software. SBP and diastolic blood pressure (DBP) were derived from the arterial pressure waveform. Pulse pressure (PP) was calculated as the difference between SBP and DBP and mean arterial pressure (MAP) as the 1 second average of the arterial pressure waveform. HR was computed from the pulse interval. The SNA waveforms were rectified, smoothed (1 second constant) and corrected for background noise by subtracting a death level of SNA activity. SNA was then normalised to the 60 second period immediately prior to PVN microinjection, setting this as 100% and the level of SNA following euthanasia as 0%. Baseline measurements of MAP, SBP, DBP, PP and HR were taken over a 5 minute period immediately prior to microinjection or OPC-21268 administration. To assess the time-course of the SBP, HR and SNA response following microinjection, 5 minute binned averages were taken for the 30 minute period immediately following microinjection.

To derive baroreflex function curves, Spike2 was used to generate an x-y plot of HR and SNA (% of baseline) against SBP. Plots were constructed from the trough of the sodium nitroprusside curve over the slow rising phase of the phenylephrine pressor curve. Each individual curve was exported into Excel (Microsoft Office, Microsoft Corporation, WA, USA) and the level of HR and SNA averaged into 10 mmHg bins across the blood pressure range of each curve. Averaged data points were fitted to a four-parameter sigmoid regression curve using GraphPad Prism software (GraphPad Prism software v6 Inc., La Jolla, CA, USA) and the following equation:

$$y = \frac{A_1}{1 + \exp[A_2(SBP - A_3)]} + A_4$$

where A_1 is the y-axis range of the curve, A_2 is the gain coefficient, A_3 the midpoint of the curve and A_4 is the lower plateau. These values were used to calculate the range of the reflex, SBP saturation (SBP_{sat}), SBP threshold (SBP_{thr}) and SBP operational range as previously described (Kent et al., 1972).

All curves used in the final analysis had an R^2 value greater than 0.93. Theoretical curves were then generated over a fixed range of 50-250 mmHg for adult Lewis, 50-350 mmHg for

adult LPK, 50-200 mmHg for juvenile Lewis and 50-250 mmHg for juvenile LPK. Baseline and post-pharmacological manipulation curves were averaged for each strain and age group. In order to detect whether sympathetic baroreflex curves had shifted in response to pharmacological manipulations, SNA was normalised to the same 60 second period before the manipulation for baroreflex curve analysis.

dP/dT_{max} was computed as the maximal rate of rise of each arterial pulse in the 5 min before and 30 min following PVN microinjection of muscimol. Spectral analysis of SBP variability was performed as previously described (Hildreth et al., 2013a) and the very low frequency (VLF; 0.04-0.2 Hz) component of SBP variability calculated for one 80 second period before PVN muscimol microinjection and two 80 second periods at 5 and 10 minutes post-microinjection.

Real time quantitative polymerase chain reaction (qPCR)

A separate cohort of adult animals (*n* = 6 Lewis and 5 LPK) were used for determination of relative gene expression levels for the NMDA (NR1 and NR2B subunits) and AMPA 1 receptor subunit in the PVN. The AMPA 1, NR1 and NR2B receptor subunits were chosen based on their known high abundance in this region of the PVN (Herman et al., 2000). Animals were euthanised using sodium pentobarbital (100 mg/kg i.p.) and then perfused by syringe with ice-cold heparinised saline (500 units heparin/100ml; Hospira Aust Pty. Ltd. VIC, Australia). The brain was removed and frozen on dry ice and coronal sections (100 μm) were cut on a cryostat. Bilateral tissue punches were taken from sections containing the PVN extending from -1.6 to -2.2 mm relative to bregma (Paxinos and Watson, 2013) with 4 punches pooled in total from two slices. Tissue punches were taken using sterile blunt 18-gauge needles. Total RNA was extracted using an ISOLATE II RNA Micro Kit (Bioline Pty Ltd, NSW, Australia) according to the manufacturer's instructions, which incorporated a step to remove contaminating DNA. First-strand cDNA was then synthesized using the Tetro cDNA Synthesis Kit (Bioline Pty Ltd) using random primers (100 ng/μl). The reverse transcription process consisted of a primer annealing step (25 ºC, 10 minutes), cDNA synthesis initiation (42 ºC, 30 minutes), cDNA synthesis termination (85 ºC, 5 minutes) and a final holding step (4 ºC). Reactions containing no reverse transcriptase or RNA functioned as negative controls. RNA extracted from punches taken from the hippocampus region of the same animals served as positive controls. RNA quality was determined by the Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, VIC, Australia) with an average 260/280 ratio of 2±0.1. Real time quantitative polymerase chain reaction (qPCR) was performed using

1 µl of cDNA mix in a 25 µl reaction with each forward and reverse primer (Table 2.1) using SYBR green mastermix following the manufacturer's instructions (Stratagene, Agilent Technologies, Inc., CA, USA). Conditions for qPCR (40 cycles) were as follows: 95°C for 30-seconds, 60 ºC for 1 minute, then 72 ºC for 1 minute. Each reaction was performed with three replicates and the average taken for each animal.

Table 2.1: Primers for real-time reverse quantitative polymerase chain reaction.

Gene	Forward Primer	Reverse primer	Size (bp)	Accession Number
NMDA NR1	AATGGTACCCATGTCATCCC	ATCACTCATTGTGGGCTTGA	150	NM_017010
NMDA NR2B (Grin2b)	ATGGCGGATAAGGATGAGTC	GGGAAGTAGGTGGTGACGAT	131	NM_012574
AMPA (Gria1)	CCACGTGATCGAAATGAAAC	TTCTGGACGCTTGAGTTGTC	120	NM_031608

Primers were designed using the GenScript Real-time PCR (TaqMan) Online Primer Design Tool (GenScript USA Inc. Piscataway, NJ, USA) and verified using the NBCI/ Primer-BLAST database (http://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi). Testing included assessment of secondary annealing, mismatching or partial annealing to sequences other than the intended target. All primers were confirmed to span an intron except for AMPA for which intron/exon information was not available. Primers for NR1 recognized all transcript variants 1 -9 excepting variant 6.

Two steps were undertaken to validate the specificity of PCR products. For all PCRs, a dissociation melt curve analysis was conducted, whereby a final and additional PCR cycle was conducted over the temperature range of 55–95 ºC and the melt curve was calculated from the first derivative of the fluorescence response. All gene products yielded a single specific peak for each primer at a temperature greater than 80 ºC, indicating the presence of a single amplicon. Amplified fragments from selected LPK and Lewis animals for all primer pairs were also run on a 2% agarose (70 V, 1 hour) gel post-stained with GelRed (Biotium, CA, USA) and viewed under a Chemidoc MP (BioRad, CA, USA) to confirm the presence of a single amplicon that corresponded to the predicted product size.

Cycle threshold (Ct) values (representing cycle number at which fluorescence emission data exceeded a threshold limit) were determined for each gene and mRNA levels were normalised to 18S ribosomal RNA (rRNA) as an endogenous control, predetermined for stability using the geNorm reference gene selection kit as per the manufacturer's instructions

(Primer Design Ltd, Southampton, United Kingdom). 18S rRNA Ct values were subtracted from the Ct value for each gene (to give ΔCt values). To determine fold variation, expression levels relative to Lewis rats (reference set to 1) was calculated using the $2^{-\Delta\Delta Ct}$ method and range values determined using the formula $2^{-(\Delta\Delta Ct \pm SD\ \Delta Ct)}$ (Livak and Schmittgen, 2001). Calculation of range values was similarly applied to the chosen reference, providing range values around the set value of 1.

Statistical analysis

All data are expressed as mean ± SEM. GraphPad Prism was used for statistical analysis. A two-way ANOVA was used to detect strain and age differences. Drug effects were detected with two-way repeated measures ANOVA. If significant drug x strain or age x strain interactions were detected with two-way ANOVA, groups were split and analysed with one-way ANOVA. Bonferroni's post-hoc test was used for all multiple comparisons. A t test was performed to test for strain differences for urinary protein parameters in adult animals and for the qPCR data. ANOVA results are presented alongside F values and t test results are presented alongside t values as per (Shafer and Zhang, 2013). Significance was set at $P \leq 0.05$.

Results

Basal renal and cardiovascular function

Adult LPK rats exhibited proteinuria and markedly greater levels of serum urea than Lewis controls and juvenile LPK rats (Table 2.2). Baseline SBP was greater in juvenile and adult LPK rats compared with age-matched Lewis rats and increased with age in LPK rats (Table 2.3).

Does increased activity of the PVN maintain hypertension and resting SNA in LPK rats?

To determine if increased PVN activity contributes to the hypertension, sympathetic overactivity and baroreflex dysfunction observed in adult LPK rats, the effects of bilateral PVN inhibition using the $GABA_A$ agonist muscimol on blood pressure, SNA and baroreflex function was examined (Figures 2.3 and 2.4). In the LPK, PVN inhibition produced an immediate, sustained reduction in SBP over time ($F_{3,17} = 33.29$, $P < 0.0001$). This contrasted to the depressor response observed in the Lewis, which smaller compared with the LPK ($F_{1,11} = 18.46$, $P < 0.01$) and not evident until 25-minutes post-microinjection (adjusted $P = 0.02$). PVN inhibition also reduced MAP and DBP to a greater extent in LPK versus Lewis rats (MAP -29 ± 3 vs. -10 ± 3 mmHg, $P < 0.0001$; DBP -25 ± 3 vs. -6 ± 2 mmHg, $P < 0.0001$). SBP was used for all subsequent analysis because this parameter most accurately represents the hypertension exhibited in LPK rats (Table 2.3).

PVN microinjection of muscimol produced a reduction in HR over time that was observed in both strains ($F_{6,66} = 10.21$, $P < 0.0001$) and was of greater in magnitude in LPK rats ($F_{3,135} = 10.81$, $P < 0.0001$). The depressor and bradycardic responses were not mirrored by changes in SNA, with a small, yet comparable ($F_{1,7} = 0.21$, ns), reduction in sSNA over time ($F_{6,60} = 4.53$, $P < 0.001$) and no change in rSNA observed over time ($F_{6,54} = 0.39$, ns).

The depressor response to PVN microinjection of muscimol was most likely due to inhibition of neurons located in the PVN rather than nearby regions because GABA microinjections lowered SBP significantly more in LPK rats when the pipette tip was centred within the PVN versus 300 µm from its dorsal boundary (-16 ± 4 vs. -2 ± 2 mmHg; $n = 6$, $t_5 = 9.9$, $P < 0.001$).

Together this work shows that the PVN is overactive in its regulation of blood pressure and HR in LPK rats, but not sympathetic outflow.

Table 2.2: Bodyweight and renal function parameters in LPK and Lewis rats.

	Juvenile		Adult		*P*-value		
	LPK (5)	**Lewis (7)**	**LPK (16)**	**Lewis (20)**	**Strain**	**Age**	**Strain x Age**
Bodyweight (g)	134 ± 3	168 ± 5	246 ± 9[ab]	356 ± 3[a]	< 0.0001	< 0.0001	<0.0001
Urinary protein-to-creatinine ratio	0.13[*]	-[*]	6.52 ± 1.52[a]	0.22 ± 0.05[*]	-	-	-
Urinary protein (g/l)	0.07[*]	-[*]	1.08 ± 0.19[a]	0.21 ± 0.03[*]	-	-	-
Urinary creatinine (g/l)	0.54 ± 0.07	0.70 ± 0.08	0.20 ± 0.01[b]	1.07 ± 0.09	< 0.0001	0.8328	0.0005
Serum urea (mmol/l)	14.13 ± 0.72[b]	6.56 ± 0.57	32.14 ± 1.47[ab]	9.65 ± 0.37	< 0.0001	< 0.0001	<0.0001

Values are expressed as mean ± SEM. minimum *n* values per group are indicated by the subscript associated with each strain column. [a], *P*<0.05 vs. strain-matched juvenile rat; [b], *P*<0.05 vs. age-matched Lewis rat. [*]urinary protein was detectable in 0/7 juvenile Lewis, 1/5 juvenile LPK and 9/21 adult Lewis. Animals where urinary protein was undetectable were not included in the analyses of urinary protein or urinary protein-to-creatinine ratio. Statistical analysis was therefore only performed for adult animals using an unpaired t test.

Table 2.3: Baseline blood pressure and heart rate in LPK and Lewis rats.

	Juvenile		Adult		*P*-value		
	LPK (7)	**Lewis (8)**	**LPK (19)**	**Lewis (23)**	**Strain**	**Age**	**Strain x Age**
SBP (mmHg)	148 ± 4[b]	109 ± 3	191 ± 6[ab]	128 ± 3	<0.0001	< 0.0001	0.0316
MAP (mmHg)	115 ± 6	87 ± 3	98 ± 3[ab]	87 ± 3	<0.0001	0.0307	0.0280
DBP (mmHg)	92 ± 7[b]	69 ± 3	67 ± 3[a]	67 ± 6	0.01	0.0016	0.0059
PP (mmHg)	57 ± 5	40 ± 3	124 ± 6[ab]	61 ± 2[a]	<0.0001	< 0.0001	<0.0001
HR (bpm)	489 ± 12[b]	399 ± 10	378 ±6[a]	373 ± 10	0.0001	< 0.0001	0.0005

Values are expressed as mean ± SEM. minimum *n* values per group are indicated by the subscript associated with each strain column. [a], *P*<0.05 vs. strain-matched juvenile rat; [b], *P*<0.05 vs. age-matched Lewis rat. MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; HR, heart rate.

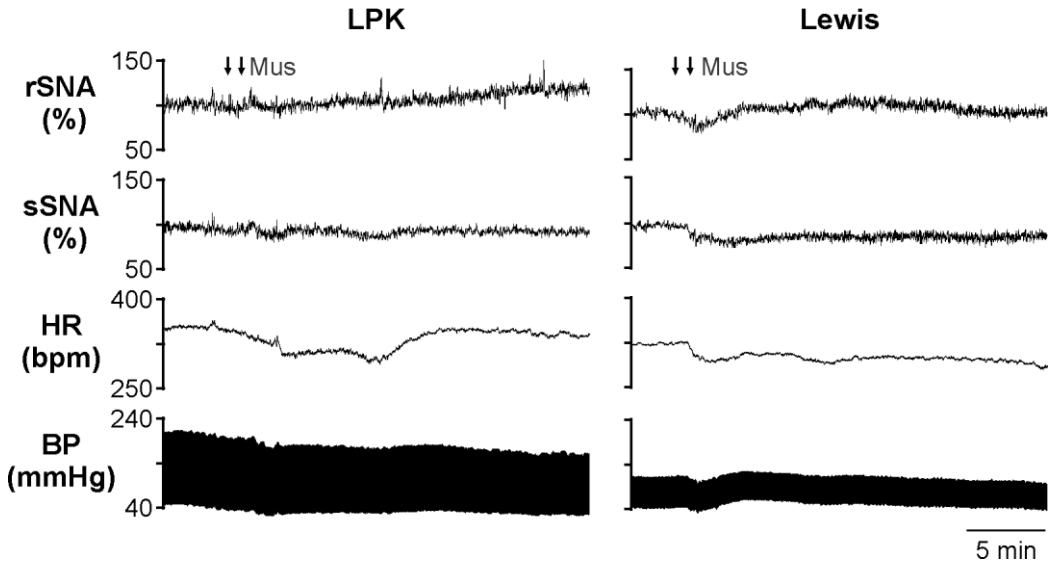

Figure 2.3. Representative recordings showing the blood pressure (BP), heart rate (HR), renal sympathetic nerve activity (rSNA) and splanchnic sympathetic nerve activity (sSNA) response to microinjection of muscimol (mus) into the PVN in an adult LPK and Lewis rat. Arrows indicate moment of microinjection.

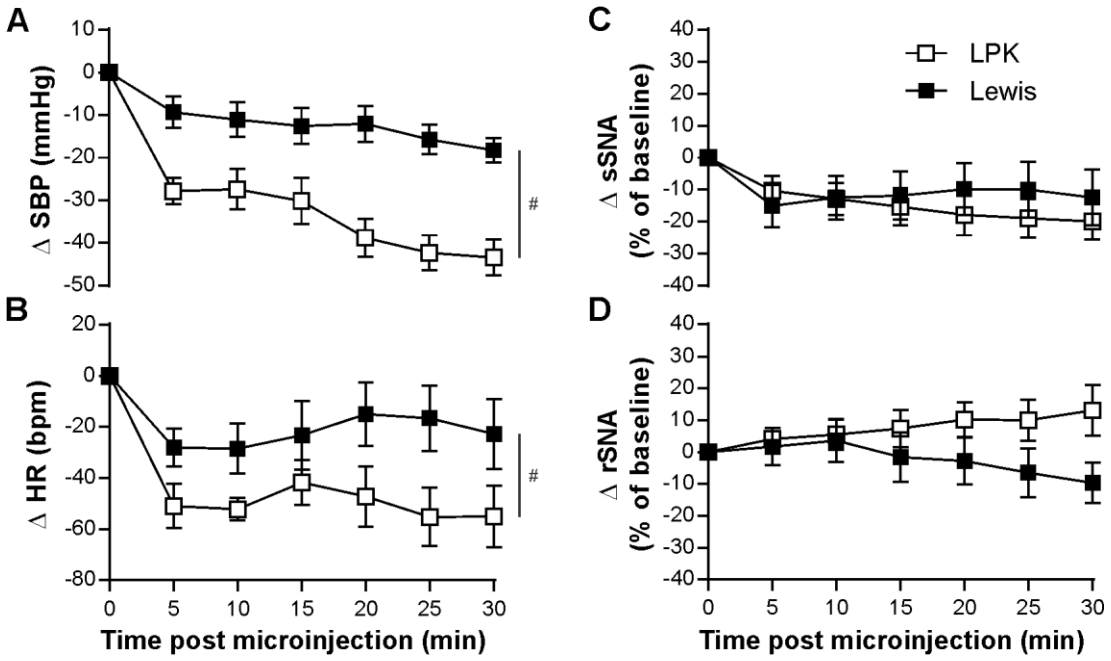

Figure 2.4. The effect of PVN microinjection of muscimol on systolic blood pressure (SBP; **A**), heart rate (HR; **B**), splanchnic sympathetic nerve activity (sSNA; **C**) and renal sympathetic nerve activity (rSNA; **D**) in adult LPK and Lewis rats. Data are expressed as a change from baseline. # indicates $P < 0.05$ difference between strains. $n \geq 5$ LPK, $n \geq 6$ Lewis.

Is altered glutamatergic tone in the PVN of LPK rats a contributing factor to hypertension, and does this develop as renal function worsens?

The main source of excitatory input to PVN neurons is glutamatergic (Li et al., 2006). To ascertain if increased PVN activity in the adult LPK is driven by increased excitatory glutamatergic input, we examined the blood pressure, sympathetic and HR responses to microinjection of kynurenic acid (Figures 2.5 and 2.6A), a non-selective ionotropic glutamate receptor antagonist. Microinjection of kynurenic acid into the PVN produced a depressor response over time in both Lewis ($F_{3,23} = 17.17$, $P < 0.0001$) and LPK ($F_{3,17} = 18.41$, $P < 0.0001$) that was of greater magnitude in the LPK ($F_{1,91} = 86.48$, $P < 0.0001$). Unlike direct inhibition of the PVN, removal of excitatory drive to the PVN using kynurenic acid did not significantly change HR over time ($F_{6,84} = 1.93$, $P = 0.09$) and produced an increase in rSNA over time ($F_{6,72} = 5.06$, $P < 0.001$) that was comparable between the strains ($F_{1,84} = 0.06$, $P > 0.05$). In a separate cohort of animals, the effect of kynurenic acid on lSNA was examined (data not shown). Kynurenic acid microinjection produced a comparable effect on lSNA in both LPK and Lewis ($F_{1,11} = 0.390$, ns) animals over time ($F_{6,66} = 2.41$, $P = 0.04$). Post-hoc analysis revealed that the time effect was only present in LPK rats, such that lSNA was reduced only at 25 minutes post-injection compared with baseline (-18±8 %; adjusted $P = 0.03$).

Enhanced glutamatergic regulation of the PVN is a feature of other disease states and may in part be contributed to by increased expression of glutamate receptors (Li et al., 2003b). To ascertain if similar mechanisms underlie the enhanced glutamatergic regulation of the PVN in adult LPK rats we examined if NMDA and AMPA receptor mRNA expression was altered. No significant difference in the relative expression of these genes between LPK and Lewis rats was noted (Table 2.4).

Collectively these results demonstrate that increased neuronal activity in the PVN is associated with hypertension in the adult LPK and that this is contributed to by increased excitatory glutamatergic regulation of the PVN. These changes are likely independent of differences in NMDA or AMPA receptor expression, noting however that mRNA expression is an imperfect measure of protein expression.

To determine if the PVN becomes overactive in its regulation of blood pressure in the LPK with the development of renal dysfunction, we examined the blood pressure, SNA and HR responses to microinjection of kynurenic acid in juvenile animals (Figures 2.5 and 2.6B)

when renal function is not overtly compromised in LPK rats (Table 2.2). At this earlier age, microinjection of kynurenic acid similarly produced a depressor response over time ($F_{6,78}$ = 32.00, $P < 0.0001$) that was of greater magnitude in the LPK compared with Lewis rats ($F_{1,91}$ = 97.34, $P < 0.0001$). Comparison of the blood pressure response to PVN microinjection of kynurenic acid in adult and juvenile LPK revealed that the PVN provides the same proportional control of blood pressure in the LPK regardless of age ($F_{1,84} = 0.93$, $P > 0.05$). Microinjection of kynurenic acid into the PVN reduced HR over time in the juvenile LPK only ($F_{2,11} = 10.47$, $P < 0.01$) and increased rSNA over time ($F_{6,54} = 3.86$, $P < 0.01$) comparably in the two strains ($F_{1,63} < 0.01$, ns). Therefore, the PVN is overactive in its regulation of blood pressure early in the disease and is directly contributing to the hypertension observed.

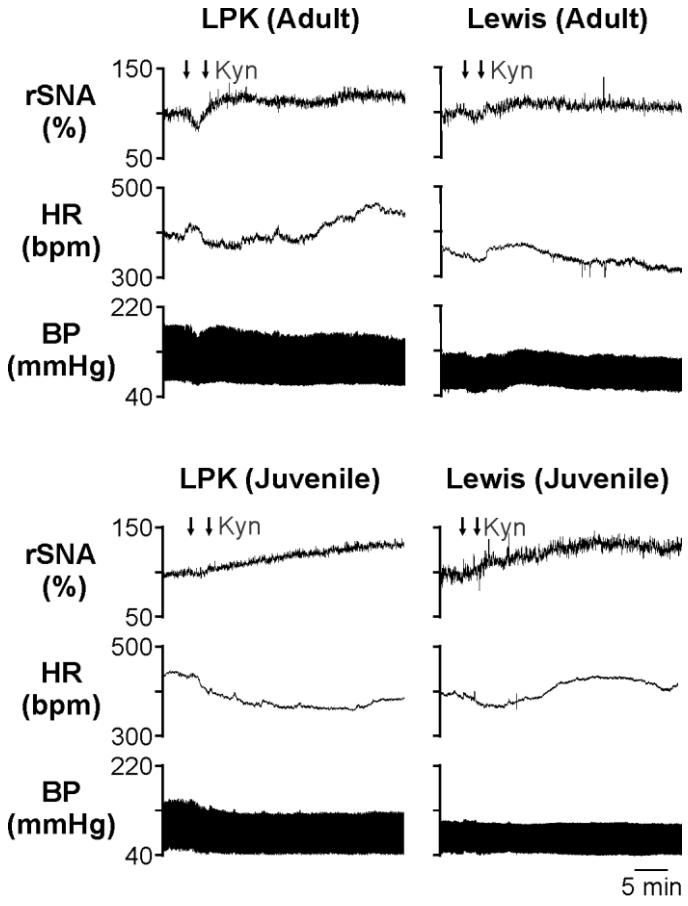

Figure 2.5. Representative recordings showing the blood pressure (BP), heart rate (HR) and renal sympathetic nerve activity (rSNA) response to microinjection of kynurenic acid (Kyn) into the PVN in adult and juvenile LPK and Lewis rats. Arrows indicate moment of microinjection.

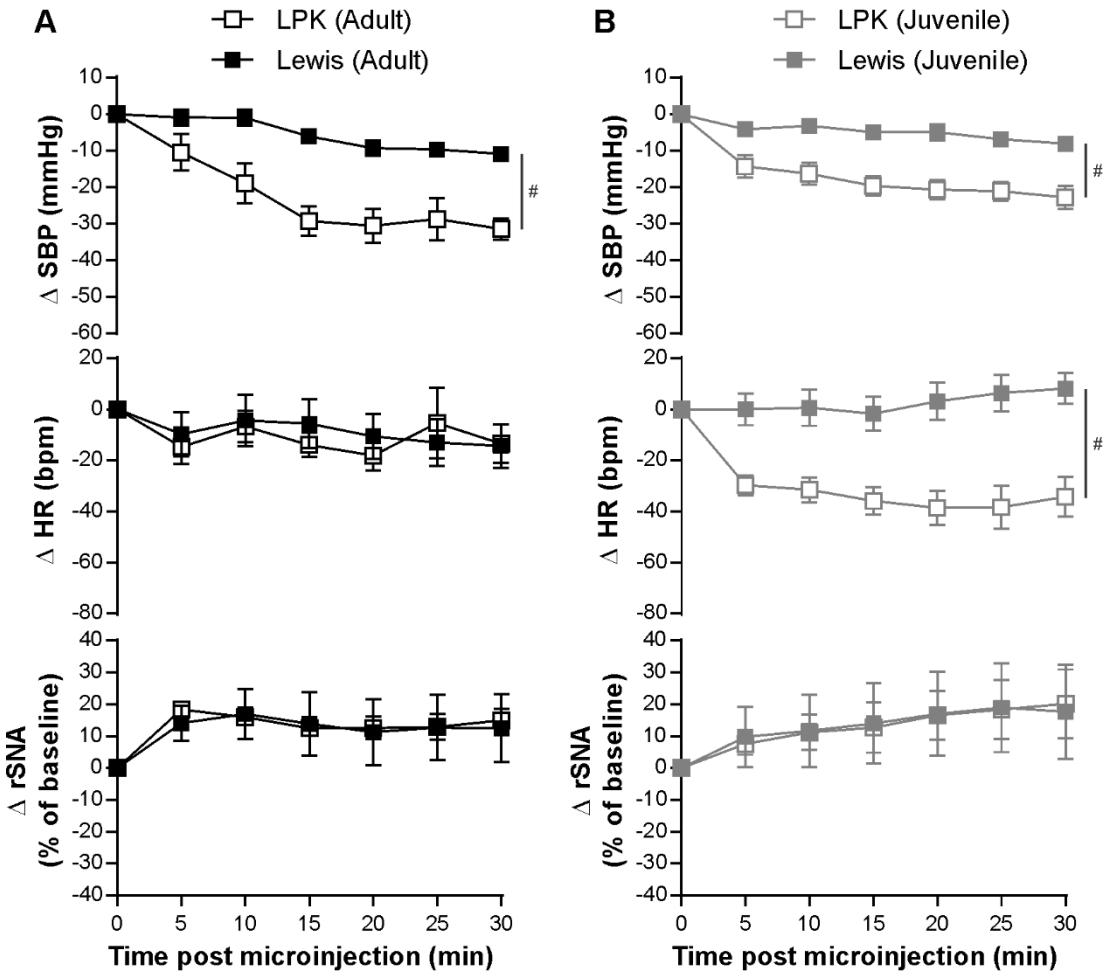

Figure 2.6. The effect of PVN microinjection of kynurenic acid on systolic blood pressure (SBP; **top**), heart rate (HR; **middle**) and renal sympathetic nerve activity (rSNA; **bottom**) in adult (**A**) and juvenile (**B**) LPK and Lewis rats. Data are expressed as a change from baseline. # indicates $P < 0.05$ difference between strains. $n \geq 7$ adult LPK, $n \geq 7$ adult Lewis, $n \geq 5$ juvenile LPK, $n \geq 6$ juvenile Lewis.

Table 2.4: ΔCt and fold difference values for NR1, NR2B and AMPA gene expression in the hypothalamic paraventricular nucleus in LPK and Lewis rats.

	ΔCt values		**Fold-difference and range values**	
Receptor	**LPK**	**Lewis**	**Lewis**	**Lewis**
NR1	7.6±0.3	7.85±0.2	1 (0.85–1.2)	1 (0.85–1.2)
NR2B	7.2±0.1	7.7±0.3	1 (0.8–1.2)	1 (0.8–1.2)
AMPA	8.6±0.7	8.1±0.2	1 (0.9–1.15)	1 (0.9–1.15)

Δ cycle threshold (ΔCt) values expressed as mean ± SEM represent threshold PCR cycle number normalised to endogenous control gene 18S rRNA, with a lower number representing a higher level of expression. Data is also expressed as normalized ΔΔCt values and fold variation relative to reference Lewis animals, calculated using the $2^{-\Delta\Delta Ct}$ method.(Livak and Schmittgen, 2001) There was no difference in gene expression level for any of the receptor subunits between strains in the PVN region (all $P > 0.05$).

Does PVN-dependent stimulation of cardiac autonomic outflow or vasopressin secretion contribute to the maintenance of hypertension in LPK rats?

Our findings that both direct and indirect inhibition of the PVN produced a profound depressor response in LPK rats that was not accompanied by a greater reduction in SNA, regardless of the nerve bed recorded, suggests that the PVN maintenance of hypertension in LPK animals is largely independent of vasomotor sympathetic outflow. The PVN can also command changes in neural input to the heart to modulate its contractile force and rate (Mendonca et al., 2018). We examined whether, in LPK rats, PVN inhibition with muscimol was associated with an enhanced reduction in dP/dT_{max} , a measure of left-ventricular contractility (Monge Garcia et al., 2018). Muscimol reduced dP/dT_{max} in both LPK (7956 ± 1038 vs. 7014 ± 875 mmHg/sec; $P < 0.01$) and Lewis (4251 ± 709 vs. 3416 ± 513 mmHg/sec; $P < 0.01$) rats, though the magnitude was similar in each strain ($P = 0.72$). In separate LPK animals, the depressor response evoked by PVN inhibition was examined under combined neural autonomic blockade with atenolol and methylatropine. After administration of atenolol and methylatropine, resting SBP was unaffected (181 ± 11 vs 189 ± 10 mmHg, baseline vs atenolol/methylatropine; $n = 4$, $t_6 = 0.05$, $P < 0.05$), whereas the depressor response to PVN inhibition was enhanced (-31 ± 2 vs -23 ± 2 %, LPK with atenolol/methylatropine pre-treatment vs LPK without pre-treatment; $t_8 = 2.8$, $P = 0.02$). The HR response to PVN inhibition was eliminated in these animals (306±5 vs 311±10 bpm, baseline vs PVN muscimol; $t_6 = 0.5$, $P > 0.05$). Therefore, neural control of cardiac output likely serves to buffer, but not drive, reductions in arterial pressure in response to PVN inhibition in the LPK.

The PVN has the potential to regulate blood pressure not only via its regulation of sympathetic outflow but also via its regulation of pituitary neurohormones, particularly vasopressin. Accordingly, we examined the effect of PVN muscimol microinjection on the very-low frequency component of SBP variability, which reflects hormonal vasomotor tone and autoregulatatory control of SBP (Stauss, 2007), and observed a marked reduction in this spectral parameter in LPK rats (2.4±0.5 vs 0.6±0.1 $mmHg^2$, baseline vs muscimol; adjusted $P < 0.01$) but not Lewis rats (0.7±0.3 vs 0.5±0.1 $mmHg^2$; adjusted $P > 0.05$).

Considering this, we therefore tested the hypothesis that the increased PVN regulation of blood pressure in LPK rats is vasopressin-dependent. Bolus administration of vasopressin produced a comparable pressor response in both strains (22±2 vs 17±5 %, $n = 7$ LPK vs 6 Lewis; $t_{11} = 1.0$, $P > 0.05$), while systemic vasopressin V_{1A} receptor blockade using OPC-

21268 produced a significant reduction in blood pressure in LPK rats only (-13±4 vs 0±2 %, n = 7 LPK vs 6 Lewis; $t_{11} = 3.4$, $P < 0.01$) suggesting that vasopressin-mediated regulation of blood pressure is enhanced in LPK rats but that the sensitivity of the vasculature to vasopressin is not different. We reasoned that if the PVN was overactive in its regulation of blood pressure due to a tonic increase in vasopressin secretion, subsequent inhibition of the PVN with bilateral microinjection of muscimol after systemic V_{1A} receptor blockade would produce no further change in blood pressure. To our surprise, subsequent inhibition of the PVN still produced a comparably exaggerated depressor response in LPK rats such that their blood pressure was normalised (Figures 2.7 and 2.8).

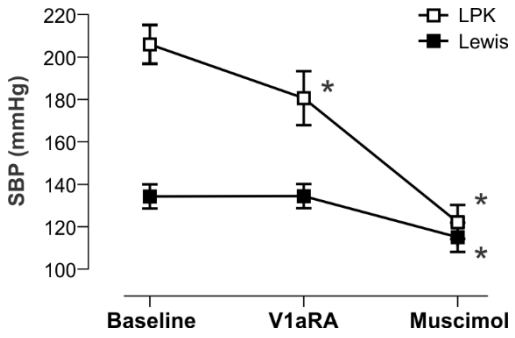

Figure 2.7. The effect of systemic V_{1A} receptor antagonism (V1aRA; n = 7 Lewis, n = 6 LPK) and subsequent PVN microinjection of muscimol (n = 5 Lewis, n = 5 LPK) on systolic blood pressure (SBP) in adult LPK and Lewis rats. * indicates $P < 0.05$ difference from baseline.

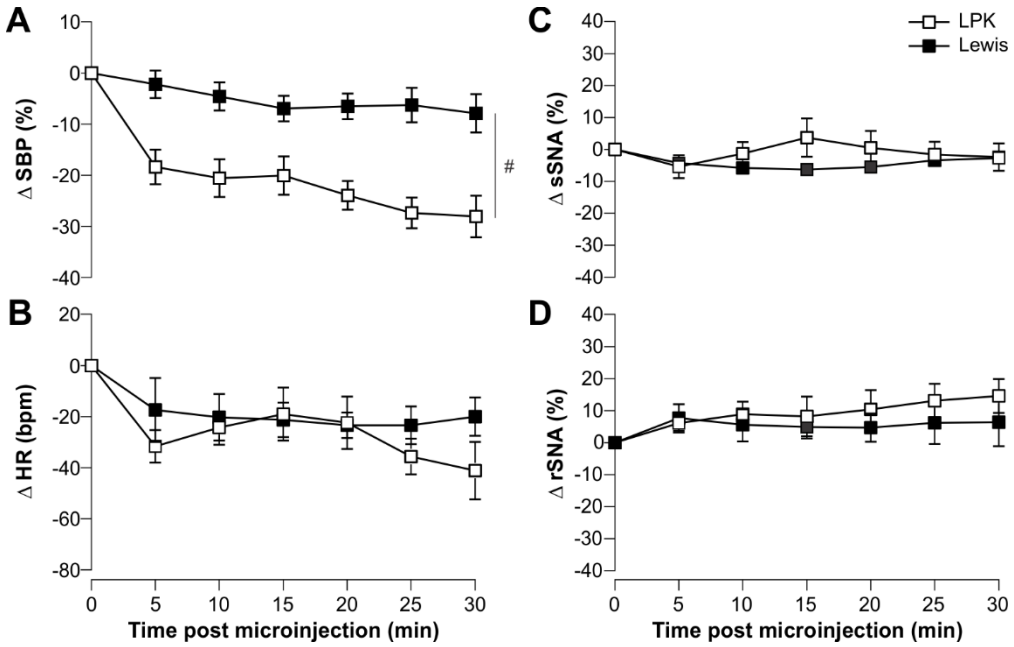

Figure 2.8 The effect of PVN microinjection of muscimol following V_{1A} receptor antagonism pre-treatment with OPC-21268 on systolic blood pressure (SBP; **A**), heart rate (HR; **B**), splanchnic sympathetic nerve activity (sSNA; **C**) and renal sympathetic nerve activity (rSNA; **D**) in adult LPK and Lewis rats. Data are expressed as a change from baseline. # indicates $P < 0.05$ difference between strains. $n \geq 4$ Lewis and $n \geq 4$ LPK.

Does increased neuronal activity within the PVN or circulating vasopressin contribute to baroreflex dysfunction in LPK rats?

Both the PVN (Michelini and Stern, 2009) and circulating vasopressin (Hasser and Bishop, 1990) contribute to the ongoing processing of the baroreceptor reflex. We therefore hypothesised that an overactive PVN or increased circulating vasopressin may contribute to the impairment in baroreflex control of HR and SNA observed in this model. Consistent with previous findings (Salman et al., 2014; Salman et al., 2015a), both the gain and range of the HR and SNA baroreflex curves was reduced in adult LPK rats at baseline (Figures 2.9 and 2.10; Tables 2.5, 2.6 and 2.7). Inhibition of the PVN with muscimol in adult animals shifted both HR and sympathetic baroreflex curves to the left in both strains, consistent with the reduction in blood pressure observed. Muscimol resulted in a comparable reduction in the gain and range of the HR, but not SNA, baroreflex (Figure 2.9; Tables 2.5 and 2.6). Blockade of excitatory input to the PVN, using bilateral microinjection of kynurenic acid, however, had no effect on either HR or sympathetic baroreflex in adult and juvenile animals (Figure 2.10). Finally, blockade of systemic V_{1A} receptors with OPC-21268 did not affect baroreflex function in adult animals of either strain (Figures 2.11). Collectively this demonstrates that neither PVN overactivity nor circulating vasopressin contribute to the baroreflex dysfunction observed in LPK rats.

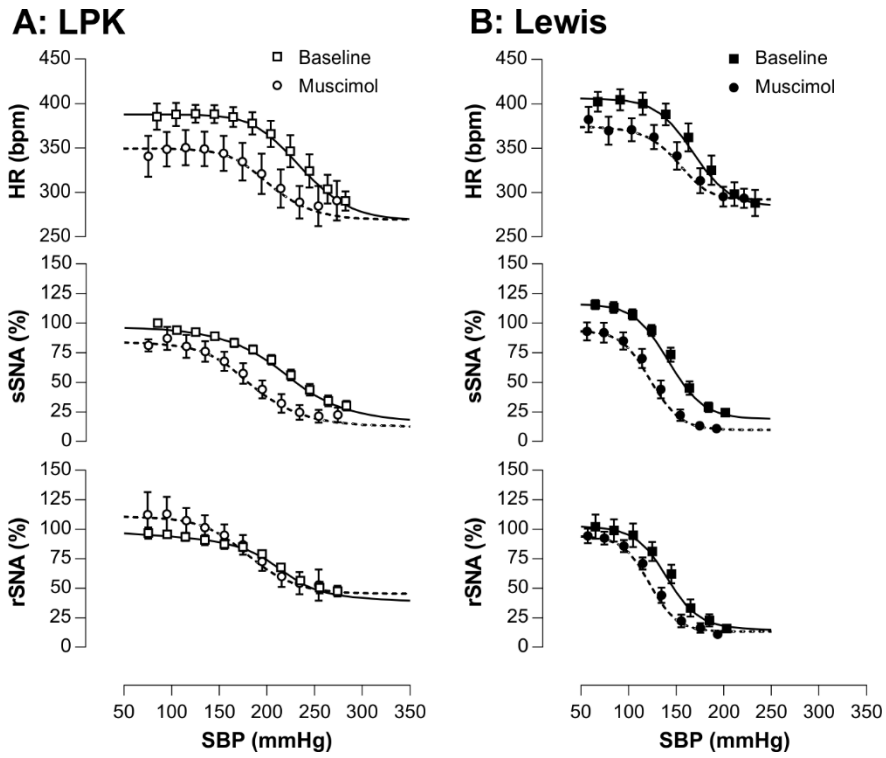

Figure 2.9. Baroreflex function curves for heart rate (HR; **top**), splanchnic sympathetic nerve activity (sSNA; **middle**) and renal sympathetic nerve activity (rSNA; **bottom**) at baseline and following PVN microinjection of muscimol in adult LPK (**A**) and Lewis (**B**) rats. Symbols represent the averaged level of HR/SNA and systolic blood pressure (SBP) in response to phenylephrine and sodium nitroprusside and lines represent sigmoid regression curves generated from these data at baseline (solid) and following muscimol (dashed). $n \geq 5$ LPK and $n \geq 6$ Lewis.

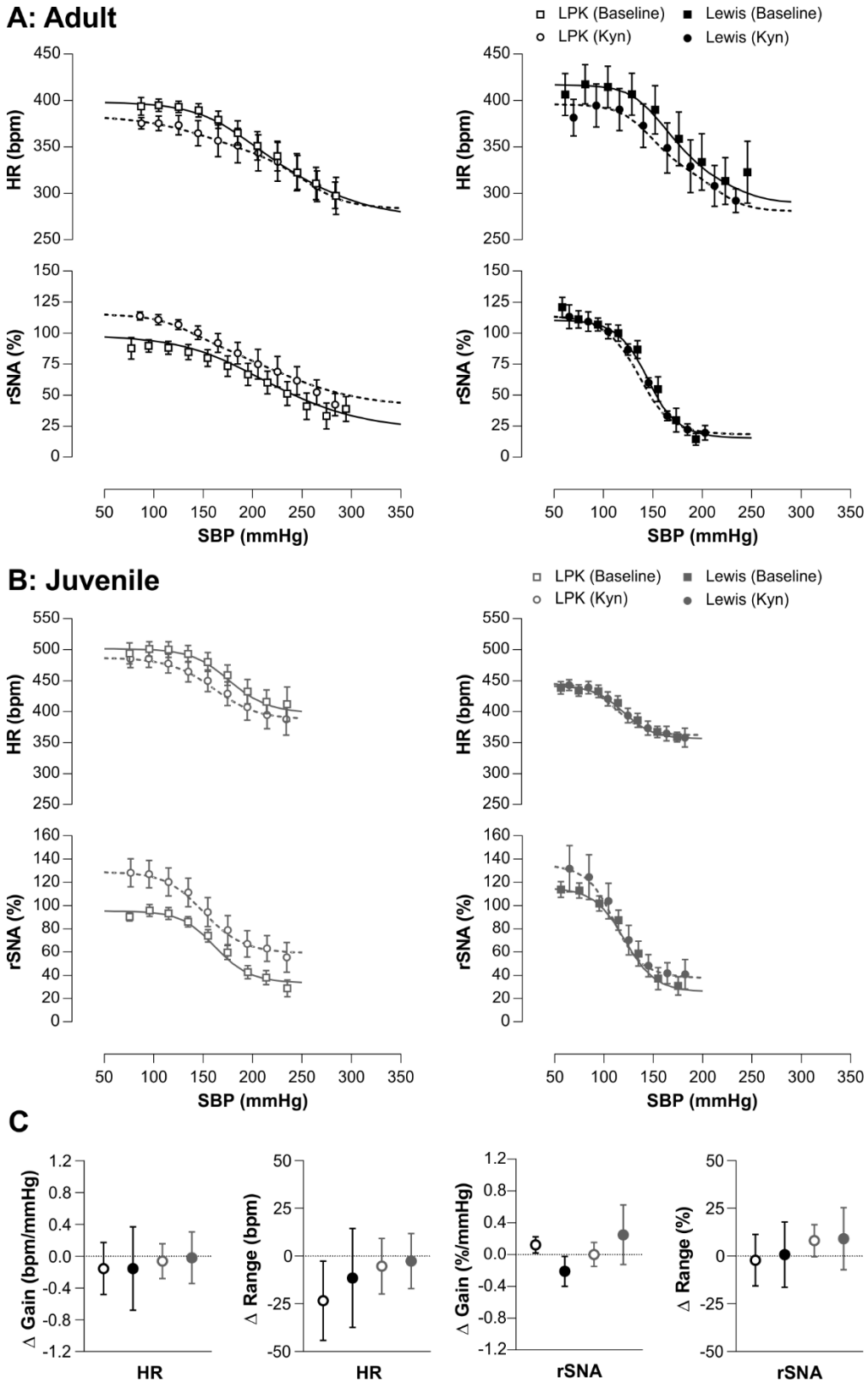

Figure 2.10. Baroreflex function curves for heart rate (HR) and renal sympathetic nerve activity (rSNA) at baseline and following PVN microinjection of kynurenic acid (Kyn) in adult (**A**) and juvenile (**B**) LPK and Lewis rats. Symbols represent the averaged level of HR/SNA and systolic blood pressure (SBP) in response to phenylephrine and sodium nitroprusside and lines represent sigmoid regression curves generated from these data at

baseline (solid) and following kynurenic acid (dashed). (**C**) The effect of PVN microinjection of kynurenic acid on the gain and range of the HR and rSNA baroreflexes. Data are expressed as a change from baseline. n ≥ 5 adult LPK, n ≥ 6 adult Lewis, n ≥ 5 juvenile LPK and n ≥ 6 juvenile Lewis.

Table 2.5: Heart rate (HR) baroreflex parameters in adult LPK and Lewis rats at baseline and following bilateral microinjection of muscimol into the paraventricular nucleus of the hypothalamus.

Group	LPK (6)		Lewis (7)		*P*-value		
Parameter	Baseline	Muscimol	Baseline	Muscimol	Strain	Treatment	Strain x Treatment
HR barocurve							
SBP_{50} (mmHg)	234 ± 9†	200 ± 9*†	149 ± 5	134 ± 5*	<0.0001	<0.0001	0.0025
SBP_{sat} (mmHg)	260 ± 11†	225 ± 11*†	168 ± 5	150 ± 4*	<0.0001	<0.0001	0.0178
SBP_{thr} (mmHg)	207 ± 8†	177 ± 7*†	130 ± 5	119 ± 6*	<0.0001	<0.0001	0.0061
SBP Range (mmHg)	53 ± 4	48 ± 7†	40 ± 4	31 ± 3	0.0102	0.1263	0.7691
Gain (bpm/mmHg)	-1.5 ± 0.2	-1.1 ± 0.2	-2.2 ± 0.3	-1.9 ± 0.3	0.0440	0.0241	0.9254
Upper plateau (bpm)	388 ± 11	349 ± 19	406 ± 11	374 ± 14	0.2288	0.0050	0.7629
Lower plateau (bpm)	269 ± 14	269 ± 13	284 ± 13	292 ± 11	0.2688	0.5369	0.5749
Range (bpm)	119 ± 17	80 ± 15*	122 ± 7	82 ± 10*	0.8864	<0.0001	0.9147

Values are expressed as mean ± SEM. (*n)* indicates number of animals per group. * $P<0.05$ vs. baseline; † $P<0.05$ vs. treatment-matched Lewis rat. SBP, systolic blood pressure; SBP_{50}, SBP at the midpoint of the curve; SBP_{sat}; SBP saturation; SBP_{thr}, SBP threshold.

Table 2.6: Sympathetic baroreflex parameters in adult LPK and Lewis rats at baseline and following bilateral microinjection of muscimol into the paraventricular nucleus of the hypothalamus.

Group	LPK (5)		Lewis (6)		*P*-value		
Parameter	Baseline	Muscimol	Baseline	Muscimol	Strain	Treatment	Strain x Treatment
sSNA barocurve							
SBP_{50} (mmHg)	219 ± 10†	185 ± 6*†	143 ± 4	123 ± 4*	<0.0001	<0.0001	0.0148
SBP_{sat} (mmHg)	260 ± 11†	217 ± 10*†	166 ± 4	142 ± 4*	<0.0001	<0.0001	0.0006
SBP_{thr} (mmHg)	178 ± 11†	152 ± 5*†	120 ± 3	105 ± 3	<0.0001	0.0015	0.3122
SBP range (mmHg)	82 ± 10†	65 ± 10	31 ± 15	37 ± 1	0.0065	0.5930	0.2274
Gain (%/mmHg)	-0.7 ± 0.1†	-0.8 ± 0.1†	-1.4 ± 0.1	-1.5 ± 0.1	<0.0001	0.4073	0.8927
Upper plateau (%)	97 ± 4	84 ± 11	116 ± 4	94 ± 8	0.0622	0.0485	0.5260
Lower plateau (%)	18 ± 3	12 ± 2	19 ± 4	10 ± 1	0.7707	0.0286	0.5199
Range (%)	79 ± 5	72 ± 10	97 ± 5	84 ± 8	0.0817	0.1441	0.6277
rSNA barocurve							
SBP_{50} (mmHg)	202 ± 10†	182 ± 6*†	143 ± 4	123 ± 3*	<0.0001	0.0004	0.9986
SBP_{sat} (mmHg)	255 ± 13†	214 ± 7*†	167 ± 7	141 ± 3	<0.0001	0.0034	0.3767
SBP_{thr} (mmHg)	181 ± 11†	154 ± 5*†	120 ± 4	105 ± 4	<0.0001	0.0023	0.2072
SBP range (mmHg)	57 ± 5	65 ± 7	46 ± 8	35 ± 3	0.0602	0.9552	0.2286
Gain (%/mmHg)	-0.6 ± 0.2†	-0.7 ± 0.1†	-1.4 ± 0.2	-1.5 ± 0.2	0.0049	0.1138	0.8205
Upper plateau (%)	93 ± 4	111 ± 11	103 ± 10	94 ± 6	0.5015	0.7439	0.0731
Lower plateau (%)	36 ± 5	45 ± 11†	14 ± 5	13 ± 4	0.0035	0.5401	0.4558
Range (%)	57 ± 5	65 ± 7	89 ± 11	81 ± 8	0.0602	0.9552	0.1406

Values are expressed as mean ± SEM. (*n)* indicates minimum number of animals per group. * $P<0.05$ vs. baseline; † $P<0.05$ vs. treatment-matched Lewis rat. SBP, systolic blood pressure; SBP_{50}, SBP at the midpoint of the curve; SBP_{sat}; SBP saturation; SBP_{thr}, SBP threshold; sSNA, splanchnic SNA; rSNA, renal SNA

Table 2.7: Comparison of baroreflex parameters at baseline in juvenile and adult LPK and Lewis rats. Baseline data is shown for the animals that received bilateral microinjection of kynurenic acid into the paraventricular nucleus of the hypothalamus.

Group	Juvenile		Adult		*P*-value		
Parameter	LPK (5)	Lewis (6)	LPK (5)	Lewis (6)	Age	Strain	Age x Strain
HR barocurve							
SBP_{50} (mmHg)	178 ± 4[b]	119 ± 5	223 ± 17[ab]	157 ± 10[a]	0.0001	<0.0001	0.6738
SBP_{sat} (mmHg)	207 ± 3[b]	137 ± 6	263 ± 20[ab]	176 ± 11[a]	<0.0001	<0.0001	0.4181
SBP_{thr} (mmHg)	149 ± 5[b]	102 ± 5	184 ± 16[ab]	138 ± 11[a]	0.0006	<0.0001	0.9554
SBP range (mmHg)	58 ± 3[b]	35 ± 2	79 ± 13[ab]	38 ± 3	0.0267	<0.0001	0.1077
Gain (bpm/mmHg)	-1.2 ± 0.2	-1.7 ± 0.2	-1.2 ± 0.2[b]	-2.3 ± 0.4	0.3007	0.0070	0.1956
Upper plateau (bpm)	502 ± 12	442 ± 9	399 ± 6[a]	417 ± 21	0.0001	0.1568	0.0105
Lower plateau (bpm)	397 ± 21	356 ± 7	274 ± 16 [a]	290 ± 14 [a]	<0.0001	0.4254	0.0737
Range (bpm)	105 ± 11	86 ± 9	125 ± 16	127 ± 17	0.0311	0.5479	0.4378
rSNA barocurve							
SBP_{50} (mmHg)	162 ± 3[b]	121 ± 4	217 ± 14[ab]	146±3	0.0001	<0.0001	0.0956
SBP_{sat} (mmHg)	184 ± 4[b]	140 ± 4	269 ± 18[ab]	164±4	<0.0001	<0.0001	0.0129
SBP_{thr} (mmHg)	140 ± 2[b]	101±4	165 ± 13[b]	127±4	0.0070	0.0002	0.9850
SBP range (mmHg)	45 ± 2	39 ± 3	103 ± 16[ab]	37±5	0.0097	0.0016	0.0062
Gain (%/mmHg)	-0.9 ± 0.1[b]	-1.5 ± 0.1	-0.5 ± 0.1[ab]	-1.8±0.1	0.4122	<0.0001	0.0119
Upper plateau (%)	95 ± 5	116 ± 6	99 ± 5	111±7	0.8964	0.0113	0.4659
Lower plateau (%)	33 ± 6	26 ± 8	21 ± 8	15±10	0.1986	0.4202	0.9296
Range (%)	62 ± 8	90 ± 6	77 ± 8	95±11	0.2468	0.0162	0.5719

Values are expressed as mean ± SEM. (*n*) indicates minimum number of animals per group. [a], $P<0.05$ vs. strain-matched juvenile rat; [b], $P<0.05$ vs. age-matched Lewis rat. HR, heart rate; SBP, systolic blood pressure; SBP_{50}, SBP at the midpoint of the curve; SBP_{sat}; SBP saturation; SBP_{thr}, SBP threshold; rSNA, renal SNA.

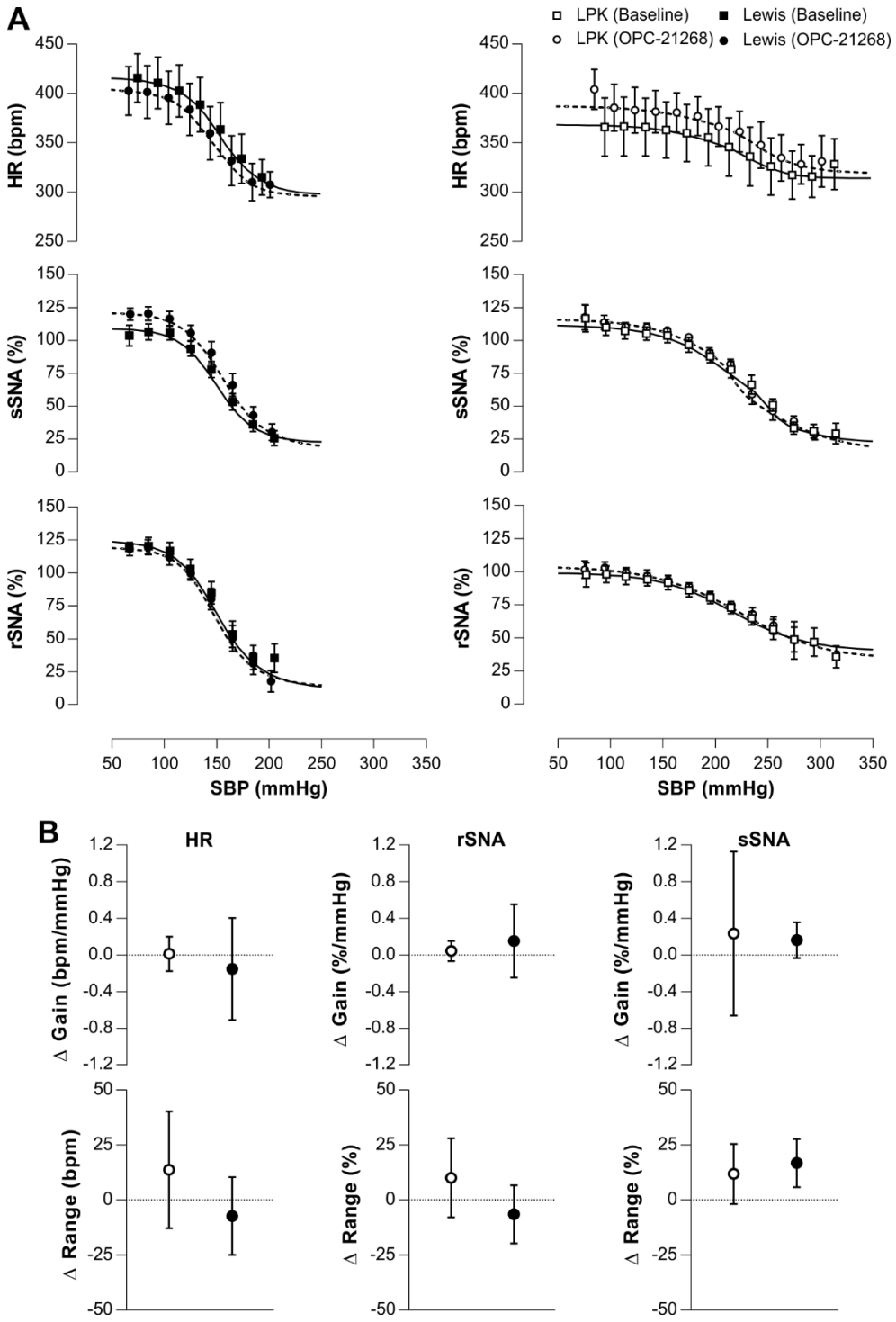

Figure 2.11. **A**: Baroreflex function curves for heart rate (HR), splanchnic sympathetic nerve activity (sSNA) and renal sympathetic nerve activity (rSNA) at baseline and after OPC-21268 (i.v.) in adult LPK and Lewis rats. Symbols represent the averaged level of HR/SNA and systolic blood pressure (SBP) in response to phenylephrine and sodium nitroprusside and lines represent sigmoid regression curves generated from these data at baseline (solid) and following OPC-21268 (dashed). **B**: The effect of OPC-21268 on the gain and range of the HR and sympathetic baroreflexes. Data are expressed as a change from baseline. *n* = 4 LPK and *n* = 6 Lewis.

Discussion

Here we show for the first time that PVN neuronal activation and circulating vasopressin result in the hypertension observed in an animal model of PKD, the LPK rat. Increased PVN activity originates early in the disease course in LPK animals from an enhancement of excitatory glutamatergic tone that drives blood pressure via mechanisms that are largely independent of sympathetic outflow or vasopressin secretion.

Our work strongly suggests that increased PVN activity contributes to hypertension in PKD, which together with previous work that implicates increased PVN neuronal outflow in several forms of hypertension (Li and Pan, 2007a; Bardgett et al., 2014a), supports the notion that maladaptive upregulation of PVN activity is a convergent feature of many neurogenic forms of hypertension. Under normal conditions, the PVN contributes little to resting blood pressure because tonic excitatory glutamatergic drive is suppressed by inhibitory GABAergic input (Li et al., 2006). We show that an enhancement of glutamatergic inputs to PVN neurons contribute to hypertension in LPK rats at both early and established stages of kidney disease.

The PVN can generate sympathetic outflow to different vascular beds and end-organs in a selective manner (Stocker et al., 2005). We found that inhibition of PVN neurons or blockade of glutamatergic inputs was not associated with an exaggerated reduction in SNA in LPK animals, as recorded from either renal, splanchnic or lumbar sympathetic beds, which is surprising given the strong reduction in blood pressure evoked using these manipulations. In addition to regulating vasomotor tone, PVN neurons regulate cardiac output via sympathetic and vagal efferents (Michelini and Stern, 2009). In LPK rats, however, the PVN control of hypertension does not appear to involve these autonomic outflows as the depressor response to PVN inhibition was greater, albeit only slightly, under cardiac autonomic blockade. This indicates that the PVN control of blood pressure in this disease model involves some other downstream effector(s). Given that circulating vasopressin levels are elevated in LPK rats (Underwood et al., 2017), as typical of human PKD (Zittema et al., 2012), we considered that the PVN was producing hypertension in LPK animals via its control over vasopressin release; however, our results showed that systemic V_{1A} receptor antagonism did not prevent the depressor response to PVN inhibition suggesting that PVN-mediated secretion of vasopressin does not directly contribute. The precise mechanism via which the PVN controls blood pressure in LPK rats remains to be determined.

In LPK animals, increased glutamatergic drive to the PVN could conceivably result from greater synaptic glutamate levels, enhanced post-synaptic excitability and/or increased density of receptors. We examined the latter possibility, and while not an absolute measure of receptor expression, our findings indicate that at least at the mRNA level, the expression of both AMPA and NMDA receptors in the PVN does not differ in LPK animals. As enhanced glutamatergic control of the PVN is evident in juvenile LPK rats, we speculate that this develops very early in the disease course and may potentially precipitate the development of hypertension in this model. What initiates the increase in excitatory drive to the PVN in LPK rats remains to be determined; however, it is prudent to note that while juvenile LPK rats do not exhibit overt renal dysfunction, as evidenced by proteinuria, they do present with renal cysts and mild renal dysfunction at this age (Phillips et al., 2007). It is therefore plausible that neural or haemal signals from the diseased kidney (Underwood et al., 2017) may be initiating and/or maintaining the enhanced excitatory regulation of this brain region in PKD.

A large body of work has demonstrated a detrimental role of vasopressin type 2 (V_2) receptor activation in accelerating renal cystogenesis (Rinschen et al., 2014). However, to date no study has examined whether V_{1A} receptor activation contributes to hypertension in PKD. Our novel data showing that acute systemic V_{1A} receptor antagonism reduced blood pressure in LPK but not Lewis rats, highlights vasopressin as a potential target for the management of hypertension in PKD. It will however be necessary to examine the effect of V_{1A} receptor antagonism in conscious LPK animals as urethane anaesthesia is known to elevate vasopressin levels (Severs et al., 1981). Nevertheless, our results suggest that the increased vasopressinergic regulation of blood pressure observed in LPK animals is unlikely to be driven by PVN neuron activation, and may therefore require the supraoptic nucleus, the other major source of vasopressin secretion.

The PVN has polysynaptic connections with cardiac sympathetic and vagal neurons (Strack et al., 1989; Standish et al., 1994). As a group, these neurons exert a cardio-stimulatory action as pharmacological excitation of the PVN rapidly increases HR (Martins-Pinge et al., 2012). In our study, we found that inhibition of the PVN with muscimol produced bradycardia, an effect that was greater in LPK rats and abolished by pharmacological denervation of the heart. These data indicate that a population of cardio-stimulatory PVN neurons are overactive in LPK rats. As we observed a similar effect with kynurenic acid in juvenile but not adult LPK rats, it possible that this neuronal population are driven by both a glutamatergic and a non-glutamatergic mechanism depending on the disease stage.

In conclusion, our study is the first to investigate the central origins of hypertension in PKD. We show that both PVN neuronal activation and circulating vasopressin are required for the maintenance of hypertension in the LPK rat model of PKD. In these animals, PVN neuronal overactivity is present early in the disease course, suggesting that changes in the neurohumoral control of blood pressure are already well established before any marked decline in renal function and need to be tackled therapeutically early if effective blood pressure control is to be achieved. Our work highlights the potential utility of V_{1A} receptor antagonism in the management of hypertension in PKD, with long-term treatment studies required to further explore this possibility.

3.

Angiotensin II Differentially Regulates Blood Pressure and Sympathetic Nerve Activity in Polycystic Kidney Disease

Underwood C.F., Kumar, N.N., McMullan, S., Rahman, A.A., Phillips J.K. & Hildreth C.M.

Abstract

Angiotensin (Ang) II exerts a hypertensive action in the hypothalamic paraventricular nucleus (PVN) by increasing sympathetic activity and vasopressin release. We examined the hypothesis that enhanced Ang II signalling in the PVN contributes to hypertension in polycystic kidney disease (PKD). PVN microinjection of Ang II produced greater increases in systolic blood pressure in urethane-anaesthetised Lewis Polycystic Kidney (LPK) versus control Lewis rats (36±5 vs. 17±2 mmHg; $P<0.01$) and was accompanied by renal and splanchnic sympathoinhibition in LPK but sympathoexcitation in Lewis. Pharmacological inhibition of peripheral V_{1A} receptors, PVN AT_1 receptors (AT1R) or astrocyte glutamate-transporters (GLT-1) abolished the Ang II pressor response in both strains (all $P<0.05$), suggesting that that PVN Ang II increases SBP by driving the release of vasopressin via AT1R and GLT-1. Examination of PVN AT1R with in-situ hybridisation revealed that AT1R mRNA was both more abundant (mean dif. 64±23%; $P=0.02$) and localised to a greater proportion of vasopressin (8±2 vs. 0.5±0.5 %; $P=0.04$) and corticotropin-releasing hormone (84±4 vs. 40±0.3 %; $P=0.04$) containing neurons in LPK vs. Lewis rats and that in both strains retrogradely-labelled presympathetic PVN neurons and the majority of astrocytes lacked AT1R. Finally, PVN inhibition of inositol trisphosphate receptors or calcineurin attenuated the Ang II pressor response by ~55% in LPK rats only ($P<0.05$). Our data suggest that the vasopressin-dependent pressor actions of Ang II are enhanced in the PVN in PKD. The mechanism is likely to involve an indirect and enhanced inhibition of astrocyte glutamate transport, possibly via inositol trisphosphate receptors and calcineurin signalling.

Introduction

Neuroendocrine and autonomic systems must be coordinated to maintain cardiovascular homeostasis and hydromineral balance. Subserving this role is a heterogeneous population of neurons contained within the hypothalamic paraventricular nucleus (PVN) that secrete vasopressin into the peripheral circulation and modulate the discharge of sympathetic nerves innervating renal and vasomotor targets in a highly coordinated manner (Son et al., 2013; Stern, 2015). Angiotensin (Ang) II is an important neuromodulator that acts directly in the PVN via its type 1 receptor (AT1R) to increase blood pressure by increasing vasopressin release (Veltmar et al., 1992; Khanmoradi and Nasimi, 2016) and sympathetic nerve activity (SNA) via projections to the rostral ventrolateral medulla (RVLM) and spinal-cord (Stern et al., 2016; Koba et al., 2018). Importantly, the cardiovascular actions of Ang II are enhanced in the PVN during perturbations in extracellular fluid homeostasis (Freeman and Brooks, 2007; Khanmoradi and Nasimi, 2017) and in several disease states, such as heart failure (Zheng et al., 2009; Han et al., 2011; Zhang et al., 2012), renovascular hypertension (Han et al., 2011) and diabetes (Zheng et al., 2009; Han et al., 2011; Patel et al., 2011; Zhang et al., 2012).

Although the transduction of Ang II in the PVN is incompletely understood, it is clear is that Ang II stimulates vasopressin-releasing and presympathetic neurons indirectly as both lack AT1R (Aguilera et al., 1995b; Oldfield et al., 2001) and require ionotropic glutamate receptors to be activated by Ang II (Latchford and Ferguson, 2004; Stern et al., 2016). Recent work suggests that the pressor and sympathetic actions of Ang II in the PVN involve an AT1R-dependent inhibition of astrocyte glutamate reuptake (Stern et al., 2016); however, evidence that the AT1R is present on PVN astrocytes is inconsistent (Lenkei et al., 1995; Stern et al., 2016), reflecting a lack of high-resolution data.

Disturbances in cardiovascular function and hydromineral balance arise in renal disease, particularly in patients with inherited polycystic kidney disease (PKD) (Chapman et al., 2010; Zittema et al., 2012). Cardinal features of PKD include elevated circulating vasopressin (Zittema et al., 2014), greater central sympathetic drive and hypertension (Klein et al., 2001), all of which are directly regulated by the excitatory actions of Ang II in the PVN (Veltmar et al., 1992; Khanmoradi and Nasimi, 2016; Stern et al., 2016). Furthermore, we have previously found that rats with PKD, Lewis Polycystic Kidney (LPK) rats, display an increased glutamatergic regulation of the PVN (Chapter 2) (Underwood et al., 2018). We therefore examined the hypothesis that enhanced Ang II signalling within the PVN

contributes to hypertension in PKD. The following questions were addressed: (1) are the pro-hypertensive actions of PVN Ang II enhanced in LPK rats?; (2) is the AT1R and astrocyte glutamate reuptake required for the blood pressure and sympathetic actions of PVN Ang II in LPK and control Lewis rats?; (3) what is the cellular distribution of the AT1R in the PVN?; and (4) are AT1R signalling pathways altered in LPK rats?

Methods

Experiments were performed in accordance with The Australian Code of Practice for the Care and Use of Animals (8th Edition, 2013) and approved by the Macquarie University Animal Ethics Committee. Male Lewis and LPK rats (13-14 weeks of age) were obtained from the Animal Resource Centre, Murdoch, Western Australia, Australia, and group-housed in standard living conditions with a 12-hour light/dark cycle and access to standard rodent chow and tap water *ad libitum*.

Acute experiments

Surgical preparation

Urethane was used to induce (1.3 g/kg i.p.) and maintain (65-130 mg/kg i.p. or i.v.) anaesthesia. The femoral artery and vein were cannulated for measurement of arterial pressure and administration of intravenous drugs and fluids (Hartmann's solution 5ml/kg/hr), respectively. A tracheostomy was performed and the animal artificially ventilated with oxygen-enriched room air adjusted to maintain arterial pH at 7.4 ± 0.5 and pCO_2 at 40 ± 5 mmHg. Animals were paralysed with cisatracurium (6mg/kg i.v. for induction and 6 mg/kg/hr i.v. for maintenance). Body temperature was assessed with a digital rectal thermometer and maintained at 37 ± 0.5 ºC with a heating mat and infrared lamp. For sympathetic nerve recordings, a dorsal flank incision was made to access the retroperitoneal cavity and expose the left kidney. The left greater splanchnic nerve and/or a branch of the left renal nerve was isolated for recording. Nerves were cut at their distal end, immersed in paraffin oil and mounted onto a silver bipolar electrode.

PVN microinjection

Animals were positioned in a stereotaxic apparatus with the head in the flat-scull position. A small burr-hole was made in the dorsal surface of the cranium to permit intracranial access for PVN microinjections. Following a stabilisation period of at least 30 minutes, animals received a bilateral PVN microinjection (100 nl/side) of either vehicle (0.1 M phosphate-buffered saline [PBS; *n* = 9 per strain] or dimethyl sulfoxide [DMSO 100%; *n* = 5 LPK and *n* = 3 Lewis]), losartan (AT1R antagonist, 500 mM (Zhu et al., 2002); Abcam, Melbourne, Australia, *n* = 6 LPK and *n* = 5 Lewis), dihydrokainic acid (DHK; glutamate transporter-1 [GLT-1] blocker, 100 mM (Stern et al., 2016); Abcam, *n* = 5 per strain), cyclosporin A (calcineurin inhibitor, 3.3 mM (Yu et al., 2013); Sigma Aldrich, *n* = 4 per strain), xestopsongin C (Inositol trisphosphate [IP3] receptor blocker, 100 µM (Wong et al., 2002); Abcam, *n* = 5 per strain) or SB203580 (p38 mitogen-activated protein kinase [MAPK]

inhibitor, 16 mM (El-Mas et al., 2013); Abcam, *n* = 5 LPK and *n* = 3 Lewis). Drugs were dissolved in either PBS (losartan and DHK) or DMSO (100% for cyclosporine A, 10% in PBS for xestospongin C and 57% in PBS for SB203580). After microinjection, blood pressure, heart rate (HR) and SNA were recorded continuously for 20 min. Immediately thereafter animals received a unilateral PVN microinjection of Ang II (10 mM in 50 nl PBS (Zhu et al., 2002); Abcam). In a separate cohort of animals, hexamethonium (8 mg/kg in saline (Ameer et al., 2014); Sigma Aldrich, *n* = 3 per strain), a ganglionic blocker, or OPC-21268 (3 mg/kg in 20 % DMSO-saline (Chapter 2) (Underwood et al., 2018); Sigma, *n* = 4 per strain), a selective V_{1A} antagonist, was administered intravenously 5-10 min prior to unilateral PVN microinjection of Ang II. All drug solutions used for microinjection contained either fluorescent blue (1/2000; Thermo Fisher Scientific, CA, USA) or red (1/1000; Sigma Aldrich) polystyrene beads to permit validation of each microinjection site. At the termination of the experimental protocol, animals were euthanised with potassium chloride (3 M, 1 ml i.v.), brains removed, post-fixed in 10 % neutral-buffered formalin for at least 12-hours and cut at 75 µm with a vibrating microtome to identify the anatomical location of each injection site relative to the rat brain atlas (Paxinos and Watson, 2013). The distribution of unilateral Ang II microinjection sites is show in Figure 3.1.

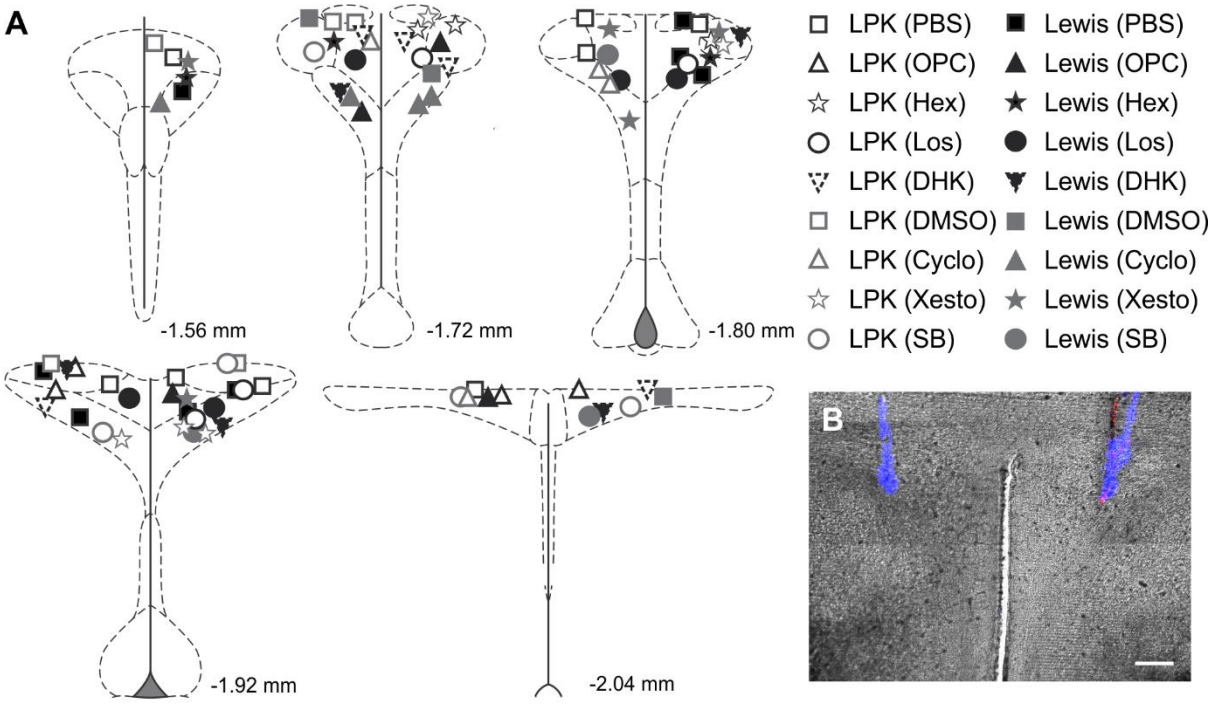

Figure 3.1. A: Summary of the distribution of unilateral Ang II microinjection sites for all experimental groups of LPK and Lewis rats. **B**: Representative injection site showing both unilateral (Ang II; red beads) and bilateral (phosphate-buffered saline in this example; blue beads) injections in the same animal. Scale bar = 200 µm. Value in mm indicates distance from bregma. PBS, phosphate-buffered saline; OPC, OPC-21268; Hex, hexamethonium; Los, losartan; DHK, dihydrokainic acid; DMSO, dimethyl sulfoxide; Cyclo, cyclosporine A; Xesto, xestospongin C; SB, SB203580.

Data acquisition and analysis

Arterial blood pressure was recorded via a pressure transducer, with the signal acquired with a CED 1401 plus (Cambridge Electronic Designs Ltd., Cambridge, UK). Heart rate and systolic blood pressure (SBP) were extracted from the arterial pressure waveform. The SNA signals were band-pass filtered (30-1000 Hz), amplified with a bio-amplifier (2000x; CWE Inc., Ardmore, PA, USA) and sampled at 5 kHz with a CED 1401 plus. All physiological data was captured and analysed using Spike2 software (Cambridge Electronic Designs Ltd.). The SNA waveforms were rectified, smoothed (1-second constant) and normalised to the 30 second period immediately prior to PVN microinjection, setting this as 100% and the level of SNA following euthanasia as 0%.

Baseline measurements of SBP, HR and SNA were taken as the average of the 60 second period immediately prior to microinjection. The Ang II response was analysed by measuring the mean level of SBP, HR and SNA in ten sequential 1 minute bins immediately following

microinjection for between strain comparisons or at the peak response for within strain comparisons. The PBS vehicle control group of Lewis and LPK animals were used for both between- and within-strain comparisons for the Ang II response. To determine the effect of each drug pre-treatment (both vehicles, losartan, DHK, cyclosporin A, xestpongin C and SB203580) on the resting level of SBP, HR and SNA, a 1 minute average was measured at baseline and 20 minutes following microinjection (i.e. immediately prior to Ang II administration).

Anatomical experiments

Retrograde tracing

Putative presympathetic PVN neurons with projections to the RVLM were identified using retrograde tracing. Male Lewis and LPK animals (12-13 weeks; $n = 4$ per strain) were anaesthetised with isoflurane and administered carprofen (2.5 mg/kg s.c.) and cephazolin (50 mg/kg i.m.) for analgesia and antibiotic prophylaxis, respectively. With rats positioned in a stereotaxic apparatus, a small craniotomy was made in the occipital plate above the left RVLM. Antidromic mapping of the facial motor nucleus was used to identify the RVLM as previously described (Turner et al., 2013). Approximately 30 nl of cholera toxin subunit B (CTB)-555 (0.25% in saline) was pressure injected 300 µm caudal and 300 µm ventral to the caudal pole of the facial motor nucleus. These coordinates were optimised according to the distribution spinally-projecting RVLM neurons and their dendrites (Farmer et al., 2019). At completion of surgery the incision site was closed with surgical staples and the animal was recovered. All animals received carprofen (2.5 mg/kg s.c.) and supplemental fluids the first post-operative day and thereafter as required. After 5-6 days, animals were deeply anaesthetised with sodium pentobarbital (100 mg/kg i.p.) and transcardially perfused with ice-cold sterile PBS (~200 ml) under aseptic conditions. The brain was rapidly removed, and the hypothalamus was blocked and snap-frozen with dry-ice. The brainstem was then removed, immersed in 10% neutral-buffered formalin for 16-24 hours, and sectioned with a vibrating microtome for confirmation of injection sites. A representative injection site is shown in Figure 3.2.

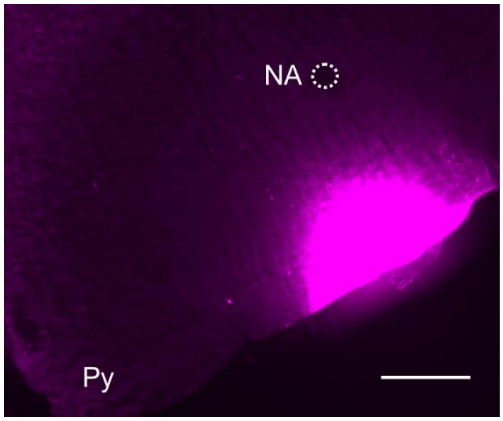

Figure 3.2. Representative CTB-555 RVLM injection site. Scale bar = 500 µm. NA, nucleus ambiguus; Py, pyramid.

Fluorescent in-situ hybridisation and immunohistochemistry

The cellular distribution and expression level of AT1R mRNA was quantified with RNAscope fluorescent in-situ hybridisation (FISH; Advanced Cell Diagnostics, Ca, USA) in the PVN of animals that received an RVLM injection of CTB-555 and an additional cohort of uninjected male Lewis and LPK animals (13 weeks of age; n = 3 per strain) that were sacrificed via cardiac exsanguination following sodium pentobarbital anaesthesia (100 mg/kg i.p.). 25 µm thick coronal sections were obtained with a cryostat, mounted onto Superfrost slides (Thermo Fisher Scientific, Ma, USA) and stored at -80 °C. RNAscope FISH was performed as per manufacturer's instructions. On the day of the assay, slides were immersed in ice-cold 4 % paraformaldehyde for 15 minutes and sequentially dehydrated with ethanol (50% for 5 minutes, 70% for 5 minutes and 100% twice for 5 minutes). After air drying the sections for ~10 minutes, a protease (Pretreatment 4; Advanced Cell Diagnostics) was applied for 30 minutes at room temperature. Thereafter the sections were rinsed in PBS (twice for 2 minutes) and incubated for 2 hours at 40 °C in either a negative control probe (DapB, a bacterial gene; Cat No. 320871), positive control probes (Cat No. 320891) or target probes against AT1R mRNA (Cat No. 422661-C1) and either glial fibrillary acidic protein mRNA (GFAP, astrocyte marker; Cat No. 407881-C3) or vasopressin mRNA (Cat No. 401421-C2) and corticotrophin-releasing hormone mRNA (CRH; Cat No. 318931-C3). Amplifications steps were performed as per manufacturer's instructions using the Alt.A protocol. Slides were then coverslipped with Prolong gold with DAPI (ThermoFisher, Macquarie Park, Australia) or underwent immunohistochemistry procedures described beneath.

Sections processed for FISH against GFAP mRNA were then processed with immunohistochemistry for GFAP protein in order to robustly identify astrocyte somata. Slides were washed in PBS (5 times for 2 minutes) and incubated in blocking solution (2 % normal horse serum in Tris-PBS with 0.2% Tween) for 1 hour at 4 °C. Then sections were incubated in a solution containing a polyclonal rabbit anti-GFAP antibody (AB_477035, Sigma; 1:400 in blocking solution) for 14 hours at 4 °C. Slides were subsequently washed (PBS; 5 times for 2 minutes) and incubated for 3 hours at room temperature in a solution containing Cy3-congugated donkey anti-rabbit IgG (1:500; Jackson ImmunoResearch). After a final series of washes (PBS; 5 times for 2 minutes), sections were coverslipped with Prolong gold with DAPI (ThermoFisher).

Image capture and analysis

Z stack images were captured at 20x magnification using an Axio Imager epiflorescence microscope (Zeiss, Germany). The step size was 1.1 µm for all images and there was 9 to 11 optical sections per image. These Z stacks were then used to generate maximal intensity projection images which were used for all analysis. The laser power and exposure time was constant for all experiments and optimised using sections hybridised with positive and negative control probes. Using these exposure times and laser powers there was a low level of fluorescence in sections hybridised with the DapB negative control probe (Figure 3.3).

With the RNAscope platform, bright puncta correspond to single RNA molecules (Wang et al., 2012). We quantified individual AT1R mRNA puncta using the spot detection plugin in Icy software (de Chaumont et al., 2012) with a sensitivity threshold that was optimised with sections that were hybridised with negative and positive control probes. This approach easily distinguished bright positive control puncta from low intensity background puncta (Figure 3.3). This plugin was used to quantify total PVN AT1R mRNA density and to identify individual AT1R mRNA puncta localised to the somata of astrocytes, vasopressin neurons and CRH neuron which were then manually counted. A cell with two or more AT1R mRNA puncta was defined as AT1R-expressing. Detection of total AT1R mRNA density and AT1R mRNA in astrocytes was performed bilaterally in two sections for each animal. Detection of AT1R mRNA in vasopressin neurons and CRH neurons was performed bilaterally in a single section between -1.6 and -1.9 mm rostral of bregma. Detection of AT1R mRNA in CTB neurons was performed ipsilateral to the injection site in one or two sections.

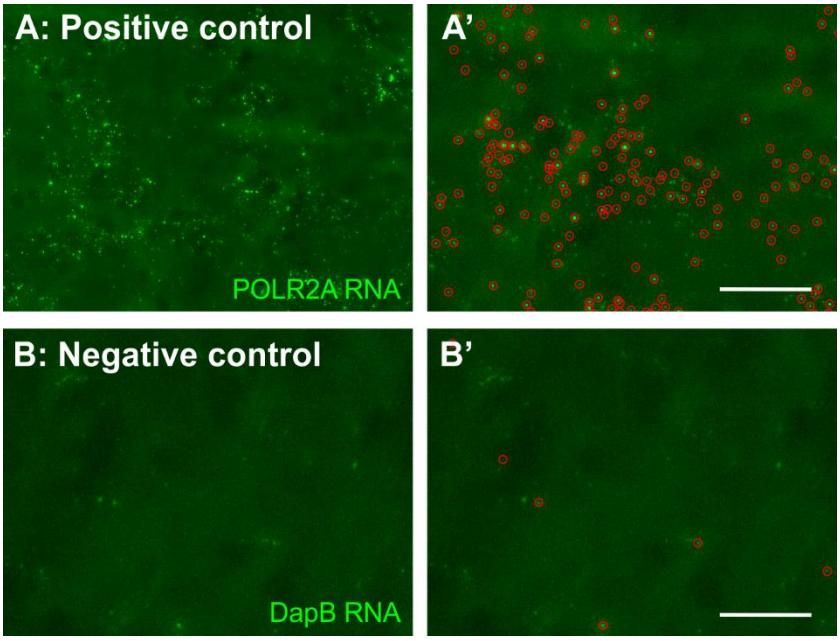

Figure 3.3. Example positive and negative control sections hybridised with POLR2A and DapB, respectively. The raw images are shown in **A** and **B**. Positive spot detections with Icy software (red circles) of the same images are shown in **A'** and **B'**. Scale bar = 20 µm.

Statistical analysis

Data are expressed as mean ± SEM unless otherwise stated. Statistical analysis was performed using GraphPad Prism version 8 (GraphPad Software, San Diego, Ca, USA). To determine if a drug significantly influenced resting SBP, HR or SNA in each strain, a one-way repeated measures ANOVA or a paired t-test was used. To determine if the drug response differed between strain or treatment groups, delta values were compared using a two-way ANOVA or an unpaired t-test. A mixed-effects models was used to compare effect of strain and Bregma level on the density of AT1R mRNA in the PVN. Strains differences in the distribution of AT1R mRNA in specific cell groups were compared with an unpaired t-test. Bonferoni's correction was used for all multiple comparisons. Statistical significance was set at $P \leq 0.05$.

Results

Ang II stimulation of the PVN generates an enhanced vasopressin-dependent pressor response and sympathoinhibition in LPK rats

Initial experiments were performed to examine whether Ang II has a greater capacity to modulate arterial pressure, HR and SNA in LPK rats. Figure 3.4 illustrates the effect of unilateral PVN microinjection of Ang II and its vehicle on SBP, HR, splanchnic SNA and renal SNA in Lewis and LPK animals. In both strains, unilateral vehicle microinjection did not affect SBP, HR or SNA (all $P > 0.05$; Figure 3.4B).

In the LPK, Ang II produced a large increase in SBP ($F_{10,80} = 135.8$, $P < 0.0001$) that did not return to baseline during the recording period (adjusted $P < 0.0001$). In contrast, in the Lewis control rats Ang II produced an increase in SBP ($F_{10,80} = 3.47$, $P < 0.001$) that was smaller in magnitude to the LPK ($F_{10,80} = 34.68$, $P < 0.0001$; Figure 3.4B) and returned to baseline by 5 min (adjusted $P = 0.06$ vs. baseline). The strain difference in the magnitude of the SBP response persisted when SBP was expressed as a percentage of baseline ($F_{1,160} = 60.98$, $P < 0.0001$; data not shown). In the LPK, PVN Ang II microinjection produced a sustained inhibition of both splanchnic ($F_{10,80} = 5.10$, $P < 0.0001$) and renal SNA ($F_{10,80} = 18.86$, $P < 0.0001$) and no change in HR ($F_{10,80} = 0.61$, ns). In the Lewis, however, Ang II produced a small but significant increase in splanchnic SNA ($F_{10,70} = 10.52$, $P < 0.0001$), renal SNA ($F_{10,80} = 18.86$, $P < 0.0001$) and HR ($F_{10,80} = 4.20$, $P < 0.001$).

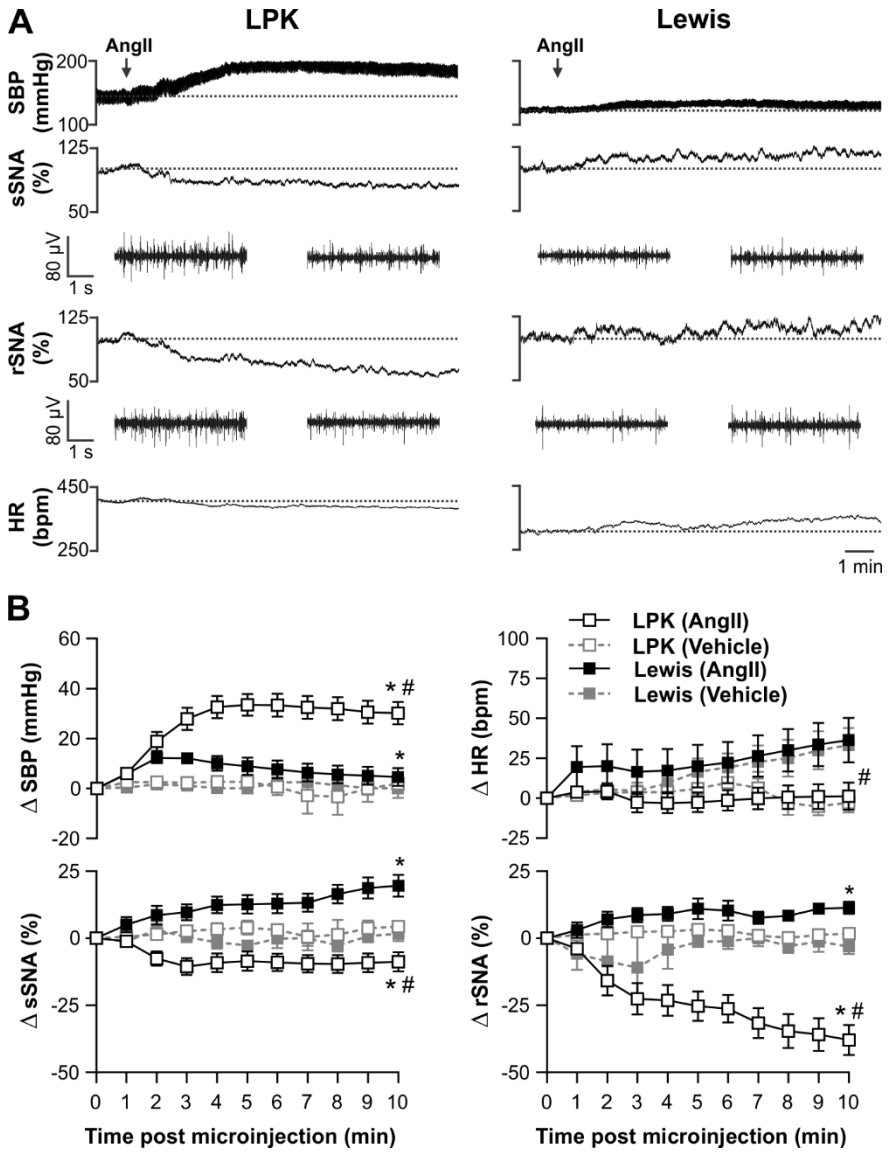

Figure 3.4. The effect of PVN microinjection of Ang II on systolic blood pressure (SBP), splanchnic sympathetic nerve activity (sSNA), renal sympathetic nerve activity (rSNA) and heart rate (HR) in LPK and Lewis rats. A: A representative recording showing a PVN Ang II response in a LPK and a Lewis rat. Broken line indicates baseline. A 5 second segment of the raw splanchnic and renal sympathetic nerve recording at baseline and at the peak response is shown underneath each respective integrated and normalised trace. B: Grouped data for PVN microinjection of Ang II and vehicle in LPK and Lewis rats. Data are shown as mean ± SEM. * indicates two-way ANOVA $P < 0.05$ difference vs. vehicle, # indicates two-way ANOVA $P < 0.05$ strain difference. $n \geq 8$ Lewis, $n = 9$ LPK for Ang II and $n = 4$ per strain for vehicle.

Given that in LPK rats we observed that PVN Ang II produced a strong increase in blood pressure yet decreased SNA, we hypothesised that the pressor response to Ang II observed in LPK rats was not mediated via the sympathetic nervous system but rather via the release of vasopressin and its actions on vascular V_{1A} receptors. To test this, we administered either hexamethonium, a ganglionic blocker, or OPC-21268, a selective V_{1A} receptor antagonist, intravenously prior to Ang II microinjection. Hexamethonium administration predictably lowered SBP in LPK (by -69 ± 10 mmHg, $t_2 = 6.59$, $P = 0.02$) and Lewis (by -50 ± 9 mmHg, $t_2 = 9.65$, $P = 0.01$) rats but the magnitude was not significantly different between the strains ($t_4 = 1.57$, $P = 0.19$). In contrast, OPC-21268 administration produced a variable and non-significant change in SBP in both LPK (-6 ± 9 mmHg, $t_3 = 0.71$, $P = 0.53$) and Lewis (7 ± 3 mmHg, $t_3 = 2.18$, $P = 0.12$) rats. Importantly, in both strains, the pressor response to PVN Ang II was unaffected by hexamethonium (adjusted $P = 0.59$ for LPK, Figure 3.5A; adjusted $P = 0.63$ for Lewis, Figure 3.5B) yet was eliminated by OPC-21268 (adjusted $P < 0.001$ for LPK, Figure 3.5A; adjusted $P < 0.01$ for Lewis, Figure 3.5B). These data therefore suggest that in both Lewis and LPK rats, the pressor response to PVN Ang II microinjection may not be primarily mediated by the sympathetic nervous system and more than likely involves vasopressin release and subsequent activation of peripheral V_{1A} receptors.

Abrupt increases in blood pressure can evoke reductions in SNA through the baroreceptor reflex. It is therefore conceivable that the sympathoinhibition evoked by PVN Ang II in LPK animals is baroreflex-mediated. In that regard, we predicted that PVN Ang II microinjection would no longer decrease SNA in LPK animals pre-treated with OPC-21268 because in these animals Ang II failed to increase blood pressure. This was, however, not observed; LPK animals pre-treated with OPC-21268 still displayed a sympathoinhibitory response to PVN Ang II that was not significantly different from naïve LPK (splanchnic SNA -13 ± 4 vs. -13 ± 4 % of baseline, $t_{11} = 0.04$, $P = 0.96$; renal SNA -21 ± 5 vs. -38 ± 6 % of baseline, $t_{11} = 1.67$, $P = 0.12$). Furthermore, the sympathoexcitatory response to Ang II in the Lewis animals was similar in OPC-21268-treated and naïve Lewis (splanchnic SNA 16 ± 7 vs. 19 ± 4 % of baseline, $t_{10} = 0.37$, $P = 0.72$; renal SNA 14 ± 5 vs. 16 ± 4 % of baseline, $t_{10} = 0.37$, $P = 0.72$). Thus, in the LPK, Ang II-evoked decreases in SNA occur independently of concurrently evoked elevations in blood pressure, suggesting that two populations of PVN neurons respond to Ang II to differentially regulate SNA and blood pressure.

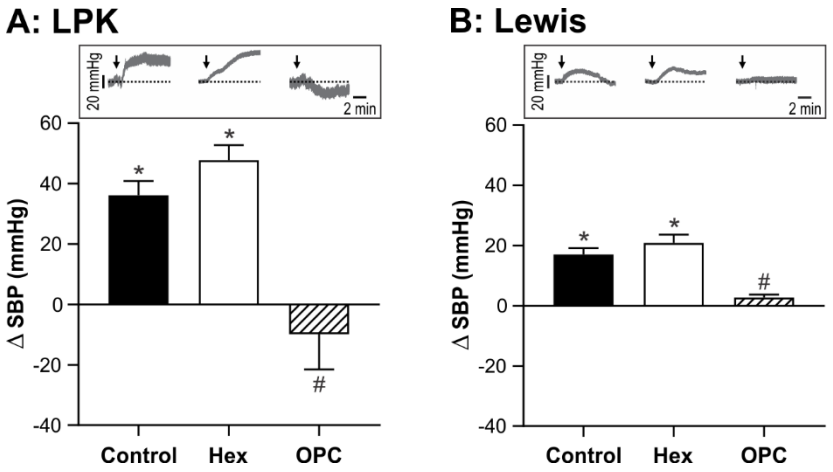

Figure 3.5. Impact of peripheral ganglionic blockade with hexamethonium (Hex) or V_{1A} receptor antagonism with OPC-21268 (OPC) on the systolic blood pressure (SBP) response to PVN microinjection of Ang II in LPK and Lewis rats. **A**: Representative recordings (top) and grouped data (bottom) for the PVN Ang II SBP response in LPK rats. **B**: Representative recordings (top) and grouped data (bottom) for the PVN Ang II SBP response in Lewis rats. Arrow and broken line indicate PVN Ang II microinjection and baseline, respectively. Data are shown as mean ± SEM. * indicates $P < 0.05$ difference vs. baseline (paired t test), # indicates Bonferroni adjusted $P < 0.05$ difference vs. control. $n = 9$ per strain for control, $n = 3$ per strain for Hex and $n = 4$ per strain for OPC.

Differential role of AT1R and GLT-1 in the blood pressure and SNA response to PVN Ang II in Lewis and LPK animals

We then sought to determine whether the AT1R and astrocyte GLT-1 were required for the pressor and SNA responses to PVN Ang II in LPK and Lewis animals. To that end, the SBP and SNA response to PVN Ang II was compared between animals that received a bilateral PVN microinjection of either losartan, a competitive AT1R antagonist, DHK, a GLT-1 blocker, or vehicle control (PBS). The effect that each of these drugs had on resting blood pressure and SNA is shown in Table 3.1. When compared to microinjection of vehicle, losartan did not affect resting SBP or SNA in either strain. In contrast, DHK produced an increase in SBP and SNA that was of a similar magnitude in each strain.

As illustrated in Figure 3.6A, in LPK rats, PVN microinjection of losartan or DHK abolished the PVN Ang II pressor response ($P = 0.55$ and $P = 0.47$ vs. baseline, respectively) and differentially affected the sympathoinhibitory Ang II response depending on the nerve bed measured: the renal sympathoinhibitory response still occurred ($P < 0.01$ vs. baseline for both drugs) though with a smaller magnitude compared to vehicle ($P < 0.01$ and $P = 0.04$, respectively), while the splanchnic sympathoinhibitory response was unaffected by both

drugs (P = 0.90 and P = 0.78 vs. vehicle, respectively). In Lewis animals, losartan and DHK eliminated both the pressor and sympathoexcitatory response to PVN Ang II (Figure 3.6B).

Table 3.1: The effect of vehicle (PBS), losartan and DHK on resting systolic blood pressure (SBP), splanchnic SNA (sSNA) and renal SNA (rSNA) in LPK and Lewis rats.

Group	Parameter (n LPK/ n Lewis)	LPK			Lewis		
		Baseline	20 min	Delta	Baseline	20 min	Delta
Vehicle (PBS)	SBP (12 / 10)	182 ± 5	183 ± 6	1 ± 4	128 ± 3	130 ± 2	2 ± 2
	sSNA (8 / 6)	102 ± 1	126 ± 7*	24 ± 7	100 ± 0.4	107 ± 6	7 ± 6
	rSNA (9 / 9)	101 ± 0.6	114 ± 14	14 ± 13	100 ± 0.3	121 ± 8*	21 ± 7
Losartan	SBP (6 / 5)	176 ± 9	167 ± 11	-8 ± 8	112 ± 6	109 ± 8	-3 ± 2
	sSNA (6 / 5)	100 ± 0.4	136 ± 10*	36 ± 10	99 ± 1	130 ± 12	31 ± 12
	rSNA (6 / 4)	99 ± 2	137 ± 10*	38 ± 10	101 ± 2	136 ± 11	35 ± 13
DHK	SBP (9 / 7)	170 ± 7	226 ± 11*	56 ± 11#	118 ± 5	162 ± 6*	43 ± 8#
	sSNA (8 / 6)	101 ± 1	234 ± 29*	133 ± 29#	100 ± 2	249 ± 19*	149 ± 19#
	rSNA (8 / 6)	101 ± 2	223 ± 31*	122 ± 31#	101 ± 2	243 ± 32*	141 ± 33#

Values are presented as mean±SEM. *, P<0.05 vs. baseline; #, P<0.05 vs. vehicle. n indicates sample size for the strain, parameter and drug indicated.

As DHK produced a significant elevation in baseline SNA, preliminary control experiments were performed to determine whether the reduction in SNA following PVN Ang II in DHK-treated LPK was specific to Ang II and not due to the washout of DHK. In these preliminary experiments, PVN microinjection of the vehicle for Ang II 20 min after DHK in n = 3 LPK was associated with a small but significant reduction in splanchnic SNA (-4 ± 0.4 % change from baseline; t_2 = 10.22, P < 0.01) and renal SNA (-5 ± 0.4 % change from baseline, n = 3; t_2 = 18.83, P < 0.01). Importantly, however, the magnitude of these SNA reductions was significantly less than that evoked by Ang II (splanchnic SNA, t_6 = 2.69, P = 0.04; renal SNA, t_6 = 4.36, P < 0.01). Together, these results indicate that both AT1R and astrocyte GLT-1 function are required for the pressor response to Ang II in both strains and the sympathoexcitatory response exhibited in the Lewis. In the LPK rats, Ang II-evoked sympathoinhibition appears to be largely – though for the renal, not exclusively – independent of GLT-1 function and therefore presumably neuronal in origin, involving AT1R-dependent (renal) and AT1R-independent (splanchnic) populations of neurons.

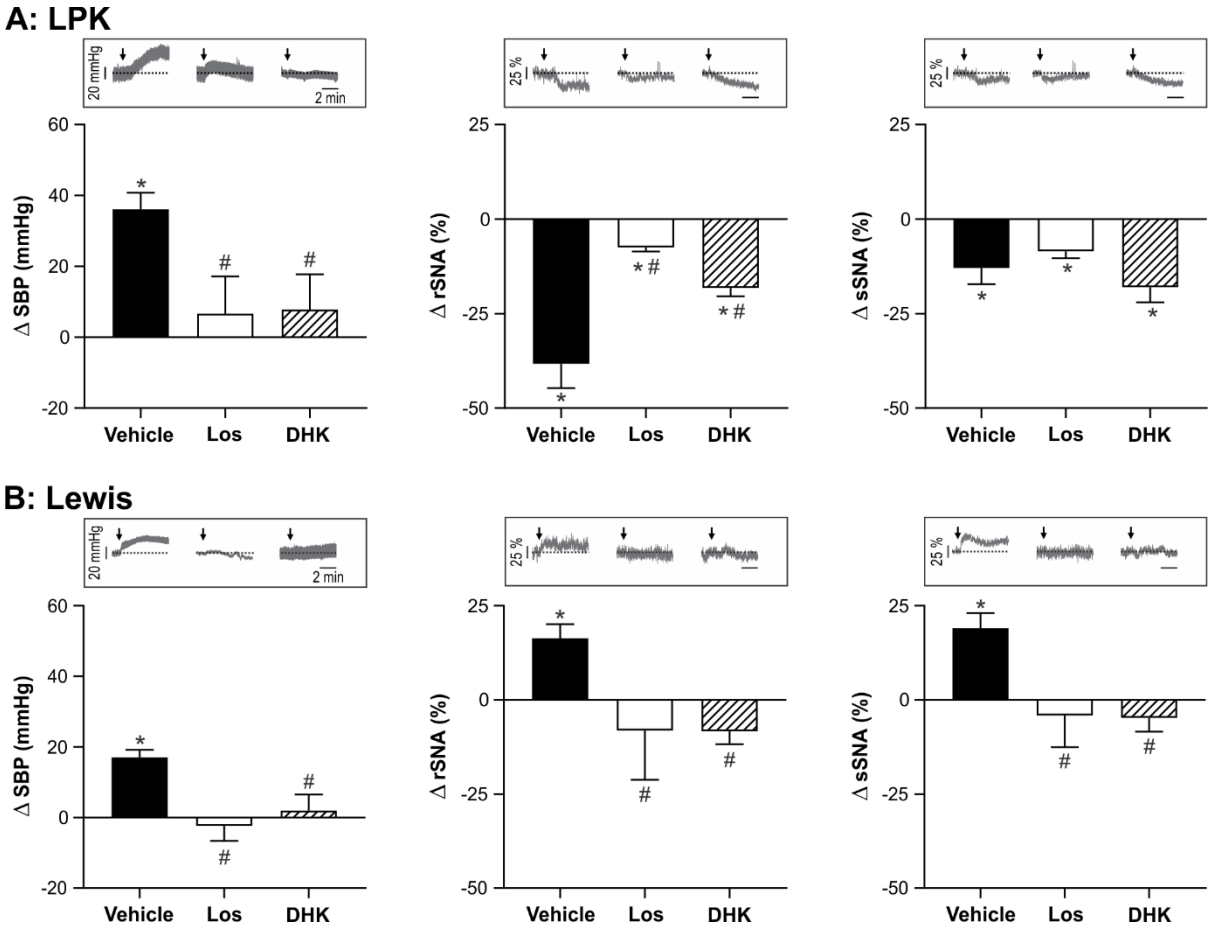

Figure 3.6. Effect of PVN AT1R inhibition with losartan (Los) and GLT-1 blockade with dihydrokainic acid (DHK) on the systolic blood pressure (SBP), renal sympathetic nerve activity (rSNA) and splanchnic sympathetic nerve activity (sSNA) response to PVN microinjection of Ang II in LPK and Lewis rats. **A**, Representative recordings (top) and grouped data (bottom) for the PVN Ang II SBP and SNA response in LPK rats. **B**, Representative recordings (top) and grouped data (bottom) for the PVN Ang II SBP and SNA response in Lewis rats. Arrow and broken line indicate PVN Ang II microinjection and baseline, respectively. Data are shown as mean ± SEM. * indicates $P < 0.05$ difference from baseline (paired t test), # indicates Bonferoni adjusted $P < 0.05$ difference between vehicle. $n = 9$ LPK and $n \geq 8$ Lewis for vehicle, $n = 5$ LPK and $n \geq 4$ Lewis for Los and $n = 5$ LPK and $n \geq 4$ Lewis for DHK.

PVN AT1R mRNA is more abundant in LPK rats but is not present in most astrocytes

FISH was used to quantify the density of AT1R mRNA in the PVN in LPK and Lewis animals. AT1R mRNA density was influenced by the rostro-caudal level of the PVN ($F_{3,11}$ = 10.58, P = 0.001) and, importantly, was 64 ± 23 % greater in LPK compared to Lewis rats ($F_{1,12}$ = 7.64, P = 0.02; Figure 3.7).

Our data indicates that there are likely two populations of cells responsible for the responses we observed following PVN Ang II in LPK and Lewis rats: 1) a population of cells that in both LPK and Lewis rats cause the release of vasopressin and thereby increase blood pressure and 2) a population of cells that increases SNA in Lewis rats but decreases SNA in LPK rats. However, AT1R are suggested to be absent on both vasopressin and pre-sympathetic PVN neurons (Aguilera et al., 1995b; Oldfield et al., 2001) and evidence that it is present on astrocytes is inconsistent (Lenkei et al., 1995; Stern et al., 2016). Therefore, what is the cellular distribution of AT1R in the PVN responsible for the responses that we observe and given the differential responses we observed in LPK rats, are these receptors similarly distributed in LPK and Lewis rats?

Using FISH combined with retrograde neuronal tracing, we found that AT1R mRNA was absent from all examined RVLM-projecting (putative pre-sympathetic) PVN neurons in both strains (Figure 3.8; Table 3.2). Likewise, AT1R mRNA was absent from virtually all (98%) GFAP-positive PVN astrocytes in both strains (Figure 3.9; Table 3.2). AT1R mRNA was present in 8% of vasopressin neurons in LPK rats but was absent from all but a single neuron in Lewis rats (Figure 3.10; Table 3.2). Many CRH neurons contained AT1R mRNA in both strains, however in LPK animals twice as many were observed and the AT1R transcript count per neuron was doubled (Figure 3.11; Table 3.2).

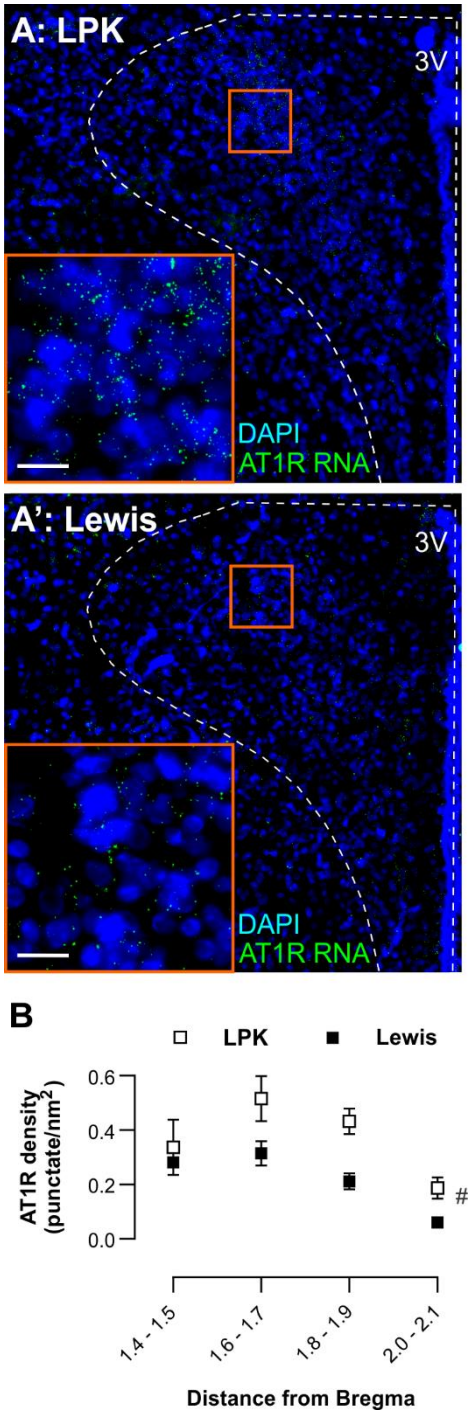

Figure 3.7. AT1R density in the PVN of LPK and Lewis rats. **A**: Representative images showing AT1R RNA puncta (green) and DAPI nuclei (blue) in a LPK (A) and a Lewis (A') rat. 3V; third cerebral ventricle. Scale = 20 µm. **B**: quantification of AT1R density throughout the rostro-caudal extent of the PVN. # indicates mixed-model $P < 0.05$ strain difference. n = 7 per strain.

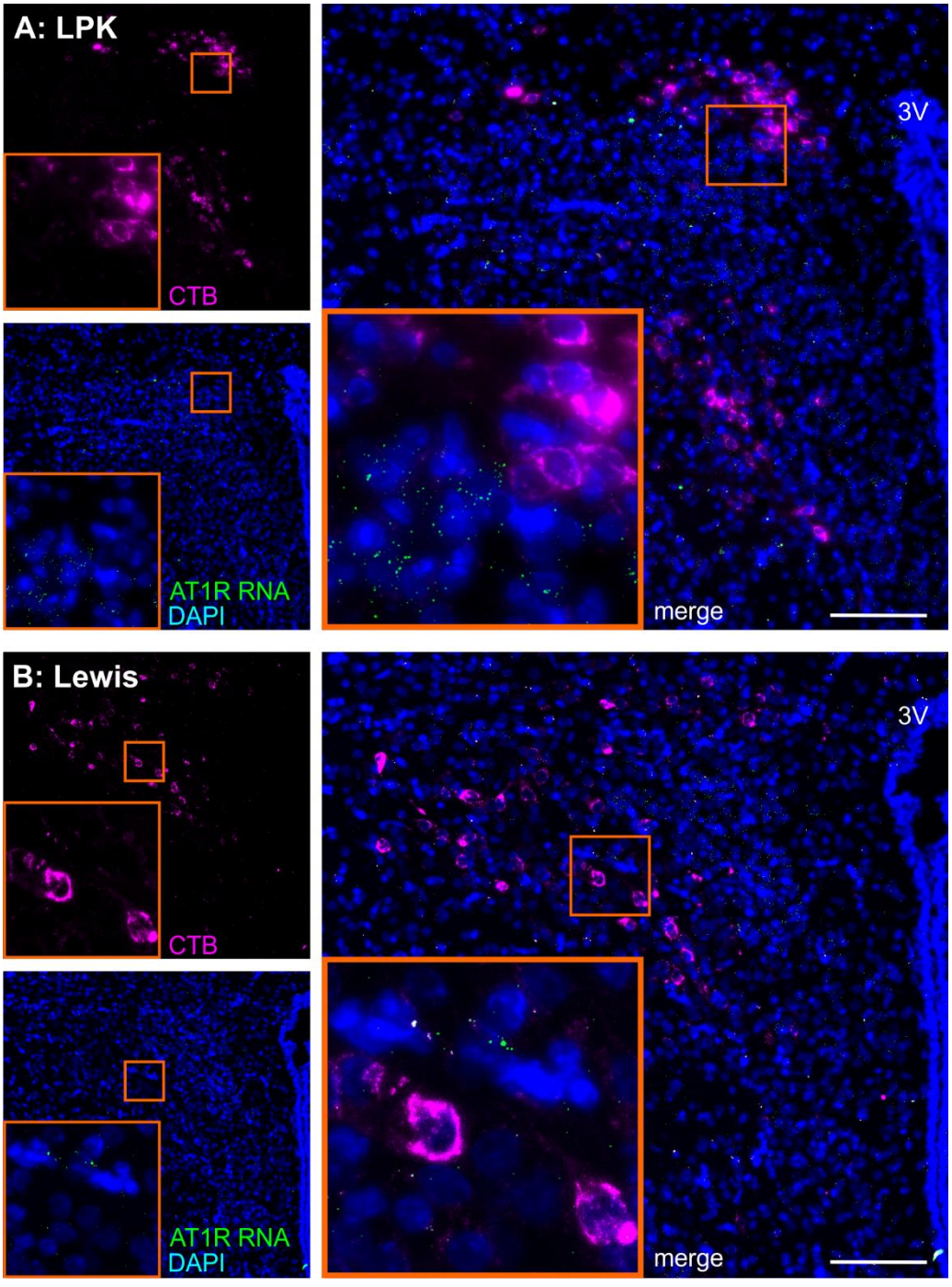

Figure 3.8. Representative images showing the distribution of CTB-labelled RVLM-projecting neurons (magenta), AT1R RNA (green) and DAPI nuclei (blue) in the PVN of a LPK (**A**) and a Lewis (**B**) rat. CTB neurons did not contain AT1R mRNA in either strain. 3V; third cerebral ventricle. Scale = 100 µm.

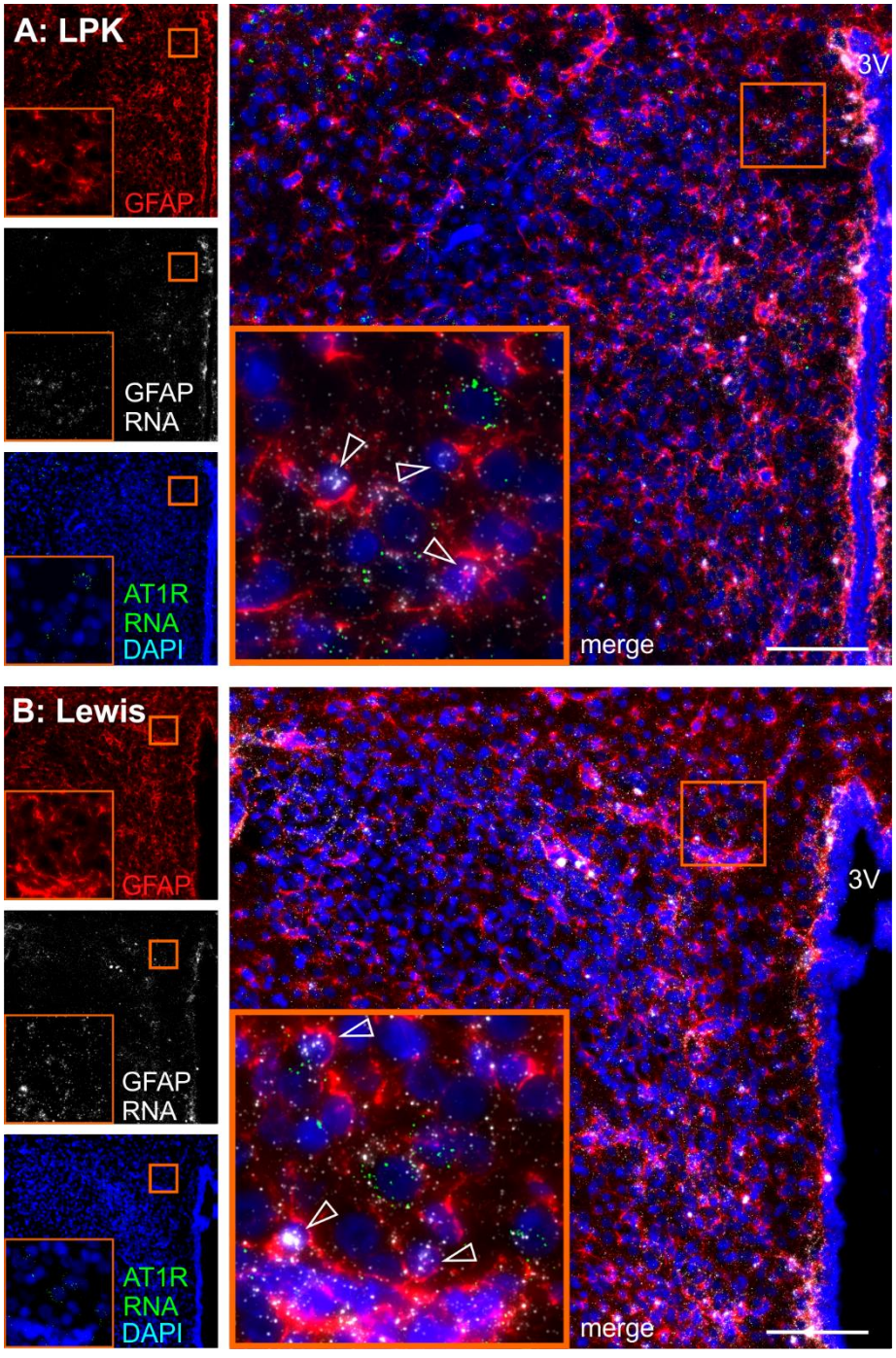

Figure 3.9. Representative images showing the distribution astrocytes (arrowheads), AT1R RNA (green) and DAPI nuclei (blue) in the PVN of a LPK (**A**) and a Lewis (**B**) rat. Astrocytes were identified by the presence of GFAP protein (red) and RNA (white), and mostly lacked AT1R RNA in both strains. 3V; third cerebral ventricle. Scale = 100 µm.

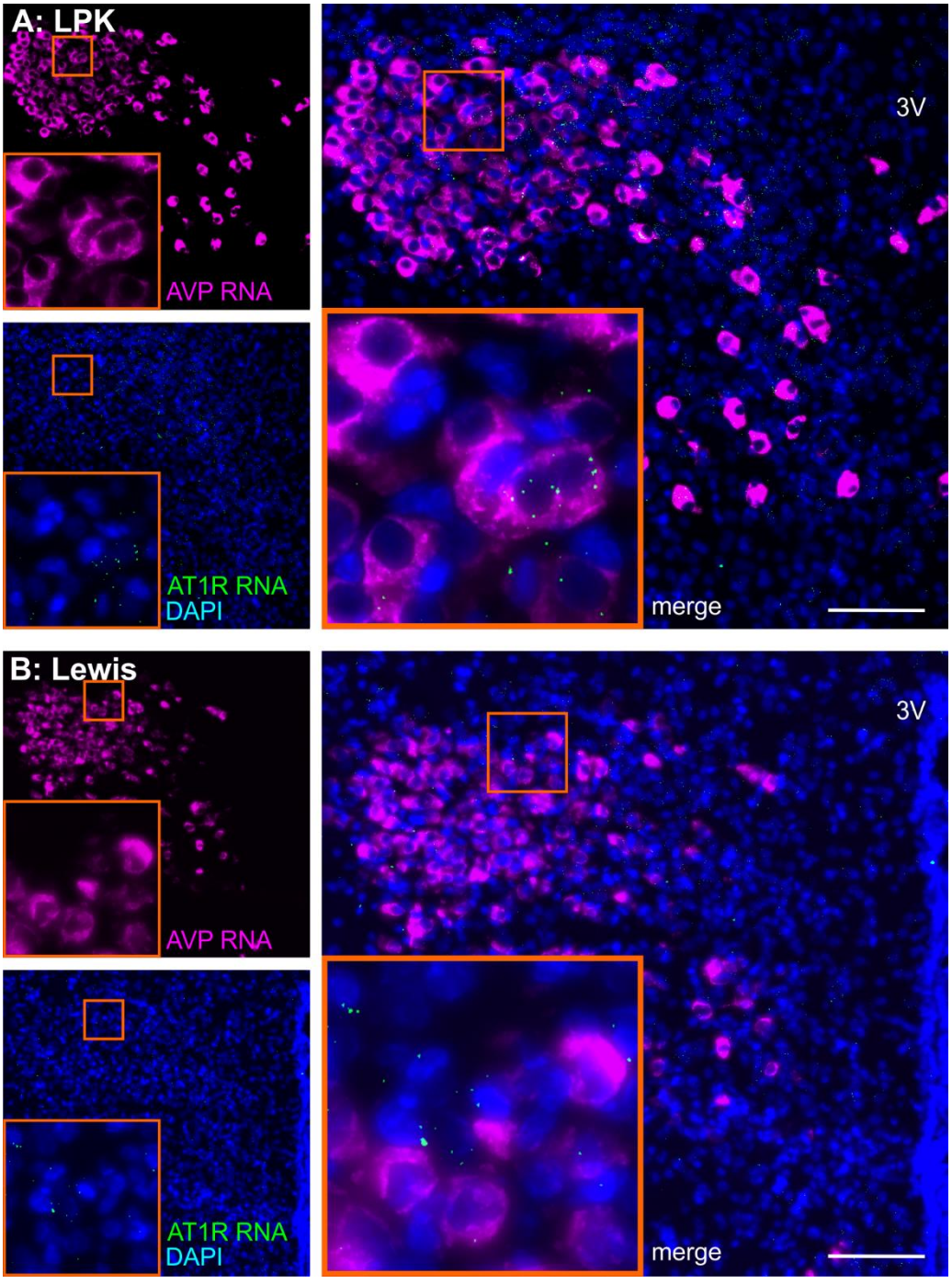

Figure 3.10. Representative images showing the distribution vasopressin (AVP) neurons (magenta), AT1R RNA (green) and DAPI nuclei (blue) in the PVN of a LPK (**A**) and a Lewis (**B**) rat. AT1R was present in a minor population of vasopressin neurons in LPK rats but not in Lewis rats. 3V; third cerebral ventricle. Scale = 100 µm.

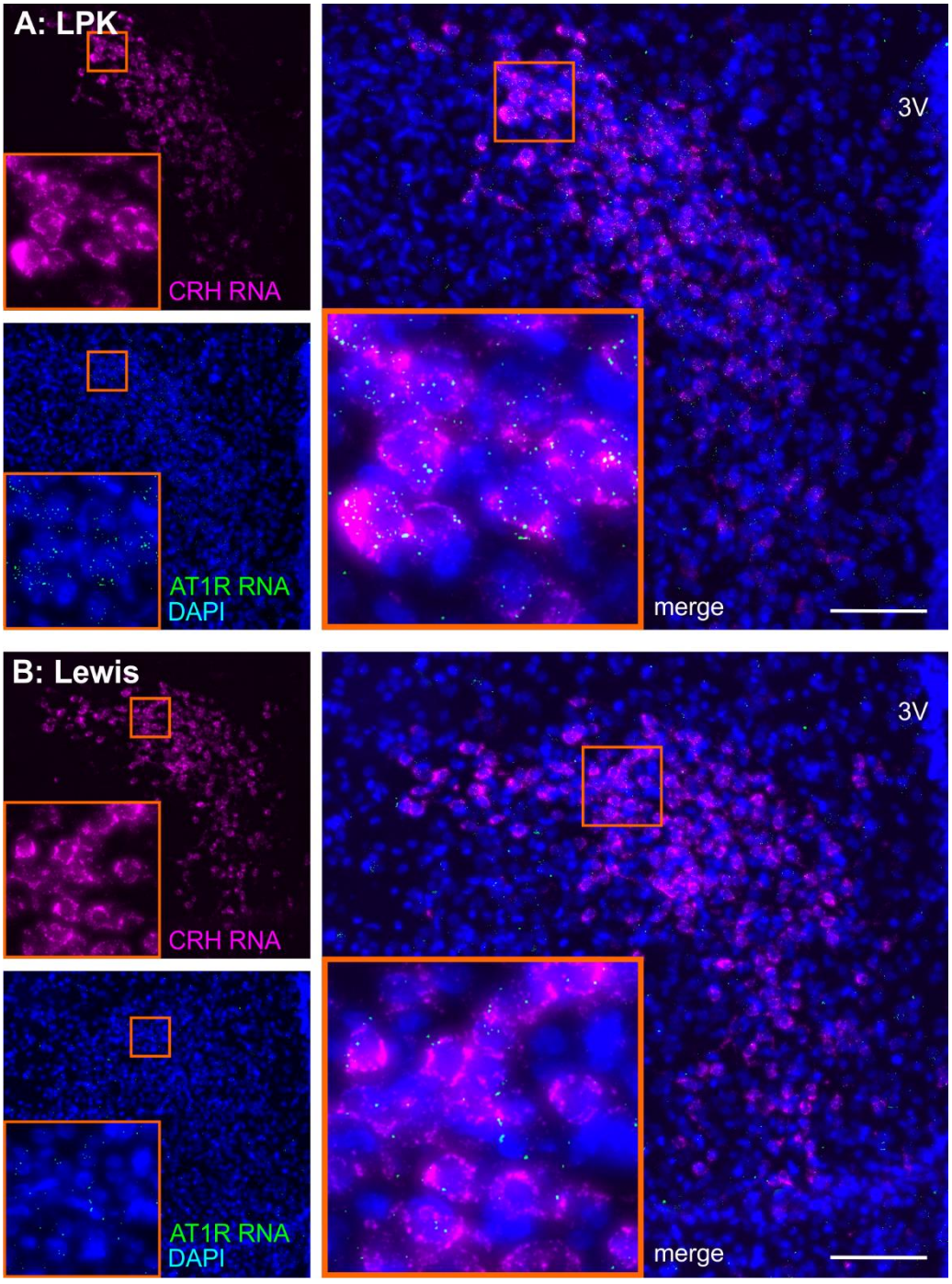

Figure 3.11. Representative images showing the distribution CRH neurons (magenta), AT1R RNA (green) and DAPI nuclei (blue) in the PVN of a LPK (**A**) and a Lewis (**B**) rat. AT1R was both more abundant and present in a larger population of CRH neurons in LPK compared to Lewis rats. 3V; third cerebral ventricle. Scale = 100 µm.

Table 3.2: Quantification of AT1R mRNA in different PVN cell types in LPK and Lewis rats.

Parameter	Strain		*P* value
	LPK	**Lewis**	
RVLM-projecting neurons			
Cells counted per animal	25 (17-33)	41 (13-71)	
AT1R+ CTB+ (% CTB)	0	0	-
AT1R puncta per AT1R+ CTB+	-	-	-
Astrocytes			
Cells counted per animal	163 (157-171)	140 (137-143)	
AT1R+ GFAP+ (% GFAP)	3 (3-4)	2 (0-5)	0.387
AT1R puncta per AT1R+ GFAP+	2.4 (2.1-2.6)	2.4 (2.0-2.8)	0.772
AVP neurons			
Cells counted per animal	71 (34-105)	155 (127-175)	
AT1R+ AVP+ (% AVP)	8 (4-12)	0 (0-1)	0.038
AT1R puncta per AT1R+ AVP+	3.9 (2.9-5.0)	-	-
CRH neurons			
Cells counted per animal	114 (97-141)	147 (101-208)	
AT1R+ CRH+ (% CRH)	84 (79-92)	41 (40-41)	<0.001
AT1R puncta per AT1R+ CRH+	5.7 (4.8-6.9)	3.0 (2.7-3.2)	0.013

Values are presented as mean (min-max). $P<0.05$ considered significant. $n = 4$ per strain for CTB experiment and $n = 3$ per strain for all other experiments. Hyphen indicates not quantified due to absence of AT1R puncta for the cell type. LPK, Lewis Polycystic Kidney rat; RVLM, rostral ventrolateral medulla; GFAP, glial fibrillary acidic protein; AVP, vasopressin; CRH, corticotrophin releasing hormone.

Enhanced Ang II pressor responses involve calcineurin and IP3 receptors in LPK rats

We reasoned that the augmented Ang II pressor response in LPK rats could be due to increased AT1R density, as indicated above, as well as an enhancement of AT1R signalling pathways. The AT1R interacts with several G proteins to stimulate, among other things, reactive oxygen species (ROS) formation, several protein kinases (e.g. p38 MAPK) and phospholipase C to produce IP3 that drives intracellular Ca^{2+} release via its intracellular receptor (Forrester et al., 2018). In addition, the AT1R is coupled to several other interacting proteins, including Ca^{2+}/calmodulin that activates calcineurin, a phosphatase that can influence cell excitability through a number of effectors (Groth et al., 2003; Forrester et al., 2018).

We sought to determine whether p38 MAPK, calcineurin or IP3 receptors were involved in the cardiovascular action of PVN Ang II in LPK and Lewis animals by comparing the SBP and SNA response to PVN Ang II between animals that received a bilateral PVN microinjection of either SB203580 (p38 MAPK inhibitor), cyclosporin A (calcineurin inhibitor), xestospongin C (IP3 receptor blocker) or vehicle (DMSO). The effect that each of these drugs had on resting blood pressure and SNA is shown in Table 3.3. When compared to vehicle, none of these drugs had an effect on resting SBP or SNA in LPK rats. In Lewis rats, cyclosporin A slightly increased SBP and renal SNA compared to vehicle, however these effects were not significantly different from LPK rats (P = 0.96 for SBP and P = 0.15 for renal SNA).

In LPK rats, while inhibition of p38 MAPK with SB203580 did not affect the PVN Ang II pressor response, inhibition of either calcineurin or IP3 receptors with cyclosporin A and xestospongin C, respectively, each attenuated the Ang II pressor response by 55% (Figure 3.12A). The attenuation was such that the Ang II pressor response in cyclosporin A pre-treated LPK (adjusted P = 0.22) and xestospongin C pre-treated LPK (adjusted P = 0.17) was no longer significantly different from Lewis animals. In Lewis rats, the pressor response to PVN Ang II was unaffected by prior microinjection of SB203580, cyclosporin A or xestospongin C (Figure 3.12B).

Neither drug pre-treatment affected the Ang II-evoked reduction in renal or splanchnic SNA in LPK rats (Figure 3.12A). In Lewis rats, however, after administration of the vehicle (DMSO), PVN Ang II failed to increase SNA and instead produced a small sympathoinhibitory response (Figure 3.12B). Therefore, although we observed that PVN Ang

II did not increase SNA in Lewis animals pre-treated with SB203580, cyclosporin A or xestospongin C (Figure 3.12B), unintended effects of the vehicle solution limit interpretation of the Lewis SNA data for this particular experiment.

Table 3.3: The effect of vehicle (DMSO), cyclosporin A, xestospongin C and SB203580 on resting systolic blood pressure (SBP), splanchnic SNA (sSNA) and renal SNA (rSNA) in LPK and Lewis rats.

Group	Parameter (*n* LPK/ *n* Lewis)	LPK			Lewis		
		Baseline	20 min	Delta	Baseline	20 min	Delta
Vehicle (DMSO)	SBP (5 / 3)	158 ± 7	167 ± 4	8 ± 5	132 ± 3	131 ± 2	-1 ± 3
	sSNA (4 / 3)	100 ± 1	129 ± 14	29 ± 13	100 ± 1	115 ± 14	15 ± 13
	rSNA (4 / 3)	101 ± 2	128 ± 23	27 ± 21	101 ± 1	106 ± 6	5 ± 5
Cyclosporin A	SBP (4 / 4)	174 ± 8	186 ± 9	12 ± 14	120 ± 7	131 ± 6*	11 ± 3$^{\#}$
	sSNA (4 / 3)	100 ± 1	145 ± 20	45 ± 21	99 ± 1	146 ± 8*	47 ± 8
	rSNA (4 / 4)	101 ± 1	138 ± 7*	37 ± 7	100 ± 2	156 ± 9*	56 ± 9$^{\#}$
Xestospongin C	SBP (5 / 5)	175 ± 9	189 ± 15	14 ± 9	109 ± 6	110 ± 9	1 ± 5
	sSNA (4 / 5)	101 ± 0.4	99 ± 14	-2 ± 13	100 ± 1	123 ± 14	23 ± 14
	rSNA (5 / 5)	98 ± 1	95 ± 15	-3 ± 15	99 ± 1	109 ± 7	10 ± 8
SB203580	SBP (5 / 3)	173 ± 2	175 ± 6	3 ± 6	118 ± 8	123 ± 8	5 ± 1
	sSNA (4 / 3)	100 ± 0.4	114 ± 7	14 ± 8	101 ± 1	130 ± 15	29 ± 15
	rSNA (3 / 3)	100 ± 2	131 ± 27	32 ± 28	100 ± 0.4	129 ± 11	29 ± 11

Values are presented as mean±SEM. *, $P<0.05$ vs. baseline; #, $P<0.05$ vs. vehicle. *n* indicates sample size for the strain, parameter and drug indicated.

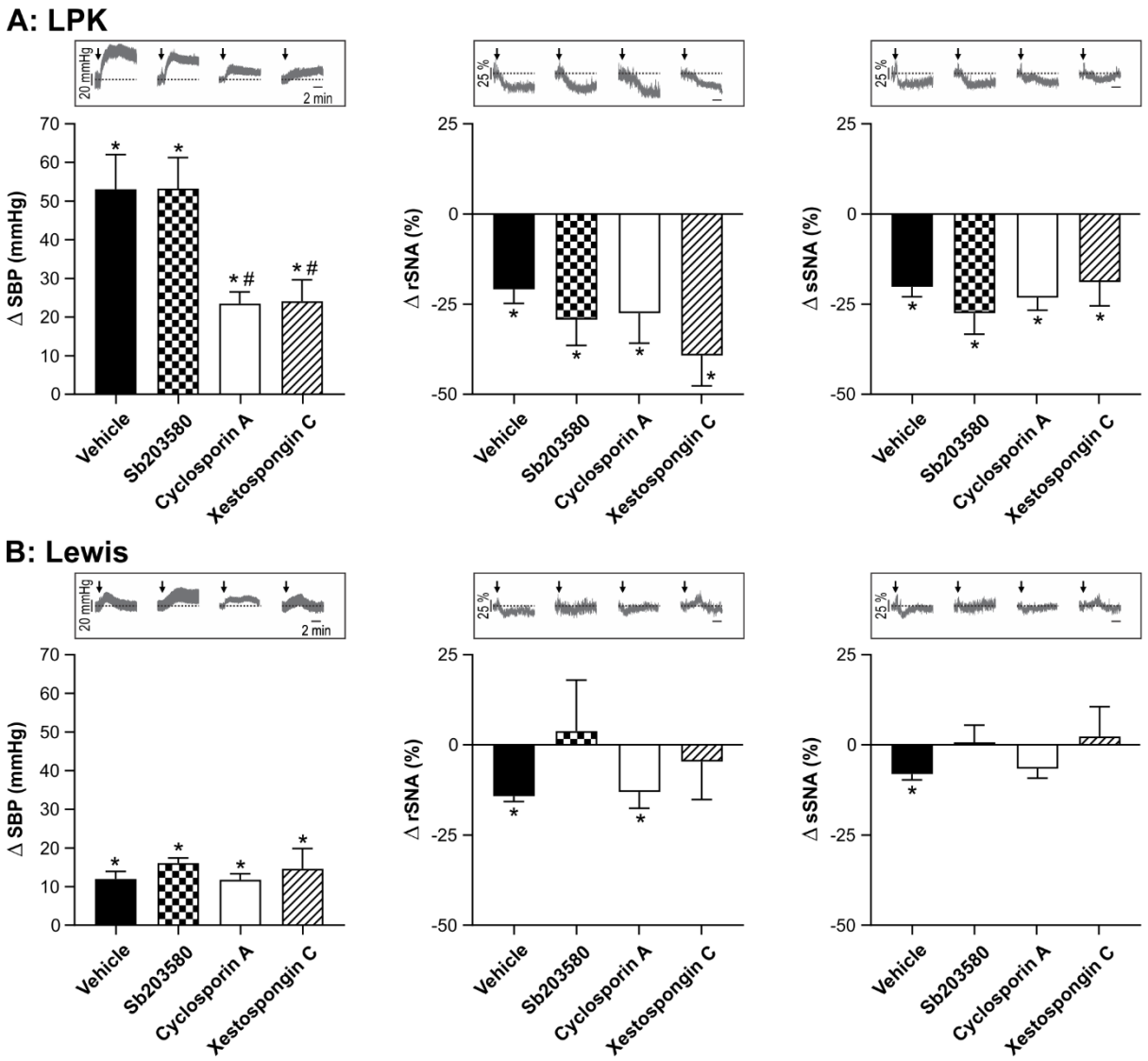

Figure 3.12. Effect of PVN p38 MAPK inhibition with SB203580, calcineurin inhibition with cyclosporin A and IP3 receptor inhibition with xestospongin C on the systolic blood pressure (SBP) response to PVN microinjection of Ang II in LPK and Lewis rats. **A**, Representative recordings (top) and grouped data (bottom) for the PVN Ang II SBP and SNA response in LPK rats. **B**, Representative recordings (top) and grouped data (bottom) for the PVN Ang II SBP and SNA response in Lewis rats. Arrow and broken line indicate PVN Ang II microinjection and baseline, respectively. Data are shown as mean ± SEM. * indicates $P \leq 0.05$ difference from baseline (paired t test), # indicates adjusted $P \leq 0.05$ difference between vehicle. $n = 5$ LPK and $n = 3$ Lewis for vehicle, $n \geq 4$ LPK and $n = 3$ Lewis for SB203580, $n = 4$ LPK and $n \geq 3$ Lewis for cyclosporin A and $n = 5$ per strain for xestospongin C.

Discussion

This study has revealed that Ang II differentially regulates divergent neuroendocrine and autonomic outflows from the PVN in the LPK rat model of PKD. We found that Ang II stimulation of the PVN evokes greater vasopressin-dependent increases in blood pressure in LPK rats compared to Lewis controls through a mechanism that involves astrocyte GLT-1. The regulation of GLT-1 by Ang II is likely to be indirect as few PVN astrocytes were observed to express the AT1R and may be enhanced in LPK rats through a greater PVN AT1R expression and the disease-related recruitment of IP3 receptor- and calcineurin-dependent pathways. Remarkably, we observed that the direction of the sympathetic response to PVN Ang II is reversed in LPK rats from an excitatory to an inhibitory response, suggesting that PVN angiotensin receptors are functionally organised to inhibit SNA in PKD. Our working model is illustrated in Figure 3.13 and is elaborated beneath.

Enhanced Ang II stimulation of vasopressin release in PKD

Elevations in blood pressure can be produced by different groups of PVN efferent neurons that generate sympathetic outflow or secrete vasopressin into the peripheral circulation (Stern, 2015). In this study we found that the pressor response to PVN Ang II microinjection was eliminated by prior systemic inhibition of V_{1A} receptors in both LPK and Lewis rats. These observations strongly suggests that PVN Ang II increases blood pressure by driving vasopressin release in both strains, consistent with previous work showing that PVN Ang II increases the discharge of magnocellular vasopressin neurons in-vitro (Latchford and Ferguson, 2004) and dose-dependently elevates circulating vasopressin concentration (Veltmar et al., 1992). While we acknowledge that it would have been valuable to measure circulating vasopressin levels in response to PVN AngII microinjection, the large volume of blood required for such an analysis would have limited the interpretation of any data gathered.

The pressor response to PVN Ang II was unaffected by ganglionic blockade in both strains, suggesting that PVN Ang II does not increase blood pressure via the sympathetic nervous system in PKD or control conditions. An experimental caveat to consider, however, is that ganglionic blockade abolishes the baroreflex which normally provides strong inhibition of vasopressin-mediated increases in blood pressure (Montani et al., 1980; Osborn et al., 1987). Therefore, pressor responses to PVN Ang II under ganglionic blockade could reflect an enhancement of the pressor action of vasopressin. Nevertheless, as V_{1A} receptor blockade

effectively eliminated the Ang II pressor response, it is likely that the PVN preferentially drives vasopressin release to increase blood pressure in both strains.

Our data suggest that the hypertensive actions of Ang II are enhanced in the PVN of LPK rats as markedly exaggerated increases in arterial pressure were observed in response to administration of Ang II into the PVN. Similar observations have been made in rat models of renovascular hypertension (Han et al., 2011) and diabetes (Patel et al., 2011; Zhang et al., 2012); however in these disease models the renal symapthoexcitatory action of PVN Ang II is also enhanced, contrasting our observations in LPK rats that exhibit SNA reductions to Ang II and further reinforcing our view that the pressor response to Ang II is vasopressin-dependent in this PKD model. We believe that greater increases in arterial pressure following PVN Ang II in LPK rats reflects an enhancement of vasopressin release rather than a sensitisation of vascular V_{1A} receptors because though we did not measure circulating vasopressin levels in our study, we have noted previously that LPK rats display normal pressor responses to an intravenous vasopressin bolus, highlighting that the sensitivity of the vasculature to vasopressin is likely normal in this disease model (Chapter 2) (Underwood et al., 2018).

In a previous study we found that ongoing PVN neuronal activity contributes to the maintenance of hypertension in LPK rats via an efferent pathway that acts independently of peripheral V_{1A} receptor activation (Chapter 2) (Underwood et al., 2018). Therefore, considering our finding that exogenous Ang II preferentially drives vasopressin release from the PVN in LPK rats, it was not surprising to observe that acute blockade of endogenous Ang II actions in the PVN with the AT1R antagonist losartan did not reduce resting blood pressure in LPK animals. It was however surprising that acute systemic inhibition of V_{1A} receptors did not lower arterial pressure in LPK rats as observed previously (Chapter 2) (Underwood et al., 2018). This discrepancy may be attributed to the small sample size in the present study and warrants further investigation into whether or not V_{1A} receptors contribute to hypertension in this PKD model.

Ang II-dependent inhibition of astrocyte GLT-1 is likely indirect

Astrocytes are critical regulators of extracellular glutamate levels in the brain through GLT-1 (Rose et al., 2017). Previous work indicates that astrocytes are required for central Ang II evoked elevations in vasopressin release (Flor et al., 2018) and sympathetic activity during normal conditions (Stern et al., 2016). Of note is the work of Stern et al. (2016) who showed

that AT1R activation reduces GLT-1 currents in PVN astrocytes in-vitro and that DHK blocked Ang II-dependent depolarising currents in RVLM-projecting PVN neurons and sympathetic drive. Our data support the concept that coupling between the AT1R and GLT-1 mediates the stimulatory action of Ang II on both SNA and vasopressin release in the PVN as we found that DHK eliminated Ang II-evoked renal and splanchnic sympathoexcitation in Lewis rats and vasopressin-dependent pressor responses in both Lewis and LPK rats (Figure 3.13). Alternatively, the inhibition of Ang II actions by DHK could be indirect via a ceiling effect as consequence of excessive extracellular glutamate levels. While we cannot discount this possibility, we consider a direct action of DHK most plausible in the context of previous work (Stern et al., 2016).

Whether Ang II inhibits GLT-1 through a direct action on AT1R in PVN astrocytes or indirectly through AT1R in another cell population has hitherto remained uncertain due to a lack of high-resolution structural data. In this study, we found that AT1R mRNA was not present in the overwhelming majority of PVN astrocytes, and in line with other studies (Aguilera et al., 1995b; Oldfield et al., 2001; de Kloet et al., 2017) was also not evident in RVLM-projecting or vasopressin neurons in control rats. Therefore, our data highlight that Ang II likely inhibits astrocyte GLT-1 function indirectly through another cell type that expresses the AT1R. While recent evidence demonstrates that GLT-1 function is dynamically regulated by local neural activity (Al Awabdh et al., 2016), the signalling mechanisms responsible await to be clarified. It is however likely that various paracrine signals are capable of influencing GLT-1 function given that astrocytes express a wide variety of G-protein coupled receptors (Hirase et al., 2014). In the PVN, it is plausible that different paracrine signals regulate GLT-1 function to shape glutamatergic transmission to specific functional neuronal populations so as to coordinate their outputs (e.g. vasopressin release and sympathetic drive). Indeed, dendritically-released vasopressin from PVN magnocellular neurons has been shown to interact with neighbouring neurons (Son et al., 2013) but also astrocytes (Haam et al., 2014). Interestingly, CRH has also recently been reported to exert local paracrine actions in the PVN (Jiang et al., 2018). Since we and others (Oldfield et al., 2001; de Kloet et al., 2017) found that CRH neurons are a major AT1R-expressing cell type in the PVN, we hypothesise that CRH neurons, via local CRH release, may serve as the intermediary between Ang II and astrocytes in the PVN (Figure 3.13). Our hypothesis is further supported by data highlighting that astrocytes express CRH receptors (Stevens et al., 2003) and reports showing that Ca^{2+}/calmodulin-dependent protein kinase II, a signalling

element downstream of CRH receptors (Bonfiglio et al., 2013; Tsuda et al., 2017), negatively regulates GLT-1 in cultured astrocytes (Underhill et al., 2015).

Alterations in PVN AT1R expression and signalling in PKD.

Our findings suggest that vasopressin secretion from the PVN is more sensitive to local Ang II levels in PKD. This difference might be attributed to a higher AT1R density as we observed that AT1R mRNA was more abundant in the PVN of LPK animals, noting however that this experiment had a low sample size and also that RNA expression is an imperfect measure of functional AT1R protein. Others have found that AT1R mRNA is more abundant in the PVN following water-deprivation or corticosteroid administration but that the gross distribution within each subdivision is unchanged (Aguilera et al., 1995a), implying that these challenges upregulate AT1R expression in neurons that normally express the receptor (e.g., CRH neurons in the medial subdivisions) but not those that do not normally express the receptor (e.g., vasopressin neurons in the lateral magnocellular portion). In LPK rats, we found that AT1R expression was robustly upregulated in CRH neurons such that AT1R mRNA was both more abundant and present in a greater proportion of the CRH neuronal population. However, whether this expression pattern is functionally related to the enhanced PVN Ang II pressor response that we observed in this PKD model will require additional studies to examine whether CRH neurons participate in this signalling process (Figure 3.13).

Another interesting observation of the current study was that a small population of PVN vasopressin neurons express the AT1R in LPK rats. To the best of our knowledge, this expression pattern has not been reported previously, the possible exception being in rats with heart failure that show greater AT1R-like immunoreactivity in the lateral magnocellular division (Wei et al., 2008; Zheng et al., 2009). The functional significance of this abnormal distribution pattern is unclear, especially considering the strong GLT-1-dependence of the vasopressin-dependent pressor response in LPK rats. However, it is worthy to note that the AT1R is normally expressed in SON vasopressin neurons (Chakfe and Bourque, 2000; Sandgren et al., 2018) where it serves to potentiate glutamate release from upstream presynaptic terminals (Stachniak et al., 2014). It is therefore reasonable to speculate that AT1R expression in this small group of vasopressin neurons might have an additive effect on extracellular glutamate levels in addition to that achieved through the negative regulation of GLT-1.

The AT1R recruits multiple interacting signalling pathways that vary according to tissue type and (patho)physiological state (Forrester et al., 2018). The formation of ROS is critical for AT1R signalling in central cardiovascular circuits (Zimmerman et al., 2002; Chen and Pan, 2007; Han et al., 2007) and within the PVN has been identified to be augmented to facilitate enhancements in the sympathoexcitatory action of Ang II in multiple disease states (Han et al., 2007; Han et al., 2011; Patel et al., 2011). ROS in turn influences multiple effectors (Forrester et al., 2018); for instance, Ang II-induced ROS production activates p38 MAPK in the RVLM (Chan et al., 2005). Here we show that p38 MAPK is not required for the acute pressor action of Ang II in the PVN in LPK or Lewis rats. Additionally, our data suggest that IP3 receptor activation and calcineurin signalling are dispensable for the acute pressor action of Ang II in control Lewis rats, implying that PVN AT1R controlling vasopressin release involves an intracellular signalling cascade that is independent of p38 MAPK, IP3 receptors and calcineurin in normal conditions. In LPK rats, however, the enhanced pressor action of Ang II was significantly reduced following inhibition of IP3 receptors and calcineurin, indicating that there is a disease-related recruitment of these signalling pathways in PKD.

While further studies will be required to determine precisely how IP3 receptors and calcineurin participate in the facilitation of Ang II signalling in the PVN in PKD, it is possible to speculate on some underlying mechanisms. The AT1R interacts with several heterotrimeric G proteins, including the G_q protein which couples with phospholipase C to produce the signalling molecule IP3 that in turn drives Ca^{2+} release from endoplasmic reticulum stores (Miyakawa et al., 2001; Forrester et al., 2018). Interestingly, IP3 receptors are known to functionally interact with the protein products of genes causative of PKD (Li et al., 2005; Mekahli et al., 2012). As there is some evidence that PKD genes are expressed in the hypothalamus (Ho et al., 2012), it is conceivable that the disease-related recruitment of IP3 receptors by Ang II in LPK rats is directly related to the PKD gene mutation. The AT1R also stimulates calmodulin-activation of calcineurin through a direct protein-protein interaction (Thomas et al., 1999) and via IP3-dependent increases in cystoplasmic Ca^{2+} (Lea et al., 2002). Thus, calcineurin activation could lie downstream of IP3 receptors in AT1R-expressing cells in the PVN of LPK rats, a possibility that is consistent with our data showing that inhibition of calcineurin and IP3 receptors reduced the Ang II pressor response to a comparable degree.

Control experiments performed in the current study raise an important methodological consideration regarding the use of DMSO as a vehicle solution for studies investigating Ang

II signalling since we observed that this vehicle alone abolished the PVN Ang II sympathoexcitatory response in Lewis rats. It is plausible that this unintended effect of DMSO relates to the antioxidant property of this chemical (Santos et al., 2003). Although we suggest that future studies should avoid using DMSO as a vehicle solution, we do not believe that the influence DMSO had on the Ang II SNA response in Lewis rats in the current study limits the interpretation of the associated pressor response because we have shown that it is not likely to be mediated by sympathetic nervous system in these animals.

Ang II-evoked sympathoinhibition in PKD

Our finding that robust decreases in SNA were evoked by PVN Ang II administration in LPK rats was surprising given that small to moderate increases in SNA are commonly reported in other strains (Zheng et al., 2009; Han et al., 2011; Stern et al., 2016) and were observed in the Lewis animals in our study. Importantly, our data demonstrate that PVN neurons are still capable of increasing SNA in LPK rats because administration of DHK into the PVN (mimicking glutamatergic activation) produced a large increase in renal and splanchnic SNA in these animals. Conceivably, Ang II-evoked sympathoinhibition in LPK rats could be mediated centrally or via the baroreflex owing to large increases in blood pressure that were concurrently produced. However, the latter seems unlikely as Ang II-evoked SNA reductions were consistently observed when pressor responses were eliminated with systemic V_{1A} antagonism or reduced with IP3 receptor or calcineurin inhibition.

Interestingly we observed that losartan inhibited the Ang II-evoked sympathoinhibition in the renal but not splanchnic nerve, indicating that Ang II recruits AT1R-dependent and independent cell populations to inhibit SNA in an end-organ selective manner. Though it was not directly tested, it is possible that the observed splanchnic sympathoinhibition is mediated by the AT_2 receptor as this angiotensin receptor subtype is expressed in neurons with terminal fields that closely appose RVLM-projecting PVN neurons (de Kloet et al., 2016a) and decreases blood pressure and urinary noradrenaline excretion when chronically stimulated with an agonist administered intracerebroventricularly (Gao et al., 2011) (Figure 3.13). The AT1R-dependent sympathoinhibition that we observed in the renal nerve was surprising given previous work showing that AT1R exerts a net renal sympathoexcitatory action in the PVN in other strains (Zheng et al., 2009; Han et al., 2011; Stern et al., 2016), and suggests that AT1Rs are functionally organised in the PVN in PKD to inhibit renal sympathetic outflow. Our data showing that the AT1R is not expressed in RVLM-projecting neurons and previous observations indicating that AT1R is excitatory in PVN neurons (de Kloet et al.,

2017), supports the possibility that AT1R-dependent sympathoinhibition in LPK rats could be mediated either through activation of presynaptic AT1R on inhibitory (e.g. GABAergic) neurons antecedent to PVN presympathetic neurons (Figure 3.13) or via an inhibitory relay through another brain region (e.g., caudal ventrolateral medulla (Geerling et al., 2010)). Alternatively, because we administered Ang II unilaterally into the PVN, it is possible that the sympathoinhibition is produced via a projection to the contralateral PVN. Further dissection of this pathway may ultimately yield a novel target to alleviate central sympathetic overdrive in PKD.

Conclusion

We have identified that Ang II differentially regulates blood pressure and SNA in a rat model of PKD. Our findings support the hypothesis that Ang II transmission is enhanced in the PVN in PKD, reflected by an increased sensitivity of vasopressin-secreting neurons to Ang II via a mechanism that involves astrocyte glutamate transport and signalling via IP3 receptors and calcineurin. We demonstrate that astrocytes in the rat PVN largely lack the AT1R, therefore raising the strong possibility that another AT1R-expressing cell type, potentially CRH neurons, serves as an intermediary between Ang II and astrocytes in the PVN.

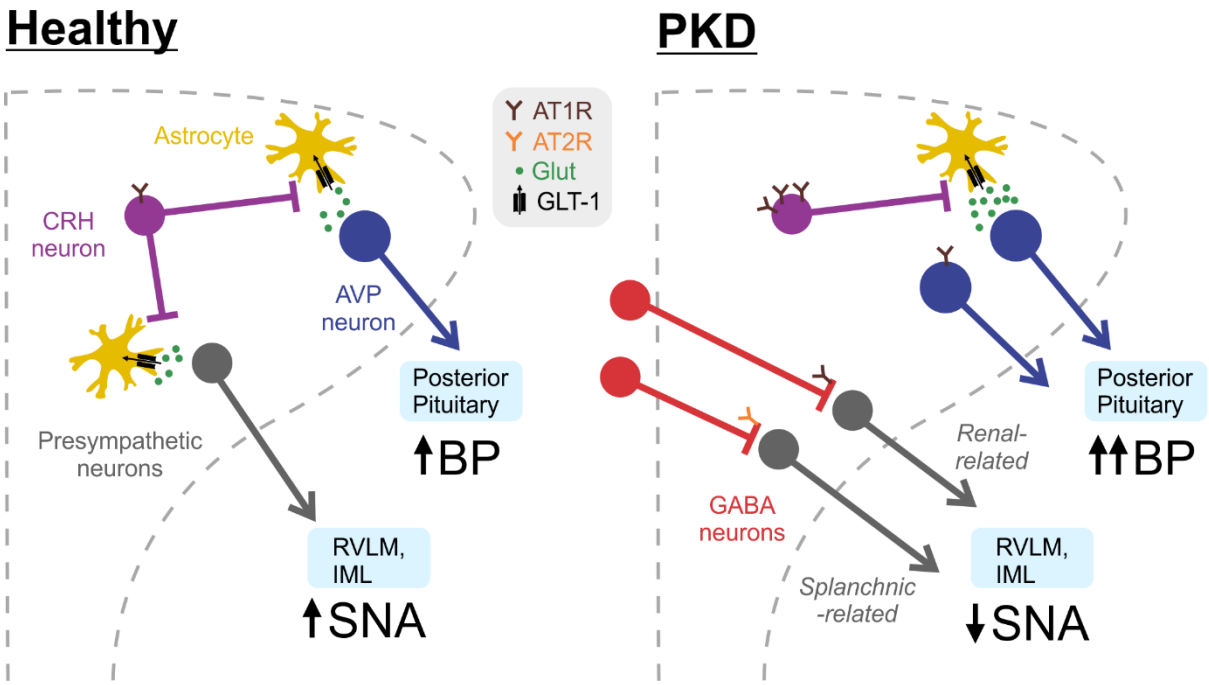

Figure 3.13. Working model describing the cardiovascular actions of Ang II in the PVN in healthy conditions and PKD. **Healthy conditions:** Ang II activates vasopressin (AVP)-releasing neurons to increase in blood pressure (BP) and presympathetic neurons to increase sympathetic nerve activity (SNA). The stimulatory action of Ang II on AVP and presympathetic neurons requires both AT1R and an inhibition of astrocyte glutamate (Glut) transporter-1 (GLT-1) to elevate extracellular glutamate. We hypothesise that CRH neurons are the AT1R-expressing cell type responsible for inhibiting GLT-1 upon increases in Ang II. **PKD:** Ang II generates a greater activation of AVP neurons to produce larger BP elevations and simultaneously inhibits presympathetic PVN neurons to reduce SNA. The enhanced Ang II activation of AVP neurons is mediated by a greater inhibition of GLT-1, possibly due to a higher density of AT1R on CRH neurons, the presence of AT1R in some AVP neurons and the activation of IP3 receptors and calcineurin (not shown). Ang II-induced sympathoinhibition in the renal nerve is AT1R-dependent, but for the splanchnic nerve is mediated by another angiotensin receptor, possibly the AT_2 receptor (AT2R). As presympathetic neurons do not express AT1R or AT2R (de Kloet et al., 2016a), Ang II evoked symapthoinhibition in LPK could be mediated by the stimulation of GABA release from presynaptic terminals antecedent to presympathetic neurons. RVLM, rostral ventrolateral medulla; IML, intermediolateral cell group of spinal cord.

4.

The Subfornical Organ Drives Hypertension in Polycystic Kidney Disease via the Hypothalamic Paraventricular Nucleus

Underwood C.F., McMullan, S., Goodchild, A.K., Phillips J.K. & Hildreth C.M.

Abstract

Hypertension is a prevalent yet poorly understood feature of polycystic kidney disease (PKD). Previously we demonstrated that increased glutamatergic neurotransmission within the hypothalamic paraventricular nucleus (PVN) produces hypertension in the Lewis Polycystic Kidney (LPK) rat model of PKD. Here we tested the hypothesis that augmented glutamatergic drive to the PVN in LPK rats originates from the forebrain lamina terminalis, a sensory structure that relays blood-borne information throughout the brain. Anatomical experiments revealed that 38% of PVN-projecting neurons in the subfornical organ (SFO) of the lamina terminalis expressed Fos/Fra, an activation marker, in LPK rats while <1% of neurons were Fos/Fra+ in Lewis control rats (P=0.01, n=8). In anaesthetized rats, SFO neuronal inhibition using isoguvacine produced a greater reduction in systolic blood pressure in the LPK versus Lewis rats (-21±4 vs. -7±2 mmHg, P<0.01; n=10), which could be prevented by prior blockade of PVN ionotropic glutamate receptors using kynurenic acid. Blockade of ionotropic glutamate receptors in the PVN produced an exaggerated depressor response in LPK relative to Lewis rats (-23±4 vs. -2±3 mmHg, P<0.001; n=13), which was corrected by prior inhibition of the SFO with muscimol but unaffected by chronic systemic angiotensin II AT1 receptor antagonism or lowering of plasma hyperosmolality through high-water intake (P>0.05); treatments that both nevertheless lowered blood pressure in LPK rats (P<0.0001). Our data reveal multiple independent mechanisms contribute to hypertension in PKD, and identify high plasma osmolality, AT1 receptor activation and, importantly, a hyperactive SFO-PVN glutamatergic pathway as potential therapeutic targets.

Introduction

Polycystic kidney disease (PKD) is a group of common monogenetic disorders characterised by the progressive accumulation of renal cysts. Hypertension occurs in ~70 % of PKD patients and is associated with poor renal and cardiovascular outcomes (Gabow et al., 1992; Chapman et al., 2010). Though its origins are unclear, the aetiology of hypertension in PKD is clearly unique from other forms of chronic kidney disease as it precedes any measurable decline in renal function (Chapman et al., 2010). Several neural, humoral and vascular changes have been identified in PKD that could potentially precipitate chronic elevations in blood pressure (Klein et al., 2001; Wang et al., 2003; Quek et al., 2018), yet whether these changes arise from a single source or develop independently remains unknown. If a single origin is predicted then the hypothalamic paraventricular nucleus (PVN) is an appealing candidate as it contains functionally-distinct neuronal populations that regulate not only multiple sympathetic outflows but also the peripheral secretion of vasopressin and other neurohormones (Stern, 2015). Using a rodent model of PKD, the Lewis Polycystic Kidney (LPK) rat, we recently identified that the PVN is in fact overactive in its control of blood pressure (Chapter 2) (Underwood et al., 2018); however, what is driving the increased activity in this brain region remains to be determined.

The PVN receives dominant excitatory inputs from the lamina terminalis, which comprises the median preoptic nucleus (MnPO), and two sensory circumventricular organs that lack a complete blood-brain barrier, the subfornical organ (SFO) and organum vasculosum of lamina terminalis (OVLT) (McKinley et al., 2001; Ulrich-Lai et al., 2011b; Llewellyn et al., 2012), which are critical for detecting haemal stimuli such as angiotensin II and plasma solutes (Bardgett et al., 2014a; Holbein and Toney, 2015). Given that sustained activation of neurons in the lamina terminalis contributes to hypertension in the spontaneously hypertensive, angiotensin II-salt sensitive , and DOCA-salt sensitive rat models (Osborn et al., 2012; Mourao et al., 2016; Collister et al., 2018), we hypothesised that upregulated neural activity within the forebrain lamina terminalis similarly contributes to the hypertension observed in PKD via its excitatory influence on the PVN. Importantly, this hypothesis is supported by the fact that blood pressure can be reduced in PKD models by suppressing two critical stimuli detected by the lamina terminalis: angiotensin II and plasma osmolality (Quek et al., 2018; Sagar et al., 2019).

We therefore addressed the following questions: (1) Are PVN-projecting neurons in the lamina terminalis chronically activated in the LPK rat? (2) Does the lamina terminalis

contribute to hypertension in the LPK rat via glutamatergic activation of the PVN? (3) Does inactivation of angiotensin type 1 receptors (AT1R) or suppression of plasma osmolality reduce glutamatergic drive to the PVN and blood pressure in the LPK rat?

Methods

Animals

Experiments were performed in accordance with The Australian Code of Practice for the Care and Use of Animals (8th Edition, 2013) and approved by the Macquarie University Animal Ethics Committee. Male Lewis and LPK rats were obtained from the Animal Resource Centre, Murdoch, Western Australia, Australia and housed in standard living conditions with a 12-hour light/dark cycle and access to standard rodent chow and water *ad libitum*. Rats were group housed except for radiotelemetry and retrograde tracing experiments in which rats were individually housed.

Study 1: Are PVN-projecting lamina terminalis neurons chronically activated in the LPK rat?

Retrograde tracing

Fluorescently-conjugated cholera-toxin subunit B (CTB-555; 0.25% in saline, ~30 nl) was microinjected into the right PVN (1.2-1.4 mm caudal of bregma, 0.2 mm lateral to the midline and 8.2 mm ventral to the dura surface) in isoflurane-anesthetised 13 week old LPK and Lewis rats as described in Chapter 3. After a period of 5 days, rats were anaesthetised with sodium pentobarbital (100 mg/kg i.p.) and transcardially perfused with ~300 ml of heparinised 0.9% saline (5000 IU/L at 4º C) followed by ~300 ml 10% neutral-buffered formalin (4 °C). Brains were harvested and immersed in the same fixative for 12-18 hours at 4º C and then sectioned using a vibrating microtome (VT1200S, Leica). Resulting PVN CTB injections were centred at -1.8 ± 0.1 mm rostral of bregma and spread with a dorsoventral and mediolateral distribution that was predominately restricted to all sub-divisions of the posterior PVN (Figure 4.1).

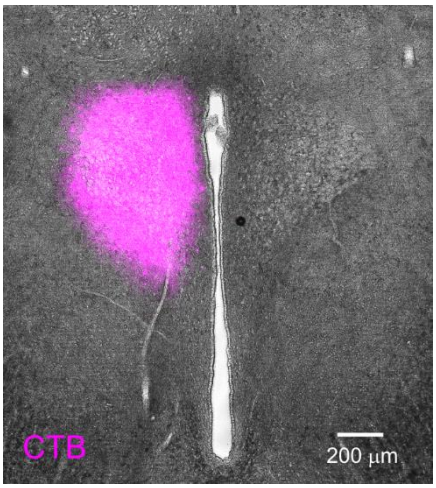

Figure 4.1. Example of a unilateral PVN CTB injection.

Fos/Fra immunohistochemistry

To assess the activation state of CTB-positive neurons within the lamina terminalis, immediate-early gene immunohistochemistry was performed using a rabbit polyclonal antibody that recognises amino acids 128–152 in the NH2- terminal region of Fos, a conserved region of the Fos, Fos-B, Fra-1, and Fra-2 (1:1000; K-25, AB_2231996, Santa Cruz Biotechnology, Santa Cruz, CA, USA). One in four sequential coronal sections (50 µm thick) were incubated in 0.1M phosphate-buffered saline (PBS) containing 0.3% hydrogen peroxide for 10 min and then washed (PBS; 3 x 15 minutes) and blocked for 1 hour at 4 ºC in a solution containing avidin block (20%; Vector Laboratories, CA, USA) and 10% normal goat serum in PBS with 10 mM Tris-HCL and 0.3% Triton-X-100 (TTPBS). Sections were then transferred to the primary antibody solution (anti-Fra primary antibody (1:1000), biotin blocking agent (20%, Vector Laboratories), 10% normal goat serum and 0.5% methiolate in TTPBS). Following primary antibody incubation for 72 h at 4 ºC, sections were washed (PBS; 3 x 15 minutes) and then incubated for 24 hours at 4 ºC in 10 mM Tris-HCL PBS containing biotin-conjugated goat anti-rabbit IgG (1:500; Jackson ImmunoResearch, West Grove, PA, USA) and 1% goat serum. Sections were subsequently washed (PBS; 3 x 15 minutes) and incubated overnight at 4 ºC in Extravidin-peroxidase (1:1000; E-2886, Sigma Aldrich, Castle Hill, NSW, Australia). After a final series of washes (PBS; 3 x 15 minutes), sections were incubated in DAB reaction solution (2% buffer, 2% H_2O_2, 4% DAB in distilled water) for 75 seconds (SK-4100; Vector Labs, Burlingame, CA, USA). Sections were mounted onto glass sides and cover-slipped with fluorescent mounting medium (Agilent, Santa Clara, CA, USA).

Image capture and analysis

Images were captured with a combined bright-field/epifluorescence microscope (Axioimager 2; Zeiss, Germany). CTB+ neurons and Fos/Fra+ cells in the same field of view were manually counted in Fiji ImageJ (Schindelin et al., 2012). Only neurons with a visible nucleus were included in the analysis. Total Fos/Fra counts were performed with the spot detection plug-in in Icy software (de Chaumont et al., 2012), with detections overlayed on the raw image and manually corrected to ensure accurate detection of nuclei. In each rat, counts were performed and then averaged in ≥ 2 sections for the OVLT and SFO and ≥ 4 sections for the MnPO. The anatomical boundaries of the OVLT, MnPO and SFO were defined as per the rat brain atlas (Paxinos and Watson, 2013).

Study 2: Does the lamina terminalis contribute to hypertension in the LPK rat via glutamatergic activation of the PVN?

Surgical preparation for acute experiments

Rats (13-14 weeks) were anaesthetised with urethane (1.3 g/kg i.p.) and prepared to measure arterial blood pressure, administer intravenous drugs and fluids and permit artificial ventilation as described in Chapter 2 (Underwood et al., 2018). Additional doses of urethane (65-130 mg/kg i.p. or i.v.) were administered as required to maintain anaesthesia throughout the experiment. Rats were paralysed with cisatracurium (6mg/kg i.v. for induction and 6 mg/kg/hr i.v. for maintenance). Body temperature was maintained at 37 ± 0.5 ºC with a heating mat and infrared lamp. A dorsal flank incision was made to access the retroperitoneal cavity for sympathetic nerve recordings. At least one of the following sympathetic nerves were isolated for recording: the left greater splanchnic nerve; a branch of the left renal nerve or a branch of the left lumbar nerve. Recordings were made with a silver bipolar electrode from nerves cut at their distal end and immersed in paraffin oil. Recordings were stabilised for ≥ 30 minutes prior to the experimental protocol.

SFO and PVN microinjections

Rats were positioned in a stereotaxic apparatus and a craniotomy made to access the SFO (0.5-0.8 mm caudal to bregma, medial and 5.2-5.5 ventral to the dura) and/or PVN 1.2-1.3 mm caudal to bregma, 0.3 lateral to the midline and 8.1 ventral to the dura). One of two microinjection protocols were performed to examine the following questions: (1) does SFO neuron activation contribute to resting cardiovascular activity in LPK and Lewis rats via a glutamatergic mechanism in the PVN? and (2) is SFO neuronal activation the source of high glutamatergic tone in LPK rats? To address the first question, isoguvacine (10 mM; 100 nl (Dutschmann and Herbert, 2006)), a short-acting $GABA_A$ receptor agonist, was injected into the SFO 30 minutes before and 20 minutes following bilateral PVN microinjection of kynurenic acid (100 mM; 100 nl (Miyawaki et al., 2002)), a non-selective ionotropic glutamate antagonist (n = 5 per strain). To address the second aim, muscimol (10 mM; 100 nl (Kim et al., 2013)), a long-acting $GABA_A$ receptor agonist, was injected into the SFO to provide sustained inhibition and then kynurenic acid (100 mM; 100 nl; n = 6 LPK rats) was injected into the PVN.

In a subset of experiments, vehicle (PBS, 100 nl; n = 4 per strain) was injected into the SFO at the commencement of the experiment and in a different subset isoguvacine (n = 3 LPK rats) was injected twice into the SFO 50 minutes apart to confirm the reproducibility of the

response. These control experiments showed that microinjection of PBS into the SFO had no effect on blood pressure, heart rate (HR) or sympathetic nerve activity (SNA) in either strain (data not shown; all $P > 0.05$) and that the depressor response to repeated SFO isoguvacine microinjections was reproducible in LPK rats when performed 50 min apart (-24 ± 6 vs. -25 ± 6 mmHg; $P = 0.81$).

Rats were euthanised at the end of the experiment with intravenous injection of potassium chloride. Fluorescent polystyrene microbeads (1/2000 blue beads from Thermo Fisher Scientific, CA, USA or 1/1000 red beads from Sigma Aldrich) were contained within the drugs solutions to label injection sites. Brains were harvested, immersed in 10% neutral-buffered formalin for > 12 hours (4 °C) and sectioned at 75 µm on a vibratome (VT1200S, Leica) to determine microinjection sites, which were subsequently mapped to the rat brain atlas (Paxinos and Watson, 2013). The microinjection sites for all acute experiments are shown in Figure 4.2.

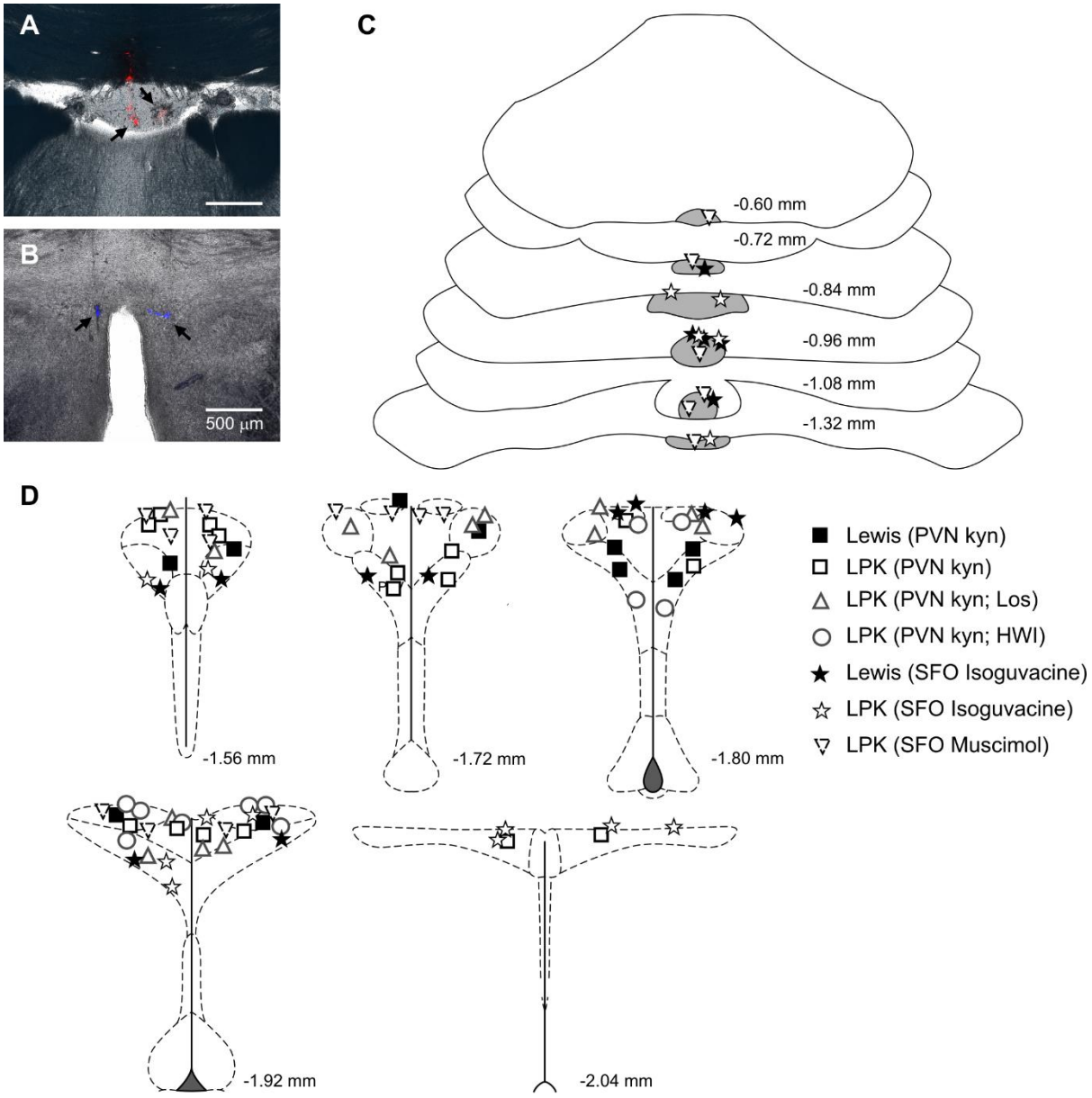

Figure 4.2. Example of microinjection sites in the SFO (**A**) and PVN (**B**). Summary of microinjection sites in the PVN for LPK and Lewis rats for the SFO (**C**) and PVN (**D**). Rostro-caudal distance from Bregma is indicated. Kyn, kynurenic acid; Los, losartan-treated; HWI, high-water intake treated.

Data acquisition and analysis

Arterial blood pressure was recorded with a pressure transducer and acquired with a CED 1401 plus (Cambridge Electronic Designs Ltd., Cambridge, UK). SNA was band-pass filtered (30-1000 Hz), amplified with a bio-amplifier (2000x; CWE Inc., Ardmore, PA, USA) and sampled at 5 kHz with a CED 1401 plus. Spike2 software was used to capture and analyse all data (Cambridge Electronic Designs Ltd.). The SNA waveforms were rectified, smoothed (1-second constant) and normalised to the 30s period immediately prior to PVN microinjection,

setting this as 100% and the level of SNA following euthanasia as 0%. Baseline measurements of systolic blood pressure (SBP), HR and SNA were taken as the average of the 1- or 5- minute period immediately prior to microinjection of isoguvacine and kynurenic acid, respectively. Drug effects were compared by measuring the average level of SBP, HR and SNA at the peak response following SFO microinjection of isoguvacine or in sequential 5 minute bins following PVN microinjection of kynurenic acid. To determine whether SFO microinjection of muscimol affected the SBP response to PVN microinjection of kynurenic acid in LPK rats, data were compared with control LPK and Lewis rats from the treatment study described beneath.

Study 3: Does inactivation of angiotensin type 1 receptors (AT1R) or suppression of plasma osmolality reduce glutamatergic drive to the PVN and blood pressure in the LPK rat?

Radiotelemetry probe implantation

Radiotelemetry probes (PAC10 or HDX10; Data Sciences International, St Paul, MN, USA) were implanted under aseptic conditions in 5- to 6-week old Lewis and LPK rats. Rats were anaesthetised with isoflurane and administered carprofen (2.5 mg/kg s.c) and cephazolin (50 mg/kg i.m.) were administered for analgesia and antibiotic prophylaxis, respectively. The tip of the catheter was inserted into the abdominal aorta via the femoral artery. The probe body was positioned subcutaneously, and the site closed with surgical staples. Rats received carprofen (2.5 mg/kg s.c.) the following day for analgesia and supplementary fluids. Recordings were made ≥ 5 days after surgery at which time rats had gained weight and resumed normal drinking behaviour.

Data were sampled continuously for 5 minutes every 15 minutes for a 24-hour period each week during the treatment period. SBP was derived from the arterial pressure waveform using Dataquest ART (Data Sciences International). All data acquired during each 24-hour recording period was averaged to obtain a single value for each animal per week.

Experimental protocol

On the day of radiotelemetry probe implantation LPK rats were randomly divided into three treatment groups: untreated (n = 8), losartan-treated (30 mg/kg/day p.o. in 1 ml/kg 70% condensed milk in water; n = 5) or high-water intake (HWI; 5% glucose in the drinking water (Hopp et al., 2015), n = 6). Lewis rats (n = 8) were not treated as our aim was to identify therapeutic interventions capable of reducing pathological levels of blood pressure and glutamatergic tone present in LPK rats (Chapter 2). Rats were treated from 6 to 13-14 weeks

of age. The untreated groups comprised animals that either received diluted condensed milk (1 ml/kg/day, vehicle for losartan; n = 4 per strain) and others that did not (control for HWI; n = 4 per strain). Since the SBP of animals that received condensed milk and those that did not was similar throughout the treatment period (LPK, $F_{1,6} = 0.11$, P =0.75; Lewis, $F_{1,6} = 2.03$, P = 0.20), the groups were combined in the final analysis.

After the treatment period, radiotelemetered animals and an additional cohort of non-radiotelemetered LPK rats (n = 1 untreated and n = 2 losartan-treated) were anaesthetised with urethane and prepared for measurement of cardiovascular parameters and stereotaxic PVN microinjection as described above. After stabilisation period of at least 30 minutes, animals received a bilateral PVN microinjection of kynurenic acid (100 mM; 100 nl; n = 5 Lewis, n = 8 LPK, n = 6 losartan-treated LPK, n = 5 high water intake-treated LPK rats).

Assessment of fluid balance

HWI-treated LPK rats and paired untreated controls (n = 3) were placed in metabolic cages weekly to measure 24-hour water intake and urine output and osmolality. All other rats were placed in metabolic cages for 24-hour measurement of water intake and urine output in the final week of treatment only. While under urethane anaesthesia, samples of arterial blood (~0.4 ml) were taken and centrifuged for 150 seconds at 15,800 rpm to separate plasma. Urine and plasma samples were stored at -20 °C. Urine and plasma osmolality was measured in duplicate with an osmometer (VAPRO 5520, Wescor, UT, USA). Blood urea nitrogen was measured using an IDEXX Vet Test (IDEXX Laboratories Pty Ltd., Rydalmere, NSW, Australia), and subtracted from plasma osmolality to assess the effective plasma osmolality (Rasouli, 2016).

Statistical analysis

Data are expressed as mean ± SEM unless otherwise stated. GraphPad Prism version 7 (GraphPad Software, San Diego, Ca, USA) was used for all statistical analysis. Repeated measures ANOVA or paired t-test was used for within group comparisons. To test for differences between strain and treatment groups, an unpaired t-test or a two-way ANOVA was used. If significant drug x strain interactions were detected with two-way ANOVA, drug effects were determined in each strain separately with one-way ANOVA. Bonferoni's correction was used for all multiple comparisons. Statistical significance was set at $P \leq 0.05$.

Results

PVN-projecting SFO and MnPO neurons are chronically active in the LPK rat

To identify chronically activated PVN-projecting neuronal populations in the lamina terminalis, neurons were retrogradely labelled with CTB-555 and immunohistochemically processed to visualise Fos/Fra immediate-early genes, a marker of long-term neuronal activation (Miyata et al., 2001). The forebrain distribution of CTB+ neurons was consistent with previous reports (Sawchenko and Swanson, 1983), and similar numbers of CTB+ neurons identified in the SFO, MnPO and OVLT of both Lewis and LPK rats (Figures 4.3, 4.4 and 4.5 and Table 4.1).

Fos/Fra+ nuclei were detected throughout the lamina terminalis in both LPK and Lewis rats. The number of Fos/Fra+ nuclei within the OVLT was comparable between LPK and Lewis rats; however, within the SFO and MnPO more Fos/Fra+ nuclei were observed in LPK rats (Table 4.1). In LPK rats, colocalization of CTB-555 and Fos/Fra was common within the SFO (38% CTB+ neurons were Fos/Fra+) and MnPO (16% CTB+ neurons were Fos/Fra+), but not OVLT (Table 4.1). In Lewis rats, colocalization between CTB and Fos/Fra was virtually non-existent in all brain regions. Thus, there were more Fos/Fra+ CTB+ neurons within the SFO and MnPO in LPK compared with Lewis rats, suggesting selective and chronic activation of SFO and MnPO neurons that project to the PVN in LPK rats.

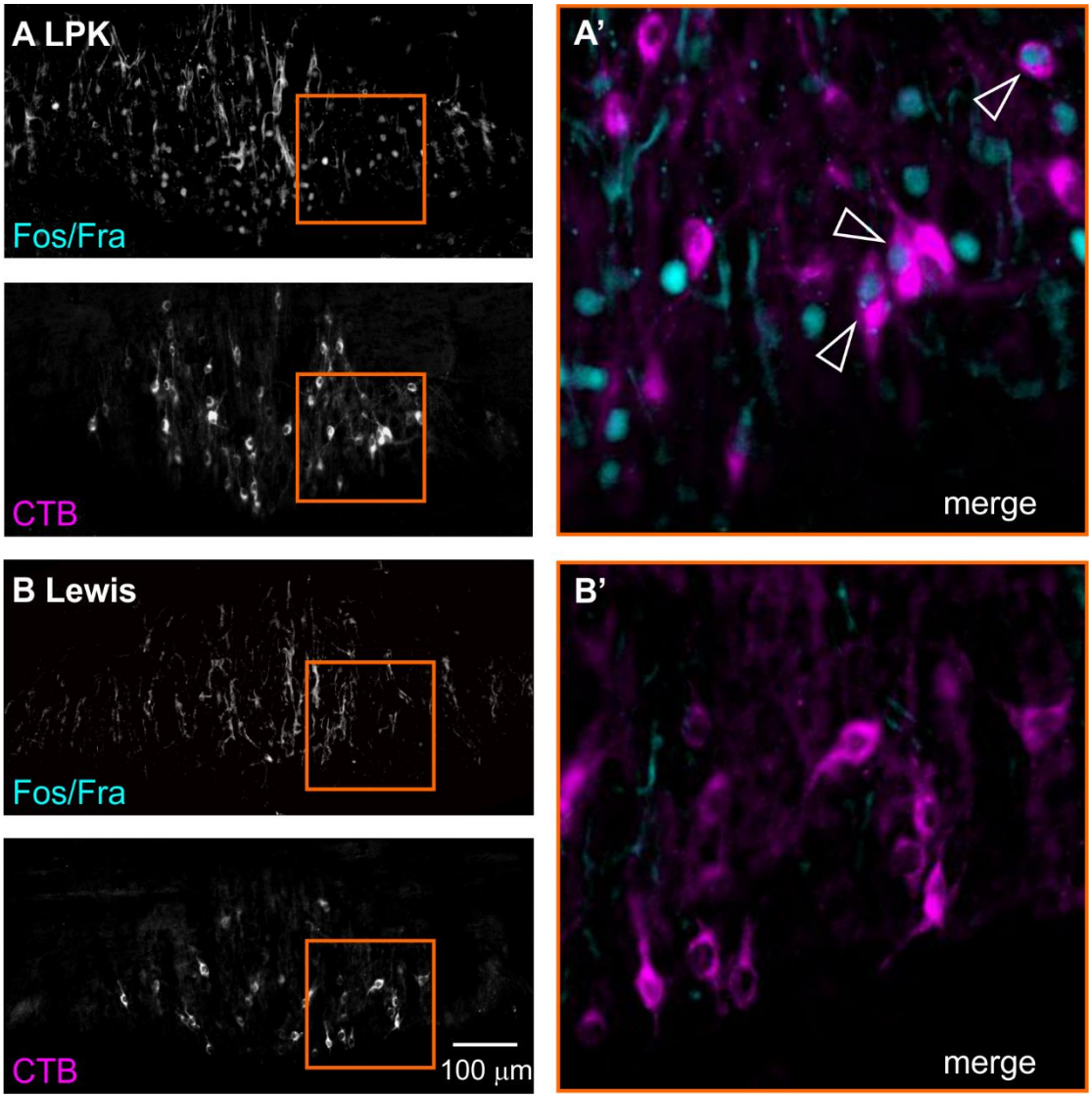

Figure 4.3. Representative images of the SFO showing the distribution of CTB+ neurons and Fos/Fra+ nuclei in a LPK (**A**) and Lewis rat (**B**). The merged image is shown in **A'** and **B'**. Fos/Fra+ nuclei have a punctate appearance and are pseudocoloured cyan, while CTB is pseudocoloured magenta. Arrows indicate CTB neuron with Fos/Fra+ nucleus. Striated structures on the Fos/Fra channel are blood vessels.

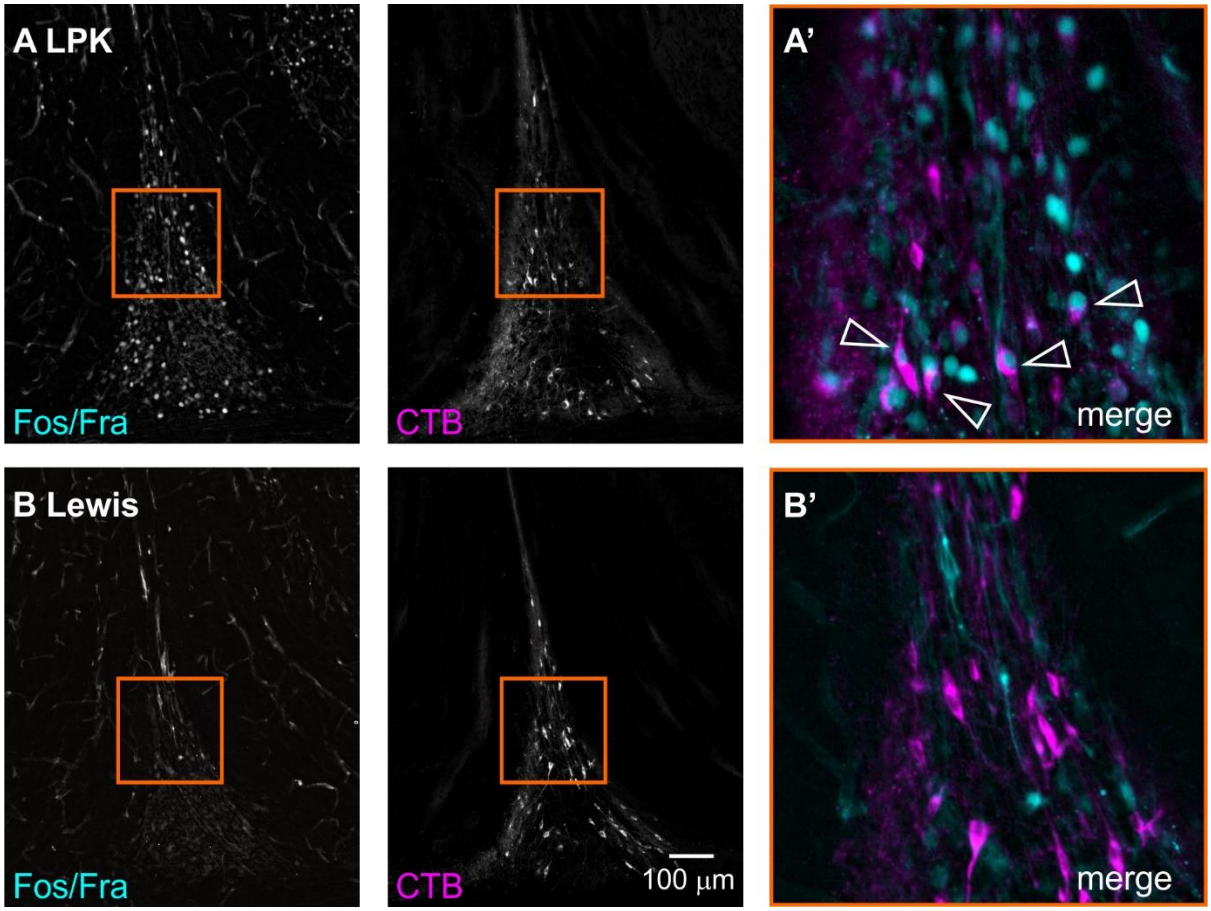

Figure 4.4. Representative images of the MnPO showing the distribution of CTB+ neurons and Fra+ nuclei in a LPK (**A**) and Lewis rat (**B**). The merged image is shown in **A'** and **B'**. Fos/Fra+ nuclei have a punctate appearance and are pseudocoloured cyan, while CTB is pseudocoloured magenta. Arrows indicate CTB neuron with Fos/Fra+ nucleus. Striated structures on the Fos/Fra channel are blood vessels.

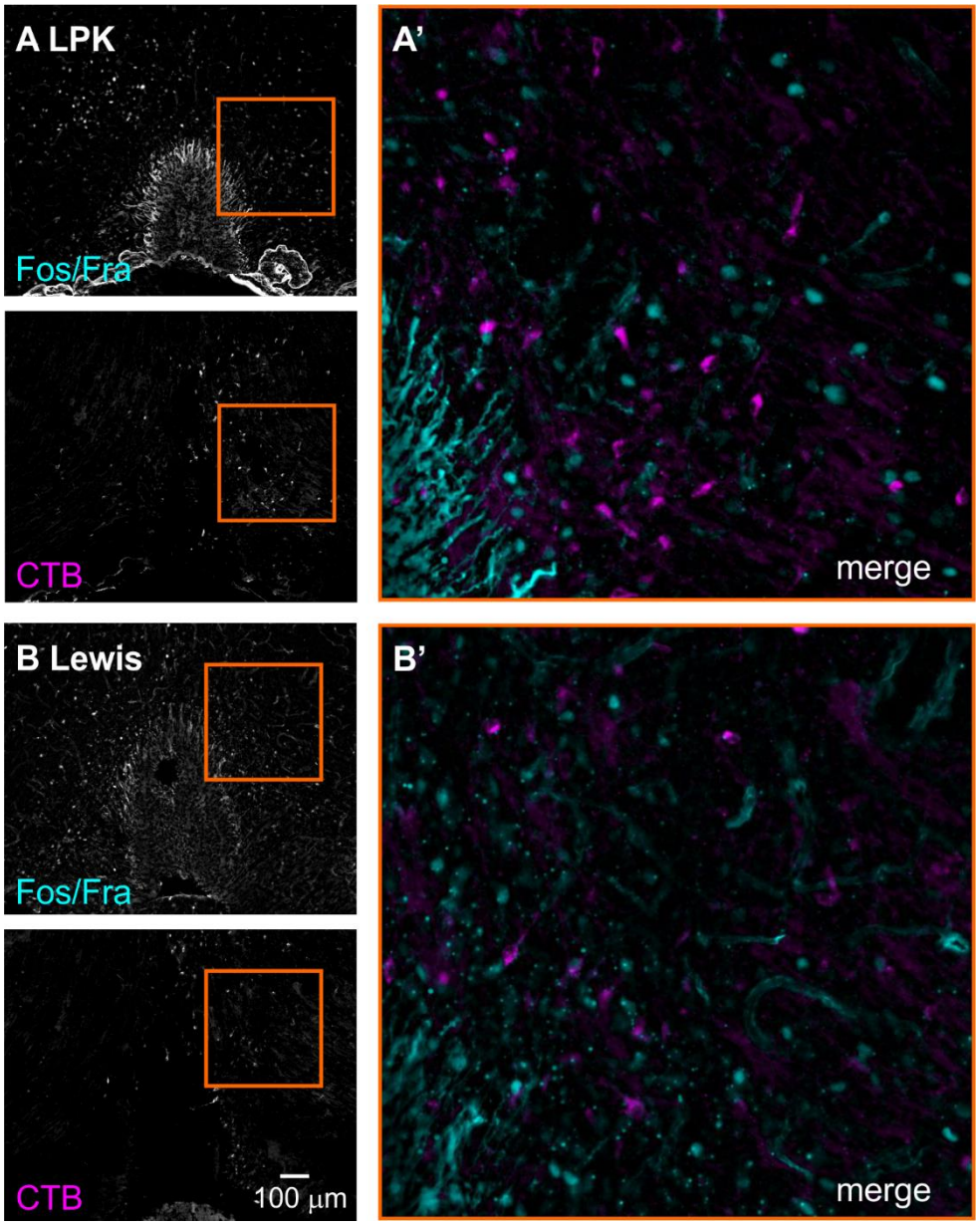

Figure 4.5. Representative images of the OVLT showing the distribution of CTB+ neurons and Fra+ nuclei in a LPK (**A**) and Lewis rat (**B**). The merged image is shown in **A'** and **B'**. Fos/Fra+ nuclei have a punctate appearance and are pseudocoloured cyan, while CTB is pseudocoloured magenta. Arrows indicate CTB neuron with Fos/Fra+ nucleus. Striated structures on the Fos/Fra channel are blood vessels.

Table 4.1: Average counts of PVN-projecting and/or chronically activated lamina terminalis neurons.

Average count per section		**Strain**		***P* value**
		Lewis (4)	**LPK (4)**	
SFO	CTB+	38 (22-71)	34 (16-41)	0.73
	Fos/Fra+	6 (0-17)	61 (16-106)	0.03
	CTB+ Fos/Fra+	0 (0-1)	13 (4-23)	0.01
MnPO	CTB+	28 (19-38)	37 (21-54)	0.33
	Fos/Fra+	34 (7-64)	91 (51-124)	0.03
	CTB+ Fos/Fra+	0 (0-0.3)	6 (2-13)	0.04
OVLT	CTB+	14 (10-21)	17 (6-25)	0.58
	Fra+	23 (0-43)	21 (12-26)	0.78
	CTB+ Fos/Fra+	0 (0-0.5)	1 (0-2)	0.09

Values are presented as means (min-max). $P < 0.05$ considered significant. *n* number for each strain indicted in parenthesis. LPK, Lewis Polycystic Kidney rat; SFO, subfornical organ; MnPO, median preoptic nucleus; OVLT, organ of the vascular lamina terminalis.

The activity of the SFO contributes to hypertension in the LPK rat via a glutamatergic synapse in the PVN

Next, we determined the contribution of lamina terminalis neurons to hypertension in the LPK rat. We focused on the SFO rather than MnPO as it had the highest proportion of Fos/Fra+ CTB+ neurons and also lies upstream of the MnPO (Matsuda et al., 2017), and therefore may be activating the PVN both directly and indirectly via a disynaptic relay in the MnPO. We reasoned that if SFO neurons contribute to hypertension in LPK rats via a glutamatergic synapse in the PVN, acute inhibition of SFO neurons with isoguvacine would produce a greater depressor response in LPK than Lewis rats, which do not show significant Fos/Fra expression in SFO, and predicted that this effect would be attenuated by prior blockade of PVN glutamate receptors.

These predictions were upheld: isoguvacine-mediated inhibition of SFO neurons caused a larger fall in systolic blood pressure (SBP) in LPK compared to Lewis rats (-21 ± 4 mmHg, n = 5 vs. -7 ± 2 mmHg, n = 5; $P < 0.01$). Interestingly, in LPK rats these effects were not accompanied by any detectable change in SNA in any of the beds measured (Figures 4.6; splanchnic SNA: -3 ± 2 % change, $P = 0.22$, n = 4; lumbar SNA: 5 ± 3 % change, $P = 0.11$, n = 5), whereas in the Lewis acute inhibition of the SFO resulted in a small but reproducible reduction in renal SNA only (Figure 4.6; splanchnic SNA: -4 ± 2 % change, $P = 0.09$, n = 5; lumbar SNA: 1 ± 7 % change, $P = 0.82$, n = 4). No changes in HR were seen following SFO inhibition in either strain (data not shown).

Prior blockade of glutamate receptors in the PVN by microinjection of kynurenic acid significantly reduced depressor responses evoked by SFO inhibition in LPK (adjusted $P < 0.01$) but not Lewis rats (adjusted $P > 0.99$; Figure 4.6), such that the magnitudes of SFO isoguvacine-mediated depressor effects were now comparable between strains ($t_8 = 0.67$, $P = 0.52$). Collectively these data demonstrate that the SFO of LPK rats is overactive and elevates blood pressure through glutamatergic input to the PVN, but this effect is not mediated via activation of sympathetic outputs.

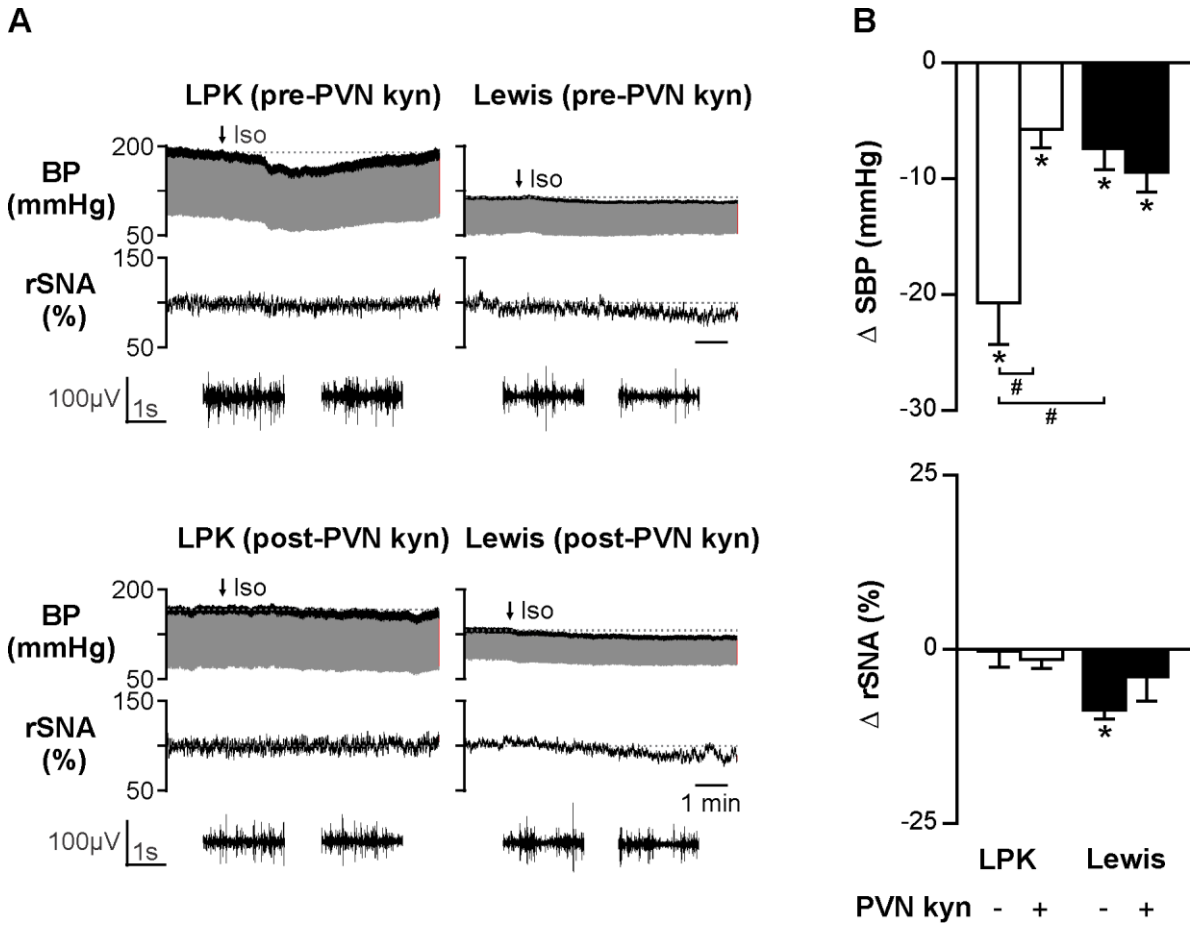

Figure 4.6. A: Representative recordings in LPK and Lewis rats showing the effect of SFO microinjection of isoguvacine (Iso; arrow) on blood pressure (BP; systolic shown in black) and renal SNA (rSNA) before and after bilateral PVN microinjection of kynurenic acid (kyn). Broken line indicates baseline value. A 1 second segment of raw renal SNA signal at baseline (left) and at the peak response (right) is shown underneath the integrated and normalised trace. **B**: Grouped data shown as change from baseline value before microinjection of isoguvacine. n = 5 per strain for SBP; n = 5 LPK and n = 4 Lewis for renal SNA. *, $P < 0.05$ significant change from basal value; #, $P < 0.05$ difference between groups indicated.

To determine whether the SFO is the primary source of increased glutamatergic drive to the PVN in LPK rats we compared depressor responses evoked by PVN microinjection of kynurenic acid in intact Lewis or LPK rats and LPK rats in which the SFO was previously silenced by microinjection of the long-acting $GABA_A$ receptor agonist muscimol (Figure 4.7). In intact LPK rats, PVN microinjection of kynurenic acid produced a marked reduction in SBP ($F_{2,9}$ = 2.30, P = 0.0001), an effect not observed in Lewis rats ($F_{2,9}$ = 2.57, P = 0.13). Prior inhibition of the SFO with muscimol reduced SBP to a similar degree as SFO isoguvacine (as described above: -24 ± 2 vs. -21 ± 4 mmHg; P = 0.52) but prevented depressor responses to subsequent PVN glutamate blockade ($F_{1,7}$ = 1.51, P = 0.27; Figure

4.7). This shows that the ongoing activity of SFO neurons is indispensable for the heightened glutamatergic drive to the PVN that contributes to hypertension in LPK rats.

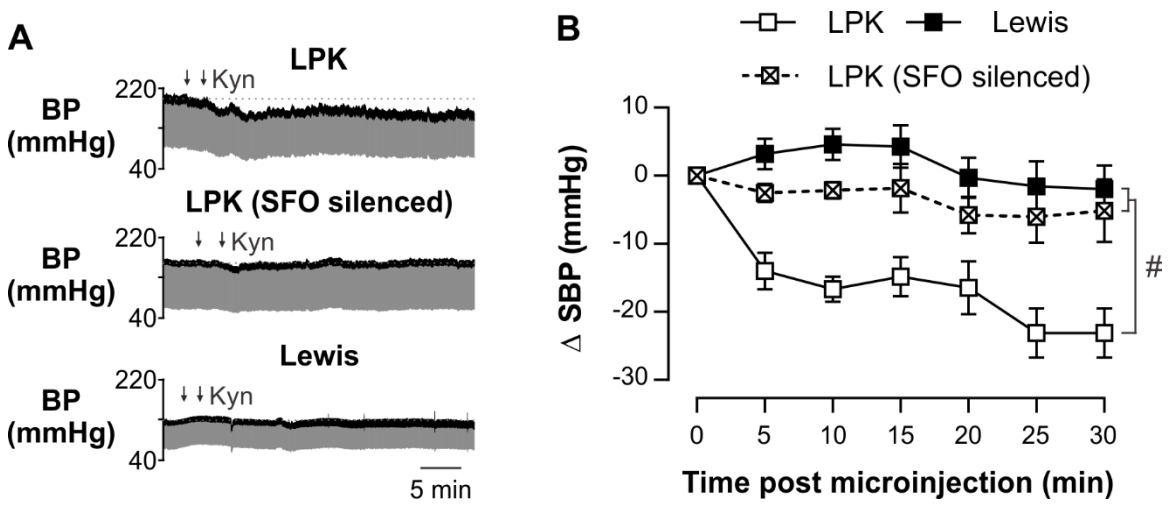

Figure 4.7. A: Representative recordings showing the effect of bilateral PVN microinjection of kynurenic acid (arrows) on blood pressure (BP; systolic shown in black) in Lewis, LPK and LPK rats in which the SFO was silenced with a microinjection of muscimol. Broken line indicates baseline value. **B:** Grouped data shown as change from baseline value before microinjection of kynurenic acid. #, $P < 0.05$ difference between groups indicated. $n = 5$ Lewis, $n = 8$ LPK and $n = 6$ LPK with SFO silenced.

Chronic AT1R antagonism and HWI do not reduce PVN glutamatergic tone in LPK rats

The SFO detects both plasma angiotensin II and osmolality levels. If the SFO-PVN pathway is driven by plasma angiotensin II or hyperosmolality in LPK rats, we predicted that long-term pharmacological inhibition of AT1R, the predominate angiotensin receptor in the SFO (Krause et al., 2008), or HWI, to suppress plasma hyperosmolality, would perturb the development of hypertension and reduce glutamatergic tone in the PVN.

Urine osmolality progressively decreased with age in LPK rats ($F_{8,23} = 49.57$, $P < 0.0001$) with a concurrent increase in water intake ($F_{8,24} = 10.73$, $P < 0.0001$) and urine output ($F_{8,24} = 21.24$, $P < 0.0001$; Figure 4.8), all of which were abnormal relative to the Lewis rats at the end of the treatment period (Lewis urine osmolality, 1913 ± 251 mmol/kg; water intake, 22 ± 1 ml/day; urine output, 13 ± 2 ml/day; all $P < 0.01$). Further, plasma osmolality was greater in untreated LPK versus Lewis rats, including after subtraction of urea levels (effective plasma osmolality; Figure 4.8D). Though largely limited to the beginning of the treatment period, HWI-treated LPK rats showed a greater increase in water consumption (at 7, 8 and 13

weeks; adjusted $P < 0.05$) and urine output (7 and 8 weeks; adjusted $P \leq 0.05$) and suppression of urine osmolality (6 to 9 weeks; adjusted $P < 0.05$) than untreated LPK rats. Importantly, HWI normalised the high plasma osmolality in LPK rats (Figure 4.8D). Losartan treatment did not influence effective plasma osmolality in LPK rats (315 ± 2 vs 330 ± 9 mmol/kg, losartan vs untreated LPK rats; $P = 0.56$).

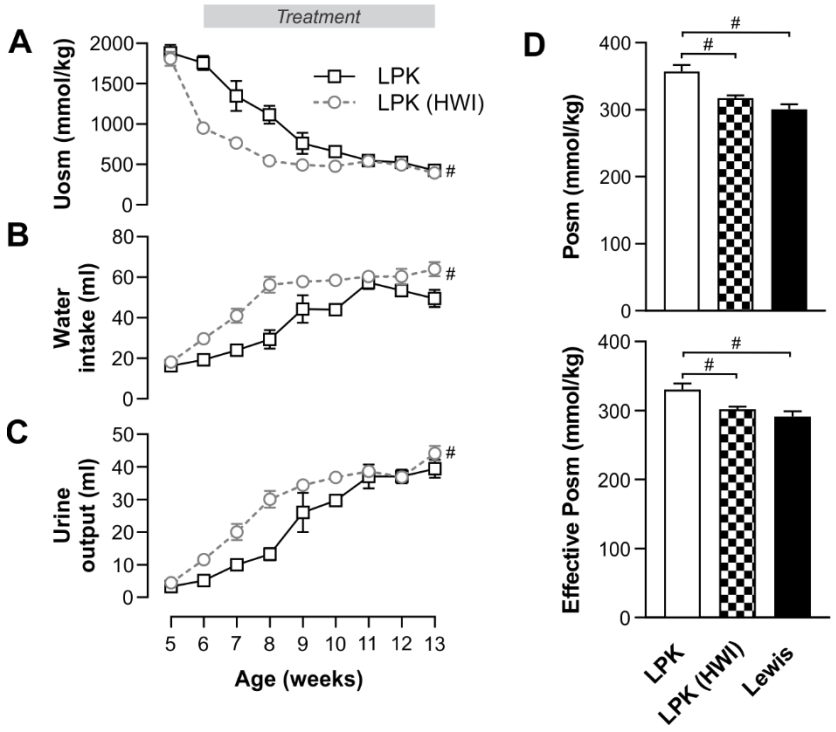

Figure 4.8. The effect of high-water intake (HWI) on 24-hour urine osmolality (**A**; Uosm), water intake (**B**), urine output (**C**) and terminal plasma osmolality (**D;** Posm, unadjusted [top] and adjusted for urea level [bottom; effective osmolality]) in LPK. Treatment (grey bar) was initiated at 6 weeks. $n = 3$ LPK and $n = 6$ high-water intake treated LPK for water consumption and urine data. $n = 8$ LPK, $n = 6$ high-water intake treated LPK and $n = 5$ Lewis for plasma osmolality. #, $P < 0.05$ difference between groups indicated.

In telemetered LPK rats blood pressure increased with age ($P < 0.001$, Figure 4.9) and was higher than Lewis rats throughout the treatment period (adjusted $P < 0.0001$; data not shown). Compared to untreated LPK rats, blood pressure was lower in LPK groups treated with losartan (by 18 ± 3 mmHg; $P < 0.0001$) or HWI (by 14 ± 3 mmHg; $P < 0.0001$). The levels of blood pressure in losartan- and HWI-treated LPK rats were comparable (adjusted $P = 0.55$).

Following treatment, the degree of glutamatergic tone within the PVN was examined under anaesthesia. Under baseline conditions, SBP was lower in both losartan- and HWI-treated rats compared with untreated LPK rats but remained elevated compared to Lewis rats (Figure

4.10). PVN microinjection of kynurenic acid lowered SBP further in LPK rats (adjusted $P < 0.01$), such that, relative to Lewis rats, the SBP of losartan-treated LPK rats was normalised (mean difference: 5 ± 9 mmHg; adjusted P > 0.99) while in the HWI-treated LPK remained elevated, albeit not significantly (mean difference: 24 ± 9 mmHg; adjusted P = 0.08, Figure 4.10). However, the magnitude of depressor responses evoked by PVN kynurenic acid microinjection were similar between LPK groups when the data were expressed as absolute (adjusted $P > 0.99$) or percentage change (adjusted $P = 0.89$). These data indicate that neither chronic AT1R antagonism nor suppression of plasma osmolality alter PVN glutamatergic tone in LPK rats.

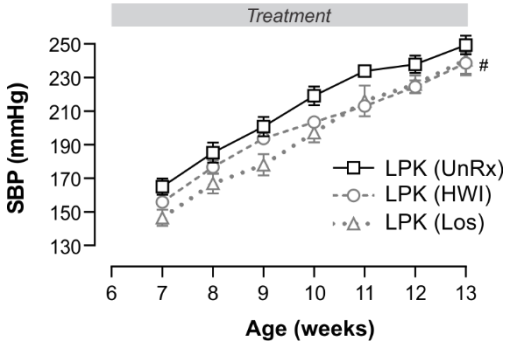

Figure 4.9. Weekly radiotelemetry recordings of systolic blood pressure (SBP) in untreated (UnRx) LPK, losartan (Los)-treated LPK and high-water intake (HWI)-treated LPK rats. Treatment (grey bar) was initiated at 6 weeks at the time of radiotelemetry probe implantation. $n \geq 7$ untreated LPK, $n = 5$ losartan-treated LPK and $n \geq 5$ high-water intake-treated LPK rats. #, $P < 0.0001$ main difference versus untreated LPK for both treatment groups.

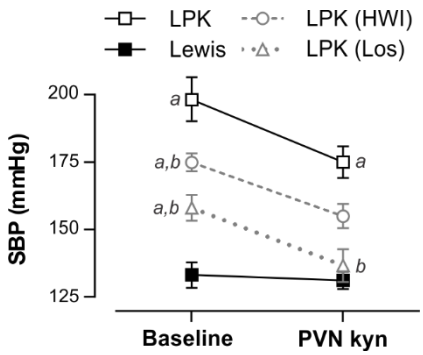

Figure 4.10. Systolic blood pressure (SBP) (under anesthesia) before and 30 min after PVN microinjection of kynurenic acid (kyn) in Lewis, untreated LPK, losartan (Los)-treated LPK and high-water intake (HWI)-treated LPK rats. $n = 5$ Lewis $n = 8$ untreated LPK, $n = 6$ losartan-treated LPK and $n = 5$ high-water intake-treated LPK rats. *a*, $P < 0.05$ versus Lewis. *b*, $P < 0.05$ versus untreated LPK.

Discussion

Effective control of hypertension is a major therapeutic goal for reduction of cardiovascular disease and kidney function decline in patients with PKD. Previously we have shown that glutamatergic inputs to the PVN are overactive in the control of blood pressure in the LPK model of PKD (Chapter 2) (Underwood et al., 2018). The current study extends this finding by showing that: (1) SFO neurons are chronically activated in LPK rats and contribute to hypertension via activation of ionotropic glutamate receptors in the PVN; (2) HWI attenuates the development of hypertension in LPK rats, a non-pharmacological intervention that was as effective as the front-line AT1R blocker losartan; (3) HWI and AT1R blockade do not affect PVN glutamatergic tone, meaning that novel therapies that disrupt PVN neurotransmission may provide additive benefits that summate with conventional treatments.

To assess the activation state of PVN-projecting neurons, we quantified the expression of Fos/Fra immediate-early genes, the latter of which has an expression profile that persists for days after stimulus presentation and therefore provides a snapshot of neural activity over a long timeframe (Miyata et al., 2001). Fos/Fra expression was present in ~ 40% of PVN-projecting SFO neurons in LPK rats but virtually absent in Lewis control rats, suggesting that these neurons display a greater level of activity in PKD. Our functional data indicate that this activity maintains hypertension in PKD rats, as SFO-targeted loss-of-function reduced SBP more in LPK compared to Lewis rats. Previous work has shown that chronic SFO lesions perturb the development of angiotensin II-induced hypertension (Hendel and Collister, 2005; Osborn et al., 2012). Our novel data therefore support the notion that SFO activation may be a feature common to different forms of hypertension and by extension, that therapeutic suppression of SFO neuronal activity may prove beneficial in the management of hypertension.

Both parvocellular and magnocellular divisions of the PVN receive dense excitatory glutamatergic (but not inhibitory GABAergic) projections from the SFO (Kawano and Masuko, 2010; Matsuda et al., 2017), and SFO activation evokes excitatory effects in the PVN, including increased action potential frequency in putative pre-autonomic and neuroendocrine PVN neurons (Ferguson et al., 1984; Bains and Ferguson, 1995), as well as elevations in blood pressure, SNA and neurohormone release (Ferguson and Kasting, 1986; Llewellyn et al., 2012; Leib et al., 2017). Our data suggest that glutamatergic inputs to the PVN are indispensable for the transmission of pro-hypertensive drive from the SFO, as blockade of PVN glutamate transmission ameliorated the exaggerated depressor effects

evoked by acute SFO inhibition in LPK rats. This finding is consistent with the work of Llewellyn et al. (2012) who showed that the pressor response to disinhibition of the SFO in normotensive rats was reduced following blockade of NMDA receptors in the PVN.

The pathological over-activation of PVN glutamate receptors in LPK rats appears to be exclusively driven by the SFO because sustained inactivation of the SFO with muscimol normalised subsequent depressor responses to PVN glutamate receptor blockade. Although we did not directly examine whether, in Lewis rats, SFO neurons contribute to an ongoing level of glutamatergic input to the PVN, it is pertinent to note that PVN glutamate receptor blockade did not affect resting blood pressure in this strain, suggesting that in these animals glutamatergic drive of PVN arising from the SFO is likely minimal or directed to non-cardiovascular outputs. Our anatomical data support the assertion that, in LPK rats, SFO-dependent glutamatergic drive to the PVN may be mediated through a direct monosynaptic projection; however, we cannot rule out the possibility that a polysynaptic pathway is involved. One such indirect pathway could involve a relay in the MnPO, an integration hub of the lamina terminalis that receives dense projections from the SFO (Matsuda et al., 2017), including axon collaterals from neurons that also project to the PVN (Duan et al., 2008), as we also found the MnPO to contain activated PVN-projecting neurons.

In PKD patients, there is causal evidence that AT1R activation contributes to hypertension (Schrier et al., 2014) and associative evidence that hypertension may also be linked with body-fluid disturbances, specifically impaired urinary concentrating ability (Seeman et al., 2004), a characteristic that predisposes patients to elevations in plasma osmolality (Zittema et al., 2012). We therefore investigated whether chronic AT1R suppression or reduced plasma osmolality could attenuate the development of hypertension. Consistent with previous studies that have assessed the development of hypertension in LPK rats treated with either an AT1R antagonist (Quek et al., 2018) or HWI (Sagar et al., 2019) using tail-cuff plethysmography, we found that both treatments lowered 24-hour ambulatory blood pressure in LPK rats as measured with the more reliable technique of radiotelemetry (Kurtz et al., 2005). Surprisingly, we found that the antihypertensive benefit of HWI is comparable to losartan, the most commonly prescribed angiotensin receptor blocker in the USA (Miller and Zodet). These data therefore indicate that HWI may provide some benefit for blood pressure management in PKD, although it remains to be seen how HWI interacts with the antihypertensive effects of other treatments in this heavily medicated cohort. With clinical trials currently underway that will assess HWI in PKD and measure blood pressure as an

outcome (El-Damanawi et al., 2018; Wong et al., 2018), our measurements of 24-hour ambulatory blood pressure substantiate the preclinical evidence that HWI may serve as an effective antihypertensive therapy in the PKD patient population. Considering the potential clinical use of HWI in PKD, it is worthy to note that we and others (Sagar et al., 2019) observed that effective reductions in blood pressure and plasma osmolality were achieved with relatively small (30%) and transient increases in water consumption, most likely indicating that urinary concentrating capacity is improved with HWI due to a modification of the renal cyst architecture (Sagar et al., 2019). However, since daily water consumption was monitored in a relatively small sample (n = 3) of untreated LPK rats in this study, additional experiments will be required to further clarify the impact of 5% glucose on water consumption in LPK rats.

A key finding of the current study is that the contribution of PVN glutamatergic tone to hypertension in LPK rats is not reduced by losartan or HWI. This indicates firstly that plasma angiotensin II and hyperosmolality alone are not responsible for producing the pro-hypertensive increase in PVN neuronal activity via interactions with the SFO or other mechanism (e.g., direct activation of AT1R in the PVN). However, we have not yet examined the effect of combined HWI and losartan treatment on PVN glutamatergic activity; consequently, we cannot rule out the possibility that isolated suppression of plasma osmolality or angiotensin II enhances the contribution of the other factor. Nevertheless, our data currently support the assertion that suppressing PVN glutamatergic drive may offer an independent therapeutic target to reduce arterial pressure in PKD. New therapies that target this neuronal pathway are therefore likely to supplement the beneficial effects offered by existent conventional (e.g. losartan) or non-pharmacological anti-hypertensives (e.g. HWI). The SFO lacks a complete blood-brain barrier and is therefore anatomically primed to respond to various blood-borne stimuli, including, but not limited to, angiotensin-II and plasma osmolality (Coble et al., 2015). This feature makes it attractive from a therapeutic standpoint, as it makes the SFO accessible to systemically administered drugs or interventions that could be used to suppress overactive circuits downstream of the SFO, including the PVN. Moreover, it is possible that the drivers of SFO-PVN glutamatergic transmission, be they pro-inflammatory cytokines (Wei et al., 2013) or local nitric oxide (Zhang et al., 2019), for example, may be common to other forms of renal disease (Chen et al., 2019), and may potentially underlie the treatment resistant hypertension commonly observed in these patients (de Beus et al., 2015).

In summary, our study has uncovered several novel mechanisms regarding the origins of hypertension in PKD. We demonstrate that activation of the SFO elevates blood pressure in LPK rats via increased glutamatergic activation of the PVN. Therapeutic suppression of this pathway may represent a novel approach to manage hypertension in PKD, particularly when combined with renin-angiotensin system inhibitors. Our finding that HWI attenuates hypertension development in LPK animals adds to a growing body of work supporting beneficial effects of HWI in PKD.

5.

High Water Intake Provides Renal and Cardiac Autonomic Benefits in a Rat Model of Polycystic Kidney Disease

Underwood C.F., Phillips J.K. & Hildreth C.M.

Abstract

High-water intake (HWI) improves renal function, hypertension and cardiac hypertrophy in polycystic kidney disease (PKD) rodents. We examined whether HWI improves baroreflex dysfunction and indices of left-ventricular mass and contractility in the Lewis Polycystic Kidney (LPK) rats. Three experimental groups were examined: HWI (5% glucose in the drinking-water)-treated LPK, untreated LPK and untreated Lewis. Using radiotelemetry, we found that cardiac baroreflex sensitivity deteriorated from 7-10 to 11-13 weeks of age in untreated LPK rats (2.7±0.1 vs. 2.3±0.1 msec/bpm; P=0.02, n=8), which was prevented by HWI (2.8±0.1 vs. 2.8±0.05 msec/bpm; P=0.72, n=5). Assessment of baroreflex function using drug-induced changes in arterial pressure under anaesthesia revealed that HWI markedly increased the range of heart rate baroreflex compared to untreated LPK rats (125±6 vs.76±12 bpm; P=0.02, n>7 per group), such that it was normalised relative to Lewis rats (113±10 bpm; P=0.47, n=7), but did not affect the gain or the range or gain of the renal sympathetic baroreflex (all P>0.05). Left-ventricular contractility was indirectly assessed in radiotelemetered animals by measuring maximal rate of rise of arterial pressure (dP/dT_{max}). The dP/dT_{max} was 75% higher in untreated LPK compared to Lewis (P<0.0001) but was reduced by 15% in HWI-LPK (P<0.001). Finally, compared to untreated LPK animals, HWI-LPK had lower left-ventricular (0.36±0.01 vs. 0.29±0.01 % bodyweight; P<0.001) and kidney mass (7.7±0.3 vs. 5.0±0.4 % bodyweight; P<0.0001) indexes, urinary protein excretion and plasma urea (both P<0.05). Across the LPK cohort, left ventricle mass was independently associated with blood pressure (R=0.62, P=0.04). Our data suggest that HWI not only provides reno-protection in PKD, but also improves cardiac baroreflex dysfunction, left-ventricular contractility and mass.

Introduction

Cardiovascular disease is the leading source of mortality in patients with chronic kidney disease (USRDS, 2016), including polycystic kidney disease (PKD) (Fick et al., 1995; Rahman et al., 2009). This high cardiovascular disease risk is partly attributable to the hypertension that develops in most individuals (Ecder and Schrier, 2001; Chapman et al., 2010), but is also likely due to cardiac autonomic dysfunction, particularly impaired baroreflex function (Johansson et al., 2005; Lal et al., 2017) as it has been shown to independently predict mortality in longitudinal cohorts (Johansson et al., 2007; John et al., 2008). Treatment options that protect against baroreflex dysfunction are currently lacking.

High-water intake (HWI) has been demonstrated to slow cyst growth in rodent models of PKD (Nagao et al., 2006; Hopp et al., 2015; Sagar et al., 2019), and is currently being trialled in patients (El-Damanawi et al., 2018; Wong et al., 2018). The premise of this treatment approach is that it suppresses the release of vasopressin, a hormone that dose-dependently accelerates cystogenesis (Wang et al., 2008; Torres et al., 2012; Boertien et al., 2013; van Gastel and Torres, 2017) and tends to be higher in patients (Danielsen et al., 1986b; Danielsen et al., 1986a; Michalski and Grzeszczak, 1996; Zittema et al., 2014), most likely due to elevations in plasma osmolality as a consequence of an inability to adequately concentrate urine (van Gastel and Torres, 2017). Importantly, the counter-regulatory response to an elevation in extracellular fluid osmolality involves not only an increase in vasopressin release, but also a change in autonomic nervous system activity and increases in arterial pressure (Brooks et al., 2005; Toney and Stocker, 2010). Furthermore, chronic experimental increases in extracellular fluid osmolality produced by high dietary-salt consumption (Miyajima and Bunag, 1985) or central hypertonic saline administration (Bunag and Miyajima, 1984) impairs baroreflex regulation of heart rate (HR) in rodents. These findings raise the possibility that chronic hyperosmotic stress may participate in some of the cardiovascular alterations observed in PKD.

The Lewis Polycystic Kidney (LPK) rat is an established model of PKD that arises from a non-orthologous mutation in *Nek8* (McCooke et al., 2012). These animals develop corticomedullary cysts from at least 3 weeks of age, early hypertension and autonomic dysfunction as renal function deteriorates (Phillips et al., 2007; Schwensen et al., 2011; Hildreth et al., 2013b; Salman et al., 2014). Recently HWI has been shown to not only attenuate renal progression in LPK rats, but also lower blood pressure and improve cardiac hypertrophy (Sagar et al., 2019), indicating that this novel therapy may also be cardio-

protective. The purpose of this study was to examine whether HWI improves baroreflex function and indices of left-ventricular mass and contractility in the LPK model. To that end, we used radiotelemetry to non-invasively record arterial pressure in HWI-treated LPK rats and untreated LPK and Lewis control rats from which measures of cardiac baroreflex sensitivity and left-ventricular contractility were derived. At the end of the treatment period, cardiac and sympathetic baroreflex function was assessed by analysing drug-induced changes in arterial pressure in anaesthetised animals.

Methods

Animals

Male LPK and Lewis control rats were acquired from the Animal Resource Centre, Murdoch, Western Australia, Australia, and housed in standard living conditions with a 12-hour light/dark cycle and access to chow and water ad libitum. Experiments were performed in accordance with The Australian Code of Practice for the Care and Use of Animals (8th Edition, 2013) and approved by the Macquarie University Animal Ethics Committee.

Radiotelemetry probe implantation, data collection and analysis

The animals reported in this Chapter are the same the cohort initially reported in Chapter 4. During each week of the treatment period, data were sampled continuously for 5 minutes every 15 minutes for a 24-hour period. For untreated LPK, the 24-hour recording period is the same data reported in Chapter 5. From these 5-minute periods, an 80 second segment was selected which contained a stable pulse interval and no ectopic beats. From the arterial pressure waveform, systolic blood pressure (SBP) and diastolic blood pressure (DBP) and HR were derived using Spike2 software (Cambridge Electronic Designs Ltd., Cambridge, UK). These 80 second segments were also used to compute spontaneous cardiac barosensitivity (BRS) and arterial dP/dT_{max}, a measure of left-ventricle contractility (Monge Garcia et al., 2018). Cardiac BRS was computed using the sequence method by a custom built script in Spike 2 (Hildreth et al., 2013a; Hildreth et al., 2013b). This script searches the arterial pressure waveform for three successive changes in blood pressure (±0.5 mmHg) that occur simultaneously with opposing changes in pulse interval (i.e., without a beat delay), and then calculates the linear regression of all sequences. We did not use the resulting regression coefficient to eliminate sequences. Arterial dP/dT_{max} was derived by measuring the maximal rate of rise of each arterial pulse within the data segment and computing the average. As we have previously demonstrated that cardiac baroreflex function rapidly deteriorates only after 10 weeks of age in LPK (Hildreth et al., 2013b; Salman et al., 2014), radiotelemetry data acquired during each 24-hour recording period between 7 to 10 weeks of age (young age group) and separately 11 to 13 weeks of age (adult age group) were averaged to obtain a single value for each animal per age group.

Treatment protocol

As described in Chapter 4, three experimental groups were examined: HWI-treated LPK rats (5% glucose in the drinking water (Hopp et al., 2015); $n = 6$), untreated LPK rats ($n = 8$) and untreated Lewis rats ($n = 8$). No treatment group was included for Lewis animals as it has

been shown previously that HWI-treatment with 5% glucose produces no noticeable renal or cardiovascular effect in this strain (Sagar et al., 2019). Rats were treated from 6 to 13-14 weeks of age.

Generation of baroreflex function curves

At the end of the treatment period, animals were anaesthetised with urethane (1.3 g/kg i.p. for induction and 65-130 mg/kg i.p. or i.v. for maintenance as required; Sigma Aldrich, Australia) and prepared for measurement of arterial pressure, HR and renal sympathetic nerve activity (SNA) as described in Chapter 2. Body temperature (37±0.5 ºC) and blood gases (arterial pH at 7.4±0.5 and pCO_2 40±5 mmHg) were maintained within a normal range throughout the experiment with external heating sources and mechanical ventilation, respectively. Cisatracurium was used to induce (6 mg/kg i.v.) and maintain (6 mg/kg/hr i.v.) muscle paralysis.

Baroreflex function curves were generated with sequential sodium nitroprusside (50 µg/ml in 0.9% saline; 5-20 µg/min i.v.) and phenylephrine (50 µg/ml in 0.9% saline; 5-20 µg/min i.v.) infusions (i.e., the Oxford method) and analysed as described in Chapter 2. Animals were euthanized at the completion of the experiment with potassium chloride (3 M, 1 ml i.v.). All curves used in the final analysis had an R^2 value greater than 0.9.

An additional cohort of untreated LPK (n = 1) and Lewis (n = 4) animals underwent the terminal baroreflex testing described above but were not implanted with radiotelemetry probes.

Assessment of renal function and tissue weights

Animals were placed in a metabolic cage for 24-hours to collect urine. Arterial blood (~0.3 ml) was collected into heparinised tubes under urethane anaesthesia and centrifuged to separate serum. Urine and serum samples were stored at -20 °C until analysis. Measurement of urinary protein and blood urea nitrogen (BUN) was performed using a VetTest chemistry analyser (IDEXX laboratories Pty Ltd., New South Wales, Australia).

Both kidneys and the left cardiac ventricle were removed following euthanasia and weighed. Tissue weights were then expressed as percentage of bodyweight to derive kidney mass index and left-ventricle mass index (LVI) (Phillips et al., 2007; Sagar et al., 2019). For both LPK groups, the relationship between LVI and adult (11 to 13 weeks of age) values of blood pressure, arterial dP/dT_{max} and cardiac BRS was determined with Pearson's correlation.

Statistical analysis

All data are expressed as mean ± SEM. Pearson's bivariate and partial (i.e., covariate adjusted) correlations were performed with SPSS Statistics v22 (IBM, New York, USA). All other statistical analysis was performed with GraphPad Prism software v8 (GraphPad Prism software Inc., La Jolla, CA, USA). Radiotelemetry data were analysed with a repeated measures mixed-effects model. A one-way ANOVA was used for all other between-group comparisons. The Holme-Sidak's correction was used for all multiple comparisons. Statistical significance was set at $P \leq 0.05$.

Results

HWI reduces blood pressure in LPK rats

The blood pressure and HR of HWI-treated LPK rats and untreated LPK and Lewis rats at the young and adult age groupings are shown in Table 5.1. The blood pressure of both LPK groups increased with age (adjusted $P < 0.0001$) and was significantly higher than Lewis rats throughout the treatment period. HWI-treated LPK rats exhibited a moderately (by 14±6 mmHg SBP; adjusted $P = 0.03$) lower level of blood pressure than untreated LPK rats throughout the treatment period.

Table 5.1: Blood pressure and heart rate of LPK treated with high water intake (HWI) and untreated (UnRx) LPK and Lewis rats at young (7 to 10 weeks) and adult (11 to 13 weeks) ages.

Parameter	Young			Adult			P value (age)	P value (group)
	LPK (UnRx)	LPK (HWI)	Lewis (UnRx)	LPK (UnRx)	LPK (HWI)	Lewis (UnRx)		
n	8	6	8	8	5	7		
SBP (mmHg)	189±6 #	178±4 #,†	124±2	236±4 #	219±5 #,†	130±2	<0.0001	<0.0001
DBP (mmHg)	124±4 #	115±3 #,†	85±1	157±3 #	146±3 #,†	90±1	<0.0001	<0.0001
HR (bpm)	382±5 #	409±4 #,†	394±3	359±3	360±5	350±4	<0.0001	0.005

Values are expressed as mean ± SEM. *n* indicates number of animals per group at each age. # $P \leq 0.05$ vs. Lewis; † $P \leq 0.05$ vs. LPK untreated. HR, heart rate; bpm, beats per minute.

HWI improves baroreflex control of HR but not renal SNA in LPK rats

Cardiac baroreflex function was assessed by analysing the relationship between HR and spontaneous changes in SBP in conscious animals with radiotelemetry. As illustrated in Figure 5.1, while cardiac BRS was similar in all groups at the young age, it declined with age in untreated LPK rats (adjusted $P = 0.02$) and increased in Lewis rats (adjusted $P = 0.01$), such that the cardiac BRS of adult untreated LPK rats was 30% lower than age-matched Lewis rats (adjusted $P < 0.01$). In contrast, HWI-treatment prevented the age-related decline in cardiac BRS in LPK rats (adjusted $P = 0.72$; young vs. adult), such that the cardiac BRS of adult HWI-treated LPK rats was higher than adult untreated LPK rats (adjusted $P = 0.02$) and now only 15% lower than adult Lewis rats (adjusted $P = 0.02$).

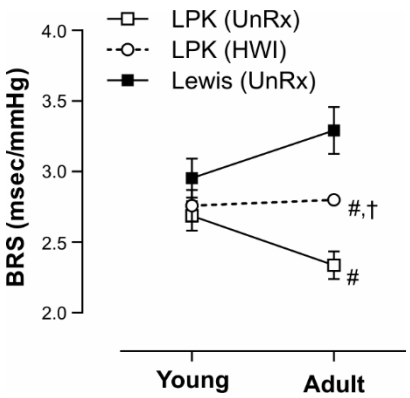

Figure 5.1: Spontaneous cardiac baroreflex sensitivity (BRS) in HWI-treated LPK rats and untreated (UnRx) LPK and Lewis rats at young (7-10 weeks) and adult (11-13 weeks) ages. # indicates $P \leq 0.05$ vs. Lewis unRx; † indicates $P \leq 0.05$ vs. LPK UnRx. Sample sizes are given in Table 5.1.

In addition to assessment of spontaneous cardiac BRS, logistic HR and sympathetic baroreflex function curves were generated at the end of treatment in anaesthetised animals by manipulating arterial pressure over a wide range with vasoactive drugs (Figure 5.2A). The HR and sympathetic baroreflexes operated around a higher arterial pressure set-point (SBP_{50}) in untreated LPK compared to Lewis rats, and both the range and gain were reduced (Figure 5.2B, Table 5.2), consistent with our previous publications (Salman et al., 2014; Salman et al., 2015a; Yao et al., 2015). In LPK animals, HWI-treatment lowered the SBP_{50} of the HR baroreflex, consistent with the lower SBP exhibited in conscious rats (Table 5.1) and under anaesthesia (157±14 vs. 190±7 mmHg, HWI vs. untreated; $P = 0.04$). Importantly, HWI-treatment increased the range and decreased the activation threshold (SBP_{thr}) of the HR baroreflex in LPK rats, such that both were normalised relative to Lewis rats (adjusted $P = 0.47$; Figure 5.2B, Table 2). No such treatment effect was noted for the HR gain (adjusted $P = 0.47$) or for the range (adjusted $P = 0.30$) or gain (adjusted $P = 0.42$) of the renal sympathetic baroreflex (Figure 5.2B, Table 5.2). HWI-treatment did not affect the operational (i.e., SBP) range of the HR (adjusted $P = 0.29$) and renal sympathetic (adjusted $P = 0.76$) baroreflexes in LPK animals.

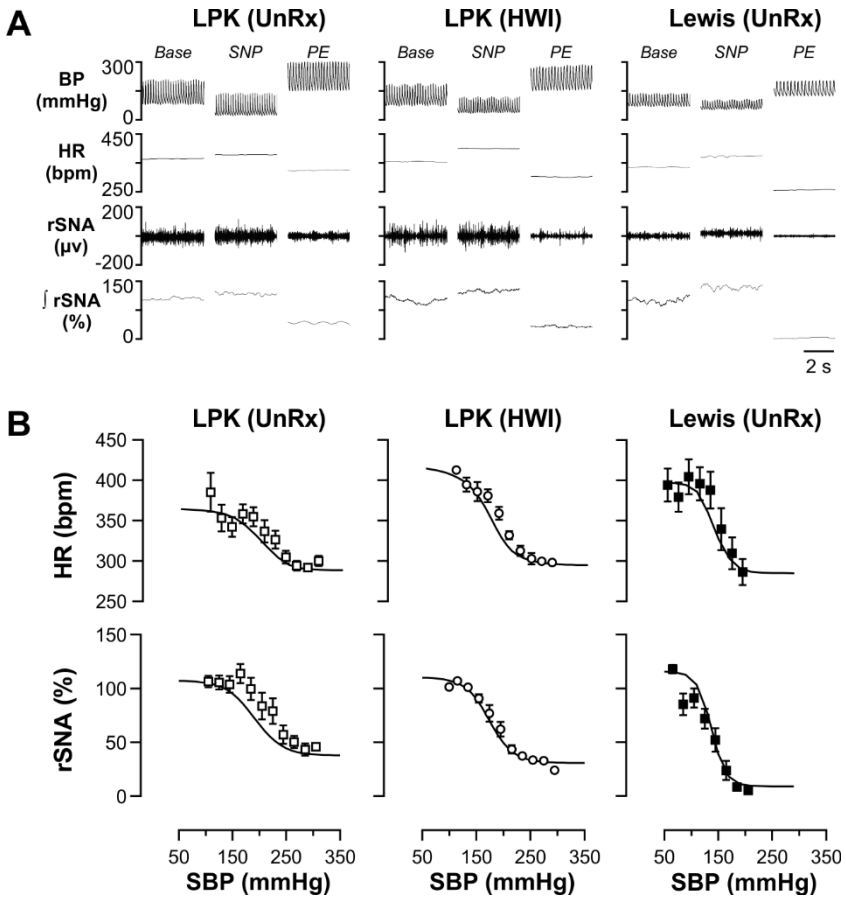

Figure 5.2. Baroreflex control of HR and renal SNA (rSNA) in HWI-treated LPK and untreated (UnRx) LPK and Lewis rats as assessed with the Oxford method. **A**: Representative recordings showing the effect of sodium nitroprusside (SNP) and phenylephrine (PE) infusion on blood pressure (BP), HR and rSNA (raw and normalised to baseline [base]). **B**: grouped logistic baroreflex curves. Sample sizes are given in Table 5.2.

Table 5.2: Baroreflex parameters in LPK rats treated with high water intake (HWI) and untreated (UnRx) LPK and untreated Lewis rats.

Parameter	Group			*P* value
	LPK (UnRx)	LPK (HWI)	Lewis (UnRx)	
HR barocurve (*n*)	8	5	7	
SBP_{50} (mmHg)	194±9 #	162±10 #,†	141±6	0.0009
Range (bpm)	76±12 #	125±6 †	113±10	0.0145
Gain (bpm/mmHg)	-1.1±0.2 #	-1.4±0.3	-2.6±0.3	0.0111
SBP Range (mmHg)	53±9	73±15 #	35±4	0.0434
SBP_{sat} (mmHg)	220±8 #	198±7 #	159±6	<0.0001
SBP_{thr} (mmHg)	167±12 #	125±17 †	123±5	0.0185
Upper plateau (bpm)	365±15	420±3	398±19	0.0771
Lower plateau (bpm)	289±6	295±4	285±16	0.8361
rSNA barocurve (*n*)	8	5	5	
SBP_{50} (mmHg)	193±9 #	174±7 #	137±4	0.0005
Range (%)	70±7 #	80±1 #	107±9	0.0048
Gain (%/mmHg)	-0.8±0.1 #	-1.0±0.1 #	-2.2±0.3	<0.0001
SBP range (mmHg)	58±6 #	55±6 #	34±4	0.0181
SBP_{sat} (mmHg)	222±8 #	201±7 #	154±4	<0.0001
SBP_{thr} (mmHg)	164±10 #	146±8	121±4	0.0111
Upper plateau (%)	108±4	111±2	116±5	0.3050
Lower plateau (%)	38±5 #	31±2 #	9±4	0.0007

Values are expressed as mean ± SEM. (*n)* indicates number of animals per group. # $P \leq 0.05$ vs. Lewis UnRX; † $P \leq 0.05$ vs. LPK UnRx. rSNA, renal SNA; SBP_{50}, SBP at the midpoint of the curve; SBP_{sat}; SBP saturation; SBP_{thr}, SBP threshold.

HWI lowers indices of left-ventricle contractility and mass in LPK rats

As illustrated in Figure 5.3A, the dP/dT_{max} of untreated and HWI-treated LPK rats was significantly greater than Lewis rats at both ages (adjusted $P < 0.0001$). The dP/dT_{max} increased with age in both untreated LPK (adjusted $P < 0.0001$) and HWI-treated (adjusted $P < 0.0001$) rats but decreased with age in Lewis rats (adjusted $P = 0.03$). HWI-treated LPK rats displayed 15% lower dP/dT_{max} than untreated LPK rats at both ages (adjusted $P < 0.001$; Figure 5.3A). Post-mortem assessment revealed that both groups of LPK animals had significantly increased LVI compared to Lewis animals (adjusted $P < 0.001$; Figure 5.3B). HWI-treatment lowered LVI by ~20% in LPK rats (adjusted $P < 0.01$).

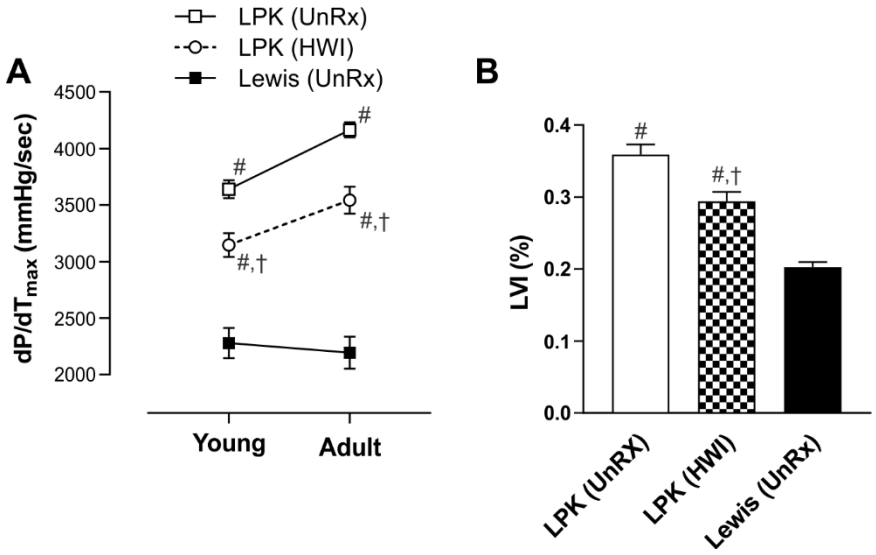

Figure 5.3. A: Radiotelemetry measurements of dP/dT_{max} in HWI-treated LPK rats and untreated (UnRx) LPK and Lewis rats at young (7-10 weeks) and adult (11-13 weeks) ages. Sample sizes for each group at each age are given in Table 5.1. **B:** Post-mortem measurements of left-ventricular mass index (LVI) in adult rats. $n = 6$ HWI-treated LPK, $n = 13$ untreated LPK and $n = 7$ Lewis. # indicates $P \leq 0.05$ vs. Lewis UnRx; † indicates $P \leq 0.05$ vs. LPK UnRx.

LVI is independently related to blood pressure but not cardiac contractility or BRS in LPK rats

Lower levels of LVI in HWI-treated LPK rats could be both causal and consequential of the persistent decrease in left-ventricular contractility or afterload (i.e., DBP) or the improvement in cardiac baroreflex function. We therefore used a Pearson's correlation to determine whether LVI was related to adult levels DBP, dP/dT_{max} or cardiac BRS in LPK rats. In the unadjusted bivariate model both dP/dT_{max} and DBP were positively correlated with LVI (Table 5.3) and with each other (R = 0.61, P = 0.03). BRS was not correlated LVI (Table 5.3) but was negatively correlated with DBP (R = -0.736, P = 0.004) and dP/dT_{max} (R = -0.592, P = 0.002) in bivariate analysis. After including DBP, dP/dT_{max} and BRS as covariates, only the relationship between DBP and LVI persisted (Table 5.3). This analysis suggests that the improvement in LVI in HWI-treated LPK rats may be related to the anti-hypertensive effect of this intervention, but not the effect on dP/dT_{max} and BRS.

Table 5.3: Pearson's correlation of LVI and DBP, dP/dT_{max} or cardiac BRS in LPK rats.

	LVI x DBP		**LVI x dP/dT_{max}**		**LVI x BRS**	
Covariate	R	P value	R	P value	R	P value
None (unadjusted)	0.729	0.005 *	0.561	0.046 *	-0.497	0.084
DBP	-	-	0.212	0.508	0.087	0.788
dP/dT_{max}	0.590	0.043 *	-	-	-0.125	0.699
BRS	0.619	0.032 *	0.323	0.306	-	-
DBP & dP/dT_{max}	-	-	-	-	0.270	0.422
DBP & BRS	-	-	0.328	0.324	-	-
dP/dT_{max} & BRS	0.621	0.041 *	-	-	-	-

* $P \leq 0.05$ significant correlation. n = 13.

HWI improves kidney size and function in LPK rats

Compared to the Lewis rats, untreated adult LPK rats had a higher kidney index, daily urinary protein excretion, BUN and a reduced bodyweight (Table 5.4). In LPK animals, HWI-treatment was associated with a 37% reduction in kidney index, greater bodyweight, lower daily protein excretion and BUN (Table 5.4).

Table 5.4: Kidney weight, body weight and renal function in adult LPK rats treated with high water intake (HWI), untreated (UnRx) LPK and untreated Lewis rats.

	Group			
Parameter	**LPK (UnRx)**	**LPK (HWI)**	**Lewis (UnRx)**	***P* value**
n	9	6	5	
Final BW (g)	287±14 #	364±6 †	370±10	0.0001
Kidney index (%)	7.72±0.34 #	4.98±0.35 #,†	1.00±0.02	<0.0001
Protein excretion (mg/day)	44±6 #	28±3 #,†	2±1	<0.0001
BUN (mmol/l)	28.0±1.9 #	15.6±0.7 #,†	8.8±0.5	<0.0001

Values are presented as mean±SEM. # $P \leq 0.05$ vs. Lewis UnRx; † $P \leq 0.05$ vs. LPK UnRx.
BW; bodyweight; BUN, blood urea nitrogen. *n* indicates minimum sample size per group.

Discussion

PKD is a chronic condition that progressively impairs renal function and markedly elevates cardiovascular disease risk (Fick et al., 1995; Rahman et al., 2009; Hildebrandt, 2010). Currently HWI is being explored as a novel therapeutic approach to slow the progression of PKD (Wong et al., 2018). In this study, we have shown that administration of glucose-sweetened drinking water to promote HWI provides not only renal but also cardiovascular benefits in the LPK rat model of PKD. Specifically, we found that HWI-treatment was associated with lower levels of arterial pressure, reduced left-ventricular contractility and mass index and improved cardiac baroreflex function. Thus, our data adds to a growing body of preclinical work demonstrating beneficial effects of HWI in PKD.

Two methodological approaches were used to assess cardiac baroreflex function in our study: 1) analysis of spontaneous changes in arterial pressure in conscious conditions and 2) analysis of drug-induced changes in arterial pressure (i.e., the Oxford method) in anaesthetised conditions. These methods are complementary; the spontaneous method when used in conjunction with radiotelemetry provides information about the sensitivity/gain of the baroreflex during its normal operation throughout the entire day, whereas the Oxford method can be used to extract additional information about the operation of the baroreflex, but typically only at a single moment in time (Hildreth et al., 2013a). Using the spontaneous method, we observed that HWI prevented the pathological decline in cardiac BRS in LPK rats. This is a salient finding since reduced spontaneous cardiac baroreflex sensitivity predicts mortality risk in renal disease patients (Johansson et al., 2007; John et al., 2008) and is currently without treatment options. Using the Oxford method, we found that HWI increased the HR baroreflex range to normal levels in LPK rats, meaning that more of the HR can be modulated to counteract changes in arterial pressure. However, using the Oxford method we did not similarly detect an improvement in the HR baroreflex gain in HWI-treated LPK rats. What underlies the disparity between the Oxford and spontaneous methods with respect to baroreflex gain/sensitivity is not clear, but could be due to inherent differences between the techniques with respect to the number of replicates (one drug-induced SBP change vs. hundreds of spontaneous changes), unintended effects of the vasoactive drugs (e.g., activation of low-pressure volume receptors) and anaesthesia that impairs cardiac baroreflex sensitivity (Hildreth et al., 2013a).

The ongoing function of the baroreflex depends on the ability of the baroreceptors to transduce arterial pressure, the processing of the baroreceptor afferent signal in the brain and

the transmission of autonomic efferents to the end-organ/s (Salman, 2016). Therefore, the improvement in cardiac baroreflex function in LPK rats could reflect a facilitation of the baroreflex neural arc at any of these different levels. However, our current and previous observations suggests that the improvement in baroreflex function in HWI-treated LPK was most likely achieved through a central mechanism because: first, HWI did not improve baroreflex control of renal SNA, indicating that this treatment did not improve baroreceptor afferent function which is common to both reflex pathways (Pilowsky and Goodchild, 2002); and second, an enhancement of cardiac efferent transmission seems unlikely as LPK rats ordinarily have normal cardiovagal efferent function (Salman et al., 2015a) and showed lower dP/dT_{max} during HWI treatment, consistent with a reduction in cardiac sympathetic tone. Considering this possibility further, previous work has shown that experimental increases in extracellular fluid osmolality impair baroreflex control of heart rate, but not renal SNA, in rats (Bunag and Miyajima, 1984; Miyajima and Bunag, 1985; Bealer, 2000), most likely via a central mechanism as the impairment can be produced by intracerebroventricular administration of hypertonic saline (Bunag and Miyajima, 1984) and is prevented by chemical lesion of the median preoptic nucleus (Bealer, 2000). While these studies fit well with our current findings, and our previous observation that immediate-early gene expression is upregulated in the median preoptic nucleus in LPK animals (Chapter 4), it is possible that the beneficial effect of HWI on cardiac baroreflex function is multifactorial, potentially involving a suppression of plasma osmolality but also an improvement in renal function. This point requires clarification because the only treatment currently approved for PKD, Tolvaptan, a V_2 receptor antagonist, elevates plasma osmolality through its potent diuretic action (Devuyst et al., 2017), and could therefore potentially have a deleterious effect on cardiac baroreflex function.

Left-ventricular hypertrophy is a major risk factor for cardiovascular mortality (Levy et al., 1990). In our study, we observed that HWI-treated LPK rats had a lower LVI, a measure of left-ventricular hypertrophy. A similar finding was reported by Sagar et al. (2019), although their analysis was restricted to whole heart weight. Our correlation analysis established an independent relationship between LVI and blood pressure in LPK rats. Although it was beyond the scope of our study to determine the exact nature of this relationship, it is conceivable that left-ventricular hypertrophy is pressure-dependent in LPK animals, such that the improvements in LVI following HWI treatment may originate from the reduction in arterial pressure observed in these animals. We cannot, however, discard the alternative

possibility that LVI may be causative of a component of the hypertension in LPK rats; for instance, left-ventricular hypertrophy may activate ischemia-sensitive symapthoexcitatory cardiac afferents (Zhu et al., 2009; Chen et al., 2015).

Our data showing that HWI reduced kidney growth and improved renal function in LPK rats is consistent with a recent report (Sagar et al., 2019) and with earlier work in another PKD model, the PCK rat (Nagao et al., 2006; Hopp et al., 2015). Importantly, earlier work in the PCK rat found that HWI is renoprotective in animals supplied with either glucose-sweetened drinking water (used herein) or a hydrated agar diet (Hopp et al., 2015), demonstrating that the benefit is due to the additional fluid intake and not a change in dietary macronutrients. While vasopressin levels were not measured in the current study, the renal improvements seen in HWI-treated LPK rats might well be due to a long-term suppression of vasopressin release and renal cAMP production as this has been noted following HWI in the PCK rat (Nagao et al., 2006; Hopp et al., 2015) and is entirely consistent with the established role of renal V_2 receptor activation in the acceleration of renal cyst growth (Wang et al., 2008; Torres et al., 2012; Boertien et al., 2013; van Gastel and Torres, 2017). It should be noted that glucose is a major plasma solute that could activate brain osmoreceptors and in turn vasopressin levels. While this possibility could be negated in future studies with saccharin, the influence of additional glucose intake on plasma tonicity in HWI-treated LPK rats is likely negligible because plasma osmolality was much lower in this cohort (Chapter 4), while Sagar et al. (2019) found that HWI via 5% glucose water did not influence plasma glucose levels in LPK rats.

In conclusion, our study supports the ongoing examination of HWI as a therapeutic option for PKD patients. Our novel results strongly indicate that the beneficial effects of HWI in PKD extend beyond renoprotection and also include improvements in cardiac structure and neuroregulation. Our study therefore warrants investigation into the long-term impact of HWI on cardiovascular morbidity and mortality in PKD patients.

6.

General Discussion

This thesis examined the hypothesis that the hypothalamus and elevations in extracellular fluid osmolality contribute to hypertension and baroreflex dysfunction in polycystic kidney disease (PKD). The major findings are:

1. An early increase in glutamatergic excitation of the hypothalamic paraventricular nucleus (PVN) contributes to hypertension, but not baroreflex dysfunction, in the Lewis Polycystic Kidney (LPK) rat model of PKD.
2. Angiotensin II regulation of vasopressin secretion from the PVN is enhanced in LPK rats through an astrocyte-dependent mechanism but the tonic activity of its type 1 receptor (AT1R) does not contribute to resting blood pressure.
3. The pro-hypertensive increases in PVN glutamatergic tone arises from neurons in the subfornical organ (SFO) in LPK rats but is not reduced by chronic inhibition of AT1R or suppression of plasma osmolality.
4. High-water intake (HWI) treatment ameliorates the development of hypertension, improves cardiac baroreflex function and lowers left-ventricular mass and contractility in LPK rats.

A central origin of hypertension in PKD

Our initial experiments identified that a significant component of the hypertension observed in LPK rats is attributable to the ongoing activity of neurons residing in the PVN. Our data strongly suggest that PVN neuronal activity is increased in LPK rats by a greater level of glutamatergic excitation that arises early in disease progression in LPK rats at an age when they are hypertensive but not overtly uraemic. It is therefore probable that early PVN excitation is an initiating event in the genesis of hypertension in PKD, although additional loss-of-function studies (e.g., acute or chronic inactivation of glutamatergic inputs to the PVN) in younger pre-hypertensive LPK rats will be required to examine this point further.

Various pre- and post-synaptic mechanisms are capable of increasing glutamatergic tone in the PVN. For example, augmented PVN glutamatergic tone is produced in the spontaneously hypertensive rat (SHR) by enhanced presynaptic glutamate release and greater NMDA receptor density, membrane trafficking and phosphorylation (Ye et al., 2012a; Qiao et al., 2017), while in water-deprived rats it occurs concurrently with increased AT1R activity (Freeman and Brooks, 2007) and reduced glutamate transporter activity (Bardgett et al., 2014b), two critical regulators of glutamatergic transmission (Stern et al., 2016). In contrast to the observations in SHR and water-deprived rats, we found that LPK rats displayed similar

levels of ionotropic glutamate receptor mRNA in the PVN and blood pressure changes following PVN microinjection of an AT1R antagonist or a glutamate transporter-1 inhibitor compared to Lewis rats. These findings highlight that enhanced PVN glutamatergic tone in LPK rats is uniquely different from SHR and water-deprived animals in that it is unlikely to be produced locally by differences in ionotropic glutamate receptor density, tonic angiotensin II actions or removal of extracellular glutamate by astrocytes. Instead, our tracing and pharmacological experiments strongly support the notion that, in LPK rats, the pro-hypertensive increase in PVN neuronal activity is produced predominately by a greater discharge of glutamatergic afferents arising from the SFO. It remains to be determined whether, in LPK rats, SFO neurons are exciting the PVN directly via a monosynaptic glutamatergic projection (Matsuda et al., 2017) and/or indirectly via polysynaptic relay with the MnPO (Leib et al., 2017) or some other structure. Further dissection of this circuit will require selective manipulations of lamina terminalis neurons (e.g., with optogenetics) according to projection target and neurochemical phenotype (i.e., glutamatergic neurons).

Effective delivery of pharmacotherapies across the blood-brain barrier is a major challenge for translational neuroscience (Shen, 2017). Thus, our finding that the SFO, a structure that lacks a complete blood-brain barrier (Morita et al., 2016), is the critical source of PVN excitation in LPK rats is salient as it may allow for peripherally-administered substances to access and interfere with this pro-hypertensive neural pathway in PKD. Conceivably this prospective therapeutic approach could operate either by interfering with the haemal stimulus (or stimuli) that is (are) driving the activity of SFO neurons or alternatively by silencing SFO neurons directly. We explored the former approach and found that while two interventions, losartan and high-water intake (HWI), perturbed the development of hypertension in LPK rats, neither effectively reduced PVN glutamatergic tone. This suggests that angiotensin II and plasma hyperosmolality are not the primary stimuli responsible for driving the activation of the SFO-PVN glutamatergic pathway in LPK rats and must therefore increase blood pressure through an independent but additive mechanism (see beneath). Importantly, however, we found that blockade of PVN glutamate receptors was required to lower the blood pressure of losartan-treated LPK rats to normotensive levels under anaesthesia. On the basis of this observation, we speculate that the stimulus responsible for increasing SFO-PVN glutamatergic transmission in LPK rats may underlie the apparent treatment-resistance of some hypertensive PKD patients to renin-angiotensin system inhibitors (Kelleher et al., 2004).

If circulating angiotensin II and osmolytes are not the source of the increased SFO-dependent glutamatergic activation of the PVN in LPK rats, then what could be driving the activity of this pathway? One possibility is that this pathway is activated by circulating pro-inflammatory cytokines as they are known to produce hypertensive effects via the SFO (Wei et al., 2013), are elevated in PKD patients (Menon et al., 2011) and predict treatment-resistant hypertension in the chronic kidney disease patient population (Chen et al., 2019). Further studies involving acute inhibition or chronic knockdown of cytokine receptors in the SFO of LPK rats would be required to examine this hypothesis. It is also possible that SFO-dependent drive of the PVN in LPK rats is produced by viscerosensory afferents, such as vagal afferents which are polysynaptically connected with the lamina terminalis neurons (Stocker and Toney, 2007) and differentially regulate cardiovascular function in LPK rats (Salman et al., 2017). Yet viscerosensory and blood-borne stimuli may not act in isolation of one another; rather, different stimuli could temporally summate during PKD progression through sensitisation (Johnson and Xue, 2018) as in-vivo recordings strongly suggest that vagal and haemal stimuli converge on individual SFO and MnPO neurons (Stocker and Toney, 2005, 2007; Zimmerman et al., 2019). The implication of this is that that early targeting of pro-hypertensive stimuli as they first arise in the progression of PKD might be required to effectively mitigate the development of hypertension later in life.

The PVN is unique among the cardiovascular-regulatory brain nuclei in that it contains multiple functionally heterogenous neuronal populations that collectively influence arterial pressure through distinct neural (i.e., cardiac autonomic innervation and sympathetic innervation of the resistance vasculature, veins, kidneys and adrenal glands) and humoral (i.e., vasopressin and corticotrophin-releasing hormone [CRH]) outflows (Porter, 1988; Kannan et al., 1989; Martin et al., 1991; Martin et al., 1997; Haselton and Vari, 1998; Deering and Coote, 2000; Kenney et al., 2001; Page et al., 2011; Ramchandra et al., 2013; Stern, 2015; Mendonca et al., 2018). Importantly, the PVN receives multiple feedback (e.g., haemal and viscerosensory stimuli) and feedforward (e.g., physiological stress) signals to ensure that its outflows are modulated in a concerted fashion to preserve, among other things, cardiovascular and extracellular fluid homeostasis (Stern, 2015). In different pathophysiological conditions in which the PVN is implicated, there appears to be a co-involvement of multiple PVN outflows with a signature that presumably reflects the underlying stressor/s of the condition. For example, chronic heart failure rats display an increased excitability of magnocellular vasopressin neurons (Reis et al., 2016) and

presympathetic neurons supplying the kidneys (Ramchandra and Barrett, 2015), while in the SHR the PVN is overactive in its control of lumbar (Allen, 2002; Li and Pan, 2007b), but not renal (Akine et al., 2003), sympathetic nerve activity (SNA).

Our experiments were designed in order to assess what functional population of PVN efferent neuron is overactive in its control of blood pressure in LPK rats. In contrast to observations in other hypertensive rat models (Allen, 2002; Li and Pan, 2007b; Bardgett et al., 2014a), our results suggest that presympathetic PVN neurons regulating renal, splanchnic and lumbar SNA are not overactive in LPK animals since *en-masse* silencing of PVN neurons directly (with a $GABA_A$ agonist) or indirectly (with an ionotropic glutamate receptor antagonist) did not produce a greater reduction in the activity of either of these nerves. Thus, we propose a model whereby the PVN preferentially regulates arterial pressure through a non-sympathetic outflow in PKD. This model is further reinforced by our finding that angiotensin II stimulation of the PVN reduces SNA in LPK rats despite large increases in arterial pressure. Further confirmation of our model will nevertheless require measurement of circulating catecholamines and ganglionic blockade studies. In addition, our loss-of-function pharmacological experiments indicate that the pro-hypertensive action of the PVN does not require cardiac autonomic outflow or peripheral V_{1A} receptor activation. Considered collectively, these unique observations, combined with our finding that PVN inhibition markedly reduces systolic blood pressure variability in the very-low frequency band, reflecting humoral and autogenic oscillations in vasomotor tone (Stauss, 2007), strongly suggest that the PVN engages a novel, most likely humoral, peripheral effector to maintain hypertension in LPK rats. Certainly the temporal profile of the depressor response to pharmacological inactivation of the PVN in LPK rats is consistent with this notion as the delay to the peak response (~20 min) is significantly longer than that observed in the SHR (~3 min (Allen, 2002; Li and Pan, 2007b)) and Dahl salt-sensitive hypertensive rat (~5 min (Gabor and Leenen, 2012)).

We consider adrenocorticotropic hormone (ACTH) as a candidate neurohormone that could potentially underlie the pro-hypertensive action of the PVN in LPK rats. ACTH is regulated by the release of CRH and vasopressin (via pituitary V_{1B} receptors) (Aguilera and Liu, 2012), both of which are under the control of the SFO (Ferguson et al., 1984; Krause et al., 2008), and can elevate arterial pressure acutely (minutes) (Sabban et al., 2009). While currently no study has measured ACTH levels in PKD patients, their cortisol levels are elevated (Tufan et al., 2010), indicating that the HPA axis may be pathologically up-regulated in this condition.

Importantly, ACTH can exert a 'pseudo-sympathetic' hypertensive action by enhancing noradrenaline release from post-ganglionic sympathetic terminals (Costa and Majewski, 1988) and the adrenal gland (Valenta et al., 1986), and could therefore underlie the heightened sympathetic vasomotor tone in PKD as suggested by ganglionic blockade studies (Phillips et al., 2007; Ameer et al., 2014). Thus, it is imperative that further work examines whether the secondary hypertension in PKD is aetiologically similar to the ACTH/glucocorticoid-dependent hypertension that arises in Cushing's disease as it might therefore be managed similarly (Isidori et al., 2015).

Altered regulation of vasopressin-releasing neurons in PKD

Magnocellular vasopressin neurons reprogram the strength of their synaptic inputs in response to chronic threats to circulatory homeostasis. For instance, glutamatergic inputs are enhanced through a potentiation of NMDA-mediated calcium signalling (Stern and Potapenko, 2013) and membrane excitability (Ferreira-Neto et al., 2017) in rats with chronic heart failure, while $GABA_A$ receptor hyperpolarising currents are weakened or reversed in rats exposed to chronic hyperosmotic stress (Kim et al., 2011; Kim et al., 2013; Choe et al., 2015a). In PKD, a differential regulation of magnocellular vasopressin neurons is suspected on the basis of observations that patients display higher circulating levels of vasopressin and/or copeptin basally (Danielsen et al., 1986b; Danielsen et al., 1986a; Michalski and Grzeszczak, 1996; Zittema et al., 2014) and in response to an acute hyperosmotic challenge (Graffe et al., 2012). Our studies indicate that, in LPK rats, magnocellular vasopressin neurons in the PVN are more sensitive to local angiotensin II through an astrocyte-dependent mechanism, while those in the SON display a greater ongoing activity (see Appendix 1). Though we found that chronic inhibition of vasopressin V_{1A} receptors did not mitigate the development of hypertension in LPK rats (see Appendix 1), the increase in SON vasopressin neuron activation described here may be potentially significant considering that vasopressin drives cyst growth in a dose-dependent manner (Wang et al., 2008; Torres et al., 2012; Boertien et al., 2013; van Gastel and Torres, 2017).

The results presented in this thesis support previous work showing that the cardiovascular actions of angiotensin II in the PVN are mediated via an inhibition of astrocyte glutamate reuptake (Stern et al., 2016), and show that this interaction is preserved in the hypertension that arises secondary to PKD. Furthermore, our high-resolution anatomical data demonstrate that the overwhelming majority of PVN astrocytes are not AT1R-expressing in rat, strongly suggesting that the interaction between local angiotensin II and PVN astrocytes is mediated

indirectly via another cell population. Consequently, further investigations concerning the signalling actions of angiotensin II in the PVN should first identify the local "angiotensin II sensor" that signals to PVN astrocytes. We hypothesise that CRH neurons detect local angiotensin II and in turn signal to distant astrocytes via long-range volume transmission as we and others (Oldfield et al., 2001; de Kloet et al., 2017) found that a large population of CRH neurons express AT1R and local CRH signalling has recently been described (Jiang et al., 2018). The idea that angiotensin II evokes the local release of CRH to ultimately activate magnocellular vasopressin and presympathetic neurons is consistent with recent data presented in abstract form by the Li and Pan laboratory which shows that administration of CRH into the PVN recapitulates the cardiovascular actions of angiotensin II in normotensive animals and is exaggerated in hypertension (Zhou et al., 2019). Our observation that, in LPK rats, more CRH neurons contain AT1R mRNA and that the AT1R transcript count per neuron is also higher raises the possibility that the detection of local angiotensin II levels in the PVN might be enhanced in PKD by both an increase in the density of CRH neurons capable of detecting angiotensin II and an increase in the sensitivity of individual CRH neurons. In future experiments we would like to utilise selective CRH receptor antagonists (Zoumakis and Chrousos, 2010) to ascertain whether local CRH signalling is required for angiotensin II regulation of arterial pressure in-vivo and astrocyte glutamate transporter-1 activity in-vitro in normal conditions and whether this is facilitated in PKD.

Our results suggest that there is a reconfiguration of angiotensin II signalling in the PVN in PKD that involves a recruitment of inositol triphosphate (IP3) receptors and calcineurin to drive greater vasopressin release. Ultimately the non-specific nature of our pharmacological manipulations precludes us from knowing in what cell population these pathways act to facilitate greater angiotensin II-evoked vasopressin release. Indeed, though we speculate that the disease-recruitment of IP3 receptors and calcineurin might occur in AT1R-expressing cells, particularly CRH neurons, as both are known to lie downstream of AT1R, these pathways are also present in astrocytes (Su et al., 2003; Xie et al., 2015) and could therefore regulate glutamate transporter-1 function basally or in response to angiotensin II. Therefore, further examination of the role of these intracellular pathways in the PVN in PKD should utilise in-vitro preparations in which IP3 receptor (Okubo et al., 2001) and calcineurin (Mehta and Zhang, 2014) dynamics can be visualised in individual cells. These preparations will be also be valuable in exploring our hypothesis that IP3 receptor signalling is altered in

PVN neurons in PKD due to functional interactions between the mutant PKD proteins and the IP3 receptor (Li et al., 2005; Mekahli et al., 2012).

Our finding that more SON vasopressin neurons express Fos/Fra immediate-early genes in LPK rats indicates that these neurons display a greater ongoing activity in PKD. Increased Fos/Fra expression is also observed in the SON of animals that have been salt-loaded or water-deprived (Sharp et al., 1991; Miyata et al., 2001; Pirnik et al., 2004) and is reflected by greater unitary discharge frequency under anaesthesia (Scott et al., 2009b; Choe et al., 2015a). In addition to discharge rate, body fluid disturbances can also affect the proportion of SON vasopressin neurons that adopt phasic firing (Leng et al., 2001) which yields quantitatively more vasopressin release (Dutton and Dyball, 1979). In future studies we would like to perform in-vivo extracellular recordings of SON vasopressin neurons in LPK rats to determine whether the basal discharge frequency or pattern is altered in PKD. These experiments will also be valuable to assess whether in PKD the discharge of SON vasopressin neurons is differentially regulated by afferent inputs (e.g., lamina terminalis neurons) or local mechanisms, such as a polarity switch in $GABA_A$ receptor function (Kim et al., 2011; Kim et al., 2013; Choe et al., 2015a).

Novel cardiovascular benefits of HWI in PKD

Suppression of extracellular fluid osmolality with HWI is an effective means of slowing cyst growth in PKD rodents (Nagao et al., 2006; Hopp et al., 2015; Sagar et al., 2019) and is currently being investigated in patients (El-Damanawi et al., 2018; Wong et al., 2018). The cardiovascular effects of HWI in PKD are however largely unknown, with interpretation of animal studies currently limited either by the use of normotensive models (Hopp et al., 2015) or tail-cuff plethysmography (Sagar et al., 2019). In our study, we found that HWI not only improved renal size and function in LPK rats but importantly, and uniquely, also improved ambulatory blood pressure, cardiac baroreflex function and indices of left-ventricular contractility and mass, thus providing clear preclinical evidence that HWI is cardioprotective in PKD.

Should HWI be shown to reduce arterial pressure in PKD patients, it will be important for further work to establish the antihypertensive mechanism as this may facilitate the optimisation of this therapy. Our results suggest that blood pressure reductions in HWI-treated LPK does not involve a reduced activation of ionotropic glutamate receptors in the PVN nor is it likely to involve a suppression of peripheral V_{1A} receptor activity. The anti-

hypertensive action of HWI may therefore be mediated by the suppression of an osmosensitive neural circuit that does not involve PVN excitation or vasopressin release or alternatively may be due to improvements entirely within the peripheral tissues. Considering the possibility of a central action of HWI, it plausible that the hypo-osmotic effect of HWI might suppress neurons in the nucleus of the solitary tract since experiments performed by Paton and colleagues using an in-situ preparation argue that this brain region maintains high sympathetic vasomotor tone independently of the PVN in dehydrated non-anaesthetised rats (Colombari et al., 2011). Going forward, determination of a central anti-hypertension action of HWI should include in-vivo experiments assessing the blood pressure response to infusions of hypotonic solutions via the intracarotid route (to preferentially inhibit forebrain osmoreceptors (Brooks et al., 2005)) or the intragastric route (to preferentially inhibit peripheral osmoreceptors (Zimmerman et al., 2019)). When considering the possibility that the anti-hypertensive mechanism of HWI is due to changes within the periphery, it is plausible that improvements in uraemia following HWI perturb arterial stiffness or endothelial dysfunction as both are improved after renal replacement therapy (Chan et al., 2003; Kaur et al., 2013).

Cardiac baroreflex dysfunction independently predicts mortality in patients with chronic kidney disease, including PKD (Johansson et al., 2007; John et al., 2008), yet is resistant to conventional antihypertensive therapy (Neumann et al., 2004). Our finding that HWI prevented the deterioration in cardiac baroreflex sensitivity in LPK rats therefore warrants clinical investigation into whether HWI protects against cardiac baroreflex dysfunction and its adverse consequences, in particular cardiac arrhythmias (La Rovere et al., 1998), in PKD patients.

Further studies will also be necessary to elucidate the mechanism underpinning the improvement in cardiac baroreflex function that we observed in HWI-treated LPK rats. These studies should firstly determine whether HWI mitigates cardiac baroreflex decline through a direct lowering of extracellular fluid osmolality or indirectly via improvements in renal function. The effect of osmolality and uraemia on baroreflex function in LPK rats could be teased apart with acute experiments involving hypotonic saline infusions but also with chronic studies that measure baroreflex function during V_2 receptor antagonism as this therapy would be expected to improve renal function but elevate plasma osmolality (Devuyst et al., 2017). Subsequent studies should also seek to determine whether HWI provides protection against cardiac baroreflex dysfunction in LPK rats via effects within the peripheral

tissues or alternatively – and perhaps more likely considering the selective nature of the improvement – by facilitating the central processing of the cardiac baroreflex. The central mechanism through which HWI might improve cardiac baroreflex function in LPK rats remains speculative at this stage; however, is unlikely to involve the PVN as our acute loss-of-function experiments demonstrated that neurons in this region do not contribute to baroreflex dysfunction in LPK rats. One possibility is that HWI suppresses the activity of a population of MnPO neurons which could be tonically suppressing the barosensitivity of cardiac-related medullary neurons in LPK rats. This hypothesis is suggested by work showing that the MnPO, while not normally required for the cardiac baroreflex (Patel and Schmid, 1988), is recruited during extracellular fluid hypertonicity to depress of the cardiac baroreflex (Bealer, 2000) and is in keeping with evidence that MnPO neurons are polysynaptically connected with the heart (Standish et al., 1994) and are both baro- and osmo-regulated (Aradachi et al., 1996; Stocker and Toney, 2005). However, currently it is not known how MnPO neurons participate in the central processing of the baroreflex and, importantly, whether they do so independently of the PVN.

Concluding Remarks

Although the high prevalence and unique early presentation of hypertension in PKD has been recognised for a century (Vollard and Fahr, 1914; Braasch, 1916; Schacht, 1931), the precise pathological events responsible have remained obscure. Research in this area has predominately focused on locally-acting renal milieu, particularly the intra-renal renin-angiotensin system (Torres et al., 1992; Loghman-Adham et al., 2004; Loghman-Adham et al., 2005; Kocyigit et al., 2013; Saigusa et al., 2015) and has dedicated less attention to neurohumoral regulatory systems, a discrepancy that reflects the permeation of Guytonian renocentric models of blood pressure control in hypertension research for half a century (Beard, 2013) and the related view that the anti-hypertensive benefit provided by renin-angiotensin system inhibitors is primarily due to renal actions (Chapman et al., 2010; Rahbari-Oskoui et al., 2014). The purpose of this thesis was to investigate another important, yet underappreciated, regulator of arterial pressure – the brain – in the production of hypertension in PKD.

The experiments contained in this thesis have succeeded in providing clear evidence that the maintenance of hypertension in LPK rats involves the excitation of a SFO-PVN neuronal circuit. This is the first description of a central nervous system circuit that exerts a pro-hypertensive action in PKD, therefore providing the foundation for a new line of inquiry

directed at understanding the genesis of hypertension in this condition with the long-term goal of enhancing its clinical management. Since our work highlights that PVN excitation is likely to occur early in the progression of PKD and is resistant to angiotensin II inhibition, it may be possible to target PVN excitation to reduce arterial pressure in young PKD patients (Fick et al., 1994; Ivy et al., 1995) and those with treatment resistance (Kelleher et al., 2004). It is also plausible that PVN excitation is an initiating event that gives rise to other seemingly disparate renal and vascular abnormalities of PKD, as a central origin of intrarenal renin-angiotensin system activation (Cao et al., 2015; Qiu et al., 2018) and vascular dysfunction (Wilbert-Lampen et al., 2006; Chung et al., 2014) has been described in other conditions. Needless to say, discovery of a common hypothalamic origin for these abnormalities would provoke a major frameshift in the understanding of PKD pathophysiology.

This thesis contains important findings that could have direct implications for the future management of comorbid cardiovascular disease in PKD. Firstly, our results show that the PVN elevates arterial pressure in LPK rats through a novel effector, potentially ACTH. The anticipated impact of identifying ACTH as hypertensive in PKD is enormous as it would not only challenge the conventional view that the PVN controls arterial pressure *only* through its pre-autonomic and vasopressin-secreting outputs but would also uncover a new therapeutic target to manage hypertension in PKD. Secondly, as the most common form of PKD, autosomal dominant PKD, is typically diagnosed in the early stages of the disease progression when patients are either asymptomatic or only recently hypertensive (Pei and Watnick, 2010; Rahbari-Oskoui et al., 2014), we envision that identification of the pro-hypertensive disease-milieu that interact with the hypothalamus in PKD would facilitate the development of anti-hypertensive interventions that could be applied early in PKD before cardiovascular damage is incurred. Finally, our finding that HWI is anti-hypertensive and protects against cardiac baroreflex dysfunction in LPK rats has significant translational potential considering that this intervention is readily accessible, inexpensive and almost certainly safe. Importantly, currently there are cohorts of PKD patients that could be studied to evaluate the long-term cardiovascular impact of HWI since there are clinical studies underway that are exploring the renal benefits of this novel intervention (El-Damanawi et al., 2018; Wong et al., 2018).

More generally, the findings of this thesis support a large body of work showing that the PVN contributes minimally to resting arterial pressure in normotension but is a critical hub for multiple hypertensive states (Earle and Pittman, 1995; Li and Pan, 2007b; Xiong et al.,

2012; Bardgett et al., 2014a). However, our unique observations in LPK rats relative to other disease models suggests that the efferent PVN neuronal populations and local signalling mechanisms responsible for generating long-term increases in arterial pressure likely vary among hypertensive conditions. Perhaps this is not surprising considering that the patterned output from the PVN is coordinated according to the specific stressor/s presented to it (Stern, 2015) and that the stressors of PKD, though not entirely clear, are likely to be different from other forms of hypertension. Ultimately, comparative investigations into the idiosyncrasies of how the PVN is reprogrammed in different hypertensive states will no doubt provide important insight into how the brain controls arterial pressure.

References

Abbott SB, Machado NL, Geerling JC, Saper CB (2016) Reciprocal control of drinking behavior by median preoptic neurons in mice. The Journal of neuroscience : the official journal of the Society for Neuroscience 36:8228-8237.

Abbott SB, Kanbar R, Bochorishvili G, Coates MB, Stornetta RL, Guyenet PG (2012) C1 neurons excite locus coeruleus and A5 noradrenergic neurons along with sympathetic outflow in rats. J Physiol 590:2897-2915.

Abdala AP, McBryde FD, Marina N, Hendy EB, Engelman ZJ, Fudim M, Sobotka PA, Gourine AV, Paton JF (2012) Hypertension is critically dependent on the carotid body input in the spontaneously hypertensive rat. The Journal of physiology 590:4269-4277.

Abe C, Inoue T, Inglis MA, Viar KE, Huang L, Ye H, Rosin DL, Stornetta RL, Okusa MD, Guyenet PG (2017) C1 neurons mediate a stress-induced anti-inflammatory reflex in mice. Nature neuroscience 20:700-707.

Adrian ED, Bronk DW, Phillips G (1932) Discharges in mammalian sympathetic nerves. The Journal of physiology 74:115-133.

Affleck VS, Coote JH, Pyner S (2012) The projection and synaptic organization of NTS afferent connections with presympathetic neurons, GABA and nNOS neurons in the paraventricular nucleus of the hypothalamus. Neuroscience 219:48-61.

Agarwal A, Anand IS, Sakhuja V, Chugh KS (1991) Effect of dialysis and renal transplantation on autonomic dysfunction in chronic renal failure. Kidney international 40:489-495.

Agassandian K, Grobe JL, Liu X, Agassandian M, Thompson AP, Sigmund CD, Cassell MD (2017) Evidence for intraventricular secretion of angiotensinogen and angiotensin by the subfornical organ using transgenic mice. American journal of physiology Regulatory, integrative and comparative physiology 312:R973-r981.

Aguilera G (1994) Regulation of pituitary ACTH secretion during chronic stress. Frontiers in neuroendocrinology 15:321-350.

Aguilera G (2011) Regulation of the hypothalamic-pituitary-adrenal axis by neuropeptides. Hormone molecular biology and clinical investigation 7:327-336.

Aguilera G, Liu Y (2012) The molecular physiology of CRH neurons. Frontiers in neuroendocrinology 33:67-84.

Aguilera G, Kiss A, Luo X (1995a) Increased expression of type 1 angiotensin II receptors in the hypothalamic paraventricular nucleus following stress and glucocorticoid administration. Journal of neuroendocrinology 7:775-783.

Aguilera G, Young WS, Kiss A, Bathia A (1995b) Direct regulation of hypothalamic corticotropin-releasing-hormone neurons by angiotensin II. Neuroendocrinology 61:437-444.

Ahmad M, White R, Tan J, Huang BS, Leenen FH (2008) Angiotensin-converting enzyme inhibitors, inhibition of brain and peripheral angiotensin-converting enzymes, and left ventricular dysfunction in rats after myocardial infarction. Journal of cardiovascular pharmacology 51:565-572.

Akine A, Montanaro M, Allen AM (2003) Hypothalamic paraventricular nucleus inhibition decreases renal sympathetic nerve activity in hypertensive and normotensive rats. Autonomic neuroscience : basic & clinical 108:17-21.

Al-Bhalal L, Akhtar M (2008) Molecular basis of autosomal recessive polycystic kidney disease (ARPKD). Advances in anatomic pathology 15:54-58.

Al-Merani SA, Brooks DP, Chapman BJ, Munday KA (1978) The half-lives of angiotensin II, angiotensin II-amide, angiotensin III, Sar1-Ala8-angiotensin II and renin in the circulatory system of the rat. The Journal of physiology 278:471-490.

Al-Nimri MA, Komers R, Oyama TT, Subramanya AR, Lindsley JN, Anderson S (2003) Endothelial-derived vasoactive mediators in polycystic kidney disease. Kidney international 63:1776-1784.

Al Awabdh S, Gupta-Agarwal S, Sheehan DF, Muir J, Norkett R, Twelvetrees AE, Griffin LD, Kittler JT (2016) Neuronal activity mediated regulation of glutamate transporter GLT-1 surface diffusion in rat astrocytes in dissociated and slice cultures. Glia 64:1252-1264.

Allen AM (2002) Inhibition of the hypothalamic paraventricular nucleus in spontaneously hypertensive rats dramatically reduces sympathetic vasomotor tone. Hypertension (Dallas, Tex : 1979) 39:275-280.

Allen AM, Chai SY, Clevers J, McKinley MJ, Paxinos G, Mendelsohn FA (1988) Localization and characterization of angiotensin II receptor binding and angiotensin converting enzyme in the human medulla oblongata. The Journal of comparative neurology 269:249-264.

Allen GV, Cechetto DF (1993) Functional and anatomical organization of cardiovascular pressor and depressor sites in the lateral hypothalamic area. II. Ascending projections. The Journal of comparative neurology 330:421-438.

Altura BM (1975) Dose-response relationships for arginine vasopressin and synthetic analogs on three types of rat blood vessels: possible evidence for regional differences in

vasopressin receptor sites within a mammal. The Journal of pharmacology and experimental therapeutics 193:413-423.

Ambuhl P, Felix D, Imboden H, Khosla MC, Ferrario CM (1992) Effects of angiotensin analogues and angiotensin receptor antagonists on paraventricular neurones. Regulatory peptides 38:111-120.

Ameer OZ, Hildreth CM, Phillips JK (2014) Sympathetic overactivity prevails over the vascular amplifier phenomena in a chronic kidney disease rat model of hypertension. Physiological Reports 2.

Ameer OZ, Boyd R, Butlin M, Avolio AP, Phillips JK (2015) Abnormalities associated with progressive aortic vascular dysfunction in chronic kidney disease. Frontiers in physiology 6:150.

Ameer OZ, Butlin M, Kaschina E, Sommerfeld M, Avolio AP, Phillips JK (2016) Long-term angiotensin II receptor blockade limits hypertension, aortic dysfunction, and structural remodeling in a rat model of chronic kidney disease. Journal of vascular research 53:216-229.

Amin MS, Wang HW, Reza E, Whitman SC, Tuana BS, Leenen FH (2005) Distribution of epithelial sodium channels and mineralocorticoid receptors in cardiovascular regulatory centers in rat brain. American journal of physiology Regulatory, integrative and comparative physiology 289:R1787-1797.

Amro OW, Paulus JK, Noubary F, Perrone RD (2016) Low-osmolar diet and adjusted water intake for vasopressin reduction in autosomal dominant polycystic kidney disease: a pilot randomized controlled trial. American journal of kidney diseases : the official journal of the National Kidney Foundation 68:882-891.

Anderson JW, Washburn DL, Ferguson AV (2000) Intrinsic osmosensitivity of subfornical organ neurons. Neuroscience 100:539-547.

Anderson JW, Smith PM, Ferguson AV (2001) Subfornical organ neurons projecting to paraventricular nucleus: whole-cell properties. Brain research 921:78-85.

Anderson WA, Bruni JE, Kaufmann A (1990) Afferent connections of the rat's supraoptic nucleus. Brain research bulletin 24:191-200.

Andersson B (1953) The effect of injections of hypertonic NaCl-solutions into different parts of the hypothalamus of goats. Acta physiologica Scandinavica 28:188-201.

Andersson B, Leksell LG, Lishajko F (1975) Perturbations in fluid balance induced by medially placed forebrain lesions. Brain research 99:261-275.

Ang SA, Harrison JL, Powers-Martin K, Reddrop C, McKitrick DJ, Holobotovskyy W, Arnolda LF, Phillips JK (2007) c-Fos activation in renal hypertension. Hypertension (Dallas, Tex : 1979) 49:1462-1481.

Antunes VR, Yao ST, Pickering AE, Murphy D, Paton JF (2006) A spinal vasopressinergic mechanism mediates hyperosmolality-induced sympathoexcitation. The Journal of physiology 576:569-583.

Appelgren BH, Thrasher TN, Keil LC, Ramsay DJ (1991) Mechanism of drinking-induced inhibition of vasopressin secretion in dehydrated dogs. The American journal of physiology 261:R1226-1233.

Aradachi H, Honda K, Negoro H, Kubota T (1996) Median preoptic neurones projecting to the supraoptic nucleus are sensitive to haemodynamic changes as well as to rise in plasma osmolality in rats. Journal of neuroendocrinology 8:35-43.

Araujo GC, Lopes OU, Campos RR (1999) Importance of glycinergic and glutamatergic synapses within the rostral ventrolateral medulla for blood pressure regulation in conscious rats. Hypertension (Dallas, Tex : 1979) 34:752-755.

Armstrong WE (1995) Morphological and electrophysiological classification of hypothalamic supraoptic neurons. Progress in neurobiology 47:291-339.

Armstrong WE, Stern JE (1997) Electrophysiological and morphological characteristics of neurons in perinuclear zone of supraoptic nucleus. Journal of neurophysiology 78:2427-2437.

Armstrong WE, Tian M, Wong H (1996) Electron microscopic analysis of synaptic inputs from the median preoptic nucleus and adjacent regions to the supraoptic nucleus in the rat. The Journal of comparative neurology 373:228-239.

Armstrong WE, Warach S, Hatton GI, McNeill TH (1980) Subnuclei in the rat hypothalamic paraventricular nucleus: a cytoarchitectural, horseradish peroxidase and immunocytochemical analysis. Neuroscience 5:1931-1958.

Augustine V, Gokce SK, Lee S, Wang B, Davidson TJ, Reimann F, Gribble F, Deisseroth K, Lois C, Oka Y (2018) Hierarchical neural architecture underlying thirst regulation. Nature 555:204-209.

Averina VA, Othmer HG, Fink GD, Osborn JW (2015) A mathematical model of salt-sensitive hypertension: the neurogenic hypothesis. The Journal of physiology 593:3065-3075.

Azar S, Ernsberger P, Livingston S, Azar P, Iwai J (1981) Paraventricular--suprachiasmatic lesions prevent salt-induced hypertension in Dahl rats. Clinical science (London, England : 1979) 61 Suppl 7:49s-51s.

Bader M, Ganten D (2008) Update on tissue renin-angiotensin systems. Journal of molecular medicine (Berlin, Germany) 86:615-621.

Badoer E, Merolli J (1998) Neurons in the hypothalamic paraventricular nucleus that project to the rostral ventrolateral medulla are activated by haemorrhage. Brain research 791:317-320.

Badoer E, Ng CW, De Matteo R (2003) Glutamatergic input in the PVN is important in renal nerve response to elevations in osmolality. American journal of physiology Renal physiology 285:F640-650.

Badoer E, McKinley MJ, Oldfield BJ, McAllen RM (1993) A comparison of hypotensive and non-hypotensive hemorrhage on Fos expression in spinally projecting neurons of the paraventricular nucleus and rostral ventrolateral medulla. Brain research 610:216-223.

Bailey TW, Jin YH, Doyle MW, Smith SM, Andresen MC (2006) Vasopressin inhibits glutamate release via two distinct modes in the brainstem. The Journal of neuroscience : the official journal of the Society for Neuroscience 26:6131-6142.

Bains JS, Ferguson AV (1995) Paraventricular nucleus neurons projecting to the spinal cord receive excitatory input from the subfornical organ. The American journal of physiology 268:R625-633.

Bains JS, Ferguson AV (1999) Activation of N-methyl-D-aspartate receptors evokes calcium spikes in the dendrites of rat hypothalamic paraventricular nucleus neurons. Neuroscience 90:885-891.

Bains JS, Potyok A, Ferguson AV (1992) Angiotensin II actions in paraventricular nucleus: functional evidence for neurotransmitter role in efferents originating in subfornical organ. Brain research 599:223-229.

Bakris G, Bursztyn M, Gavras I, Bresnahan M, Gavras H (1997) Role of vasopressin in essential hypertension: racial differences. Journal of hypertension 15:545-550.

Balanescu S, Kopp P, Gaskill MB, Morgenthaler NG, Schindler C, Rutishauser J (2011) Correlation of plasma copeptin and vasopressin concentrations in hypo-, iso-, and hyperosmolar States. The Journal of clinical endocrinology and metabolism 96:1046-1052.

Banek CT, Gauthier MM, Baumann DC, Van Helden D, Asirvatham-Jeyaraj N, Panoskaltsis-Mortari A, Fink GD, Osborn JW (2018) Targeted afferent renal denervation reduces

arterial pressure but not renal inflammation in established DOCA-salt hypertension in the rat. American journal of physiology Regulatory, integrative and comparative physiology 314:R883-r891.

Bankir L (2001) Antidiuretic action of vasopressin: quantitative aspects and interaction between V1a and V2 receptor-mediated effects. Cardiovascular research 51:372-390.

Bankir L, Bichet DG (2012) Polycystic kidney disease: An early urea-selective urine-concentrating defect in ADPKD. Nature reviews Nephrology 8:437-439.

Banks D, Harris MC (1984) Lesions of the locus coeruleus abolish baroreceptor-induced depression of supraoptic neurones in the rat. The Journal of physiology 355:383-398.

Bardgett ME, Holbein WW, Herrera-Rosales M, Toney GM (2014a) Ang II-salt hypertension depends on neuronal activity in the hypothalamic paraventricular nucleus but not on local actions of tumor necrosis factor-alpha. Hypertension (Dallas, Tex : 1979) 63:527-534.

Bardgett ME, Chen QH, Guo Q, Calderon AS, Andrade MA, Toney GM (2014b) Coping with dehydration: sympathetic activation and regulation of glutamatergic transmission in the hypothalamic PVN. American journal of physiology Regulatory, integrative and comparative physiology 306:R804-R813.

Barney CC, Threatte RM, Fregly MJ (1983) Water deprivation-induced drinking in rats: role of angiotensin II. The American journal of physiology 244:R244-248.

Barone FC, Wayner MJ, Scharoun SL, Guevara-Aguilar R, Aguilar-Baturoni HU (1981) Afferent connections to the lateral hypothalamus: a horseradish peroxidase study in the rat. Brain research bulletin 7:75-88.

Barrett CJ, Bolter CP (2006) The influence of heart rate on baroreceptor fibre activity in the carotid sinus and aortic depressor nerves of the rabbit. Experimental physiology 91:845-852.

Bavanandan S, Ajayi S, Fentum B, Paul SK, Carr SJ, Robinson TG (2005) Cardiac baroreceptor sensitivity: a prognostic marker in predialysis chronic kidney disease patients. Kidney international 67:1019-1027.

Bealer SL (2000) Central control of cardiac baroreflex responses during peripheral hyperosmolality. American journal of physiology Regulatory, integrative and comparative physiology 278:R1157-1163.

Beard DA (2013) Tautology vs. physiology in the etiology of hypertension. Physiology (Bethesda, Md) 28:270-271.

Belibi FA, Reif G, Wallace DP, Yamaguchi T, Olsen L, Li H, Helmkamp GM, Jr., Grantham JJ (2004) Cyclic AMP promotes growth and secretion in human polycystic kidney epithelial cells. Kidney international 66:964-973.

Bell PE, Hossack KF, Gabow PA, Durr JA, Johnson AM, Schrier RW (1988) Hypertension in autosomal dominant polycystic kidney disease. Kidney international 34:683-690.

Benarroch EE (1994) Neuropeptides in the sympathetic system: presence, plasticity, modulation, and implications. Annals of neurology 36:6-13.

Benicky J, Hafko R, Sanchez-Lemus E, Aguilera G, Saavedra JM (2012) Six commercially available angiotensin II AT1 receptor antibodies are non-specific. Cellular and molecular neurobiology 32:1353-1365.

Berecek KH, Barron KW, Webb RL, Brody MJ (1982) Vasopressin-central nervous system interactions in the development of DOCA hypertension. Hypertension (Dallas, Tex : 1979) 4:131-137.

Bergamaschi C, Campos RR, Schor N, Lopes OU (1995) Role of the rostral ventrolateral medulla in maintenance of blood pressure in rats with Goldblatt hypertension. Hypertension (Dallas, Tex : 1979) 26:1117-1120.

Bhatt SR, Lokhandwala MF, Banday AA (2014) Vascular oxidative stress upregulates angiotensin II type I receptors via mechanisms involving nuclear factor kappa B. Clinical and experimental hypertension (New York, NY : 1993) 36:367-373.

Biancardi VC, Son SJ, Ahmadi S, Filosa JA, Stern JE (2014a) Circulating angiotensin II gains access to the hypothalamus and brain stem during hypertension via breakdown of the blood–brain barrier. Hypertension (Dallas, Tex : 1979) 63:572-579.

Biancardi VC, Son SJ, Ahmadi S, Filosa JA, Stern JE (2014b) Circulating angiotensin II gains access to the hypothalamus and brain stem during hypertension via breakdown of the blood-brain barrier. Hypertension (Dallas, Tex : 1979) 63:572-579.

Bichet DG (2016) Vasopressin at central levels and consequences of dehydration. Annals of nutrition & metabolism 68 Suppl 2:19-23.

Bicknell RJ (1988) Optimizing release from peptide hormone secretory nerve terminals. The Journal of experimental biology 139:51-65.

Blair-West JR, Gibson AP, Sheather SJ, Woods RL, Brook AH (1987) Vasopressin release in sheep following various degrees of rehydration. The American journal of physiology 253:R640-645.

Blaustein MP (2018) The pump, the exchanger, and the holy spirit: origins and 40-year evolution of ideas about the ouabain-Na(+) pump endocrine system. American journal of physiology Cell physiology 314:C3-c26.

Bockman CS, Jeffries WB, Pettinger WA, Abel PW (1992) Reduced contractile sensitivity and vasopressin receptor affinity in DOCA-salt hypertension. The American journal of physiology 262:H1752-1758.

Boertien WE, Meijer E, Li J, Bost JE, Struck J, Flessner MF, Gansevoort RT, Torres VE (2013) Relationship of copeptin, a surrogate marker for arginine vasopressin, with change in total kidney volume and GFR decline in autosomal dominant polycystic kidney disease: results from the CRISP cohort. American journal of kidney diseases : the official journal of the National Kidney Foundation 61:420-429.

Boger RS, Glassman HN, Cavanaugh JH, Schmitz PJ, Lamm J, Moyse D, Cohen A, Kleinert HD, Luther RR (1990) Prolonged duration of blood pressure response to enalkiren, the novel dipeptide renin inhibitor, in essential hypertension. Hypertension (Dallas, Tex : 1979) 15:835-840.

Bonfiglio JJ, Inda C, Senin S, Maccarrone G, Refojo D, Giacomini D, Turck CW, Holsboer F, Arzt E, Silberstein S (2013) B-Raf and CRHR1 internalization mediate biphasic ERK1/2 activation by CRH in hippocampal HT22 Cells. Molecular endocrinology (Baltimore, Md) 27:491-510.

Bonjour JP, Malvin RL (1970) Stimulation of ADH release by the renin-angiotensin system. The American journal of physiology 218:1555-1559.

Bouby N, Bachmann S, Bichet D, Bankir L (1990) Effect of water intake on the progression of chronic renal failure in the 5/6 nephrectomized rat. The American journal of physiology 258:F973-979.

Boudaba C, Tasker JG (2006) Intranuclear coupling of hypothalamic magnocellular nuclei by glutamate synaptic circuits. American journal of physiology Regulatory, integrative and comparative physiology 291:R102-111.

Boulter C, Mulroy S, Webb S, Fleming S, Brindle K, Sandford R (2001) Cardiovascular, skeletal, and renal defects in mice with a targeted disruption of the Pkd1 gene. Proceedings of the National Academy of Sciences of the United States of America 98:12174-12179.

Bourque CW (2008) Central mechanisms of osmosensation and systemic osmoregulation. Nature Reviews Neuroscience 9:519-531.

Bourque CW, Ciura S, Trudel E, Stachniak TJE, Sharif-Naeini R (2007) Neurophysiological characterization of mammalian osmosensitive neurones. Experimental physiology 92:499-505.

Braasch W (1916) Clinical data of polycystic kidney. Surgery 23:697-702.

Braga DC, Mori E, Higa KT, Morris M, Michelini LC (2000) Central oxytocin modulates exercise-induced tachycardia. American journal of physiology Regulatory, integrative and comparative physiology 278:R1474-1482.

Briet M, Burns KD (2012) Chronic kidney disease and vascular remodelling: molecular mechanisms and clinical implications. Clinical science (London, England : 1979) 123:399-416.

Brizzee BL, Walker BR (1990) Vasopressinergic augmentation of cardiac baroreceptor reflex in conscious rats. The American journal of physiology 258:R860-868.

Brooks VL, Keil LC (1992) Vasopressin and angiotensin II in reflex regulation of ACTH, glucocorticoids, and renin: effect of water deprivation. The American journal of physiology 263:R762-769.

Brooks VL, Keil LC, Reid IA (1986) Role of the renin-angiotensin system in the control of vasopressin secretion in conscious dogs. Circulation research 58:829-838.

Brooks VL, Freeman KL, Clow KA (2004) Excitatory amino acids in rostral ventrolateral medulla support blood pressure during water deprivation in rats. American journal of physiology Heart and circulatory physiology 286:H1642-1648.

Brooks VL, Qi Y, O'Donaughy TL (2005) Increased osmolality of conscious water-deprived rats supports arterial pressure and sympathetic activity via a brain action. American journal of physiology Regulatory, integrative and comparative physiology 288:R1248-1255.

Brosnahan GM, Abebe KZ, Moore CG, Bae KT, Braun WE, Chapman AB, Flessner MF, Harris PC, Hogan MC, Perrone RD, Rahbari-Oskoui FF, Steinman TI, Torres VE, The Halt Pkd I (2018) Determinants of progression in early autosomal dominant polycystic kidney disease: is it blood pressure or renin-angiotensin-aldosterone-system blockade? Current hypertension reviews 14:39-47.

Brown CH, Bull PM, Bourque CW (2004) Phasic bursts in rat magnocellular neurosecretory cells are not intrinsically regenerative in vivo. The European journal of neuroscience 19:2977-2983.

Brown CH, Bains JS, Ludwig M, Stern JE (2013) Physiological regulation of magnocellular neurosecretory cell activity: integration of intrinsic, local and afferent mechanisms. Journal of neuroendocrinology 25:678-710.

Brown DA, Constanti A, Marsh S (1980) Angiotensin mimics the action of muscarinic agonists on rat sympathetic neurones. Brain research 193:614-619.

Brown DL, Guyenet PG (1985) Electrophysiological study of cardiovascular neurons in the rostral ventrolateral medulla in rats. Circulation research 56:359-369.

Brunner HR, Gavras H, Waeber B, Kershaw GR, Turini GA, Vukovich RA, McKinstry DN, Gavras I (1979) Oral angiotensin-converting enzyme inhibitor in long-term treatment of hypertensive patients. Annals of internal medicine 90:19-23.

Buggy J, Jonhson AK (1977) Preoptic-hypothalamic periventricular lesions: thirst deficits and hypernatremia. The American journal of physiology 233:R44-52.

Buijs RM (1978) Intra- and extrahypothalamic vasopressin and oxytocin pathways in the rat. Pathways to the limbic system, medulla oblongata and spinal cord. Cell and tissue research 192:423-435.

Bulley S, Fernández-Peña C, Hasan R, Leo MD, Muralidharan P, Mackay CE, Evanson KW, Moreira-Junior L, Mata-Daboin A, Burris SK, Wang Q, Kuruvilla KP, Jaggar JH (2018) Arterial smooth muscle cell PKD2 (TRPP1) channels regulate systemic blood pressure. eLife 7:e42628.

Bunag RD, Miyajima E (1984) Baroreflex impairment precedes hypertension during chronic cerebroventricular infusion of hypertonic sodium chloride in rats. The Journal of clinical investigation 74:2065-2073.

Burke SL, Lukoshkova EV, Head GA (2016) Characteristics of renal sympathetic nerve single units in rabbits with angiotensin-induced hypertension. Experimental physiology 101:50-66.

Burnstock G (2004) The Autonomic Neuroeffector Junction. In: Primer on the Autonomic Nervous System: Second Edition, pp 29-33.

Burrell LM, Phillips PA, Stephenson JM, Risvanis J, Rolls KA, Johnston CI (1994) Blood pressure-lowering effect of an orally active vasopressin V1 receptor antagonist in mineralocorticoid hypertension in the rat. Hypertension (Dallas, Tex : 1979) 23:737-743.

Bursztyn M, Bresnahan M, Gavras I, Gavras H (1990) Pressor hormones in elderly hypertensive persons. Racial differences. Hypertension (Dallas, Tex : 1979) 15:I88-92.

Bussien JP, Waeber B, Nussberger J, Schaller MD, Gavras H, Hofbauer K, Brunner HR (1984) Does vasopressin sustain blood pressure of normally hydrated healthy volunteers? The American journal of physiology 246:H143-147.

Buttler L, Ribeiro IM, Ferreira-Neto HC, Antunes VR (2016) Angiotensin II acting on PVN induces sympathoexcitation and pressor responses via the PI3K-dependent pathway. Autonomic neuroscience : basic & clinical 198:54-58.

Byrum CE, Guyenet PG (1987) Afferent and efferent connections of the A5 noradrenergic cell group in the rat. The Journal of comparative neurology 261:529-542.

Cadnapaphornchai MA, McFann K, Strain JD, Masoumi A, Schrier RW (2008) Increased left ventricular mass in children with autosomal dominant polycystic kidney disease and borderline hypertension. Kidney international 74:1192-1196.

Camacho A, Phillips MI (1981) Horseradish peroxidase study in rat of the neural connections of the organum vasculosum of the lamina terminalis. Neuroscience letters 25:201-204.

Cannesson M, Jian Z, Chen G, Vu TQ, Hatib F (2012) Effects of phenylephrine on cardiac output and venous return depend on the position of the heart on the Frank-Starling relationship. Journal of applied physiology (Bethesda, Md : 1985) 113:281-289.

Cao W, Li A, Wang L, Zhou Z, Su Z, Bin W, Wilcox CS, Hou FF (2015) A salt-induced reno-cerebral reflex activates renin-angiotensin systems and promotes CKD progression. Journal of the American Society of Nephrology : JASN 26:1619-1633.

Cao WH, Morrison SF (2006) Glutamate receptors in the raphe pallidus mediate brown adipose tissue thermogenesis evoked by activation of dorsomedial hypothalamic neurons. Neuropharmacology 51:426-437.

Carillo BA, Oliveira-Sales EB, Andersen M, Tufik S, Hipolide D, Santos AA, Tucci PJ, Bergamaschi CT, Campos RR (2012) Changes in GABAergic inputs in the paraventricular nucleus maintain sympathetic vasomotor tone in chronic heart failure. Autonomic neuroscience : basic & clinical 171:41-48.

Carlsson S, Skarphedinsson JO, Delle M, Hoffman P, Thoren P (1992) Differential responses in post- and pre-ganglionic adrenal sympathetic nerve activity and renal sympathetic nerve activity after injection of 2-deoxy-D-glucose and insulin in rats. Acta physiologica Scandinavica 145:169-175.

Carrara F, Azzollini N, Nattino G, Corna D, Villa S, Cerullo D, Zoja C, Abrante B, Luis-Lima S, Porrini E, Cannata A, Ferrari S, Fois M, Stucchi N, Gaspari F (2016) Simplified method to measure glomerular filtration rate by iohexol plasma clearance in conscious rats. Nephron 133:62-70.

Carrive P, Gorissen M (2008) Premotor sympathetic neurons of conditioned fear in the rat. The European journal of neuroscience 28:428-446.

Cassaglia PA, Hermes SM, Aicher SA, Brooks VL (2011) Insulin acts in the arcuate nucleus to increase lumbar sympathetic nerve activity and baroreflex function in rats. The Journal of physiology 589:1643-1662.

Cassaglia PA, Shi Z, Li B, Reis WL, Clute-Reinig NM, Stern JE, Brooks VL (2014) Neuropeptide Y acts in the paraventricular nucleus to suppress sympathetic nerve activity and its baroreflex regulation. The Journal of physiology 592:1655-1675.

Casteleijn NF, de Jager RL, Neeleman MP, Blankestijn PJ, Gansevoort RT (2014) Chronic kidney pain in autosomal dominant polycystic kidney disease: a case report of successful treatment by catheter-based renal denervation. American journal of kidney diseases : the official journal of the National Kidney Foundation 63:1019-1021.

Casteleijn NF, Messchendorp AL, Bae KT, Higashihara E, Kappert P, Torres V, Meijer E, Leliveld AM (2017a) Polyuria due to vasopressin V2 receptor antagonism is not associated with increased ureter diameter in ADPKD patients. Clinical and experimental nephrology 21:375-382.

Casteleijn NF, Zittema D, Bakker SJ, Boertien WE, Gaillard CA, Meijer E, Spithoven EM, Struck J, Gansevoort RT (2015) Urine and plasma osmolality in patients with autosomal dominant polycystic kidney disease: reliable indicators of vasopressin activity and disease prognosis? American journal of nephrology 41:248-256.

Casteleijn NF, van Gastel MD, Blankestijn PJ, Drenth JP, de Jager RL, Leliveld AM, Stellema R, Wolff AP, Groen GJ, Gansevoort RT (2017b) Novel treatment protocol for ameliorating refractory, chronic pain in patients with autosomal dominant polycystic kidney disease. Kidney international 91:972-981.

Cato MJ, Toney GM (2005) Angiotensin II excites paraventricular nucleus neurons that innervate the rostral ventrolateral medulla: an in vitro patch-clamp study in brain slices. Journal of neurophysiology 93:403-413.

Cechetto DF, Saper CB (1988) Neurochemical organization of the hypothalamic projection to the spinal cord in the rat. The Journal of comparative neurology 272:579-604.

Cerasola G, Vecchi M, Mule G, Cottone S, Mangano MT, Andronico G, Contorno A, Parrino I, Renda F, Pavone G (1998) Sympathetic activity and blood pressure pattern in autosomal dominant polycystic kidney disease hypertensives. American journal of nephrology 18:391-398.

Chakfe Y, Bourque CW (2000) Excitatory peptides and osmotic pressure modulate mechanosensitive cation channels in concert. Nature neuroscience 3:572-579.

Chan CT, Harvey PJ, Picton P, Pierratos A, Miller JA, Floras JS (2003) Short-term blood pressure, noradrenergic, and vascular effects of nocturnal home hemodialysis. Hypertension (Dallas, Tex : 1979) 42:925-931.

Chan SH, Hsu KS, Huang CC, Wang LL, Ou CC, Chan JY (2005) NADPH oxidase-derived superoxide anion mediates angiotensin II-induced pressor effect via activation of p38 mitogen-activated protein kinase in the rostral ventrolateral medulla. Circulation research 97:772-780.

Chapman AB (2008) Approaches to testing new treatments in autosomal dominant polycystic kidney disease: insights from the CRISP and HALT-PKD studies. Clinical journal of the American Society of Nephrology : CJASN 3:1197-1204.

Chapman AB, Stepniakowski K, Rahbari-Oskoui F (2010) Hypertension in autosomal dominant polycystic kidney disease. Advances in chronic kidney disease 17:153-163.

Chapman AB, Johnson A, Gabow PA, Schrier RW (1990) The renin-angiotensin-aldosterone system and autosomal dominant polycystic kidney disease. The New England journal of medicine 323:1091-1096.

Chapman AB, Johnson AM, Gabow PA, Schrier RW (1994) Overt proteinuria and microalbuminuria in autosomal dominant polycystic kidney disease. Journal of the American Society of Nephrology : JASN 5:1349-1354.

Chapman AB, Johnson AM, Rainguet S, Hossack K, Gabow P, Schrier RW (1997) Left ventricular hypertrophy in autosomal dominant polycystic kidney disease. Journal of the American Society of Nephrology : JASN 8:1292-1297.

Chen A, Huang BS, Wang HW, Ahmad M, Leenen FH (2014) Knockdown of mineralocorticoid or angiotensin II type 1 receptor gene expression in the paraventricular nucleus prevents angiotensin II hypertension in rats. The Journal of physiology 592:3523-3536.

Chen C-Y, Bonham AC (2005) Glutamate suppresses GABA release via presynaptic metabotropic glutamate receptors at baroreceptor neurones in rats. The Journal of physiology 562:535-551.

Chen J, Bundy JD, Hamm LL, Hsu CY, Lash J, Miller ER, 3rd, Thomas G, Cohen DL, Weir MR, Raj DS, Chen HY, Xie D, Rao P, Wright JT, Jr., Rahman M, He J (2019) Inflammation and apparent treatment-resistant hypertension in patients with chronic kidney disease. Hypertension (Dallas, Tex : 1979) 73:785-793.

Chen NX, Moe SM, Eggleston-Gulyas T, Chen X, Hoffmeyer WD, Bacallao RL, Herbert BS, Gattone VH, 2nd (2011) Calcimimetics inhibit renal pathology in rodent nephronophthisis. Kidney international 80:612-619.

Chen Q-H, Toney GM (2010) In vivo discharge properties of hypothalamic paraventricular nucleus neurons with axonal projections to the rostral ventrolateral medulla. Journal of neurophysiology 103:4-15.

Chen Q, Pan HL (2007) Signaling mechanisms of angiotensin II-induced attenuation of GABAergic input to hypothalamic presympathetic neurons. Journal of neurophysiology 97:3279-3287.

Chen QH, Toney GM (2001) AT(1)-receptor blockade in the hypothalamic PVN reduces central hyperosmolality-induced renal sympathoexcitation. American journal of physiology Regulatory, integrative and comparative physiology 281:R1844-1853.

Chen QH, Toney GM (2003) Identification and characterization of two functionally distinct groups of spinal cord-projecting paraventricular nucleus neurons with sympathetic-related activity. Neuroscience 118:797-807.

Chen QH, Toney GM (2009) Excitability of paraventricular nucleus neurones that project to the rostral ventrolateral medulla is regulated by small-conductance Ca2+-activated K+ channels. The Journal of physiology 587:4235-4247.

Chen QH, Haywood JR, Toney GM (2003) Sympathoexcitation by PVN-injected bicuculline requires activation of excitatory amino acid receptors. Hypertension (Dallas, Tex : 1979) 42:725-731.

Chen WW, Xiong XQ, Chen Q, Li YH, Kang YM, Zhu GQ (2015) Cardiac sympathetic afferent reflex and its implications for sympathetic activation in chronic heart failure and hypertension. Acta physiologica (Oxford, England) 213:778-794.

Chen YL, Chan SH, Chan JY (1996) Participation of galanin in baroreflex inhibition of heart rate by hypothalamic PVN in rat. The American journal of physiology 271:H1823-1828.

Choe KY, Han SY, Gaub P, Shell B, Voisin DL, Knapp BA, Barker PA, Brown CH, Cunningham JT, Bourque CW (2015a) High salt intake increases blood pressure via BDNF-mediated downregulation of KCC2 and impaired baroreflex inhibition of vasopressin neurons. Neuron 85:549-560.

Choe KY, Han SY, Gaub P, Shell B, Voisin DL, Knapp BA, Barker PA, Brown CH, Cunningham JT, Bourque CW (2015b) High salt intake increases blood pressure via

BDNF-mediated downregulation of KCC2 and impaired baroreflex inhibition of vasopressin neurons. Neuron 85:549-560.

Choukroun G, Itakura Y, Albouze G, Christophe JL, Man NK, Grunfeld JP, Jungers P (1995) Factors influencing progression of renal failure in autosomal dominant polycystic kidney disease. Journal of the American Society of Nephrology : JASN 6:1634-1642.

Chung I-M, Kim Hee Y, Lee Youn J (2014) Psychological Stress Impairs Both Endothelial Function and Insulin Sensitivity via Activation of Inflammation and ER Stress Pathways Mediated by Corticotropin-Releasing Hormone. Arterioscler Thromb Vasc Biol 34:A340-A340.

Ciriello J, Kline RL, Zhang TX, Caverson MM (1984) Lesions of the paraventricular nucleus alter the development of spontaneous hypertension in the rat. Brain research 310:355-359.

Ciura S, Bourque CW (2006) Transient receptor potential vanilloid 1 is required for intrinsic osmoreception in organum vasculosum lamina terminalis neurons and for normal thirst responses to systemic hyperosmolality. The Journal of neuroscience : the official journal of the Society for Neuroscience 26:9069-9075.

Ciura S, Liedtke W, Bourque CW (2011) Hypertonicity sensing in organum vasculosum lamina terminalis neurons: a mechanical process involving TRPV1 but not TRPV4. The Journal of neuroscience : the official journal of the Society for Neuroscience 31:14669-14676.

Ciura S, Prager-Khoutorsky M, Thirouin ZS, Wyrosdic JC, Olson JE, Liedtke W, Bourque CW (2018) Trpv4 mediates hypotonic inhibition of central osmosensory neurons via taurine gliotransmission. Cell reports 23:2245-2253.

Clarkson J, Han SY, Piet R, McLennan T, Kane GM, Ng J, Porteous RW, Kim JS, Colledge WH, Iremonger KJ, Herbison AE (2017) Definition of the hypothalamic GnRH pulse generator in mice. Proceedings of the National Academy of Sciences of the United States of America 114:E10216-e10223.

Clifford PS (2011) Local control of blood flow. Advances in physiology education 35:5-15.

Coble JP, Grobe JL, Johnson AK, Sigmund CD (2015) Mechanisms of brain renin angiotensin system-induced drinking and blood pressure: importance of the subfornical organ. American journal of physiology Regulatory, integrative and comparative physiology 308:R238-R249.

Coldren KM, Brown R, Hasser EM, Heesch CM (2015) Relaxin increases sympathetic nerve activity and activates spinally projecting neurons in the paraventricular nucleus of

nonpregnant, but not pregnant, rats. American journal of physiology Regulatory, integrative and comparative physiology 309:R1553-1568.

Collaborators GRF (2018) Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet (London, England) 392:1923-1994.

Collister JP, Olson MK, Nahey DB, Vieira AA, Osborn JW (2013) OVLT lesion decreases basal arterial pressure and the chronic hypertensive response to AngII in rats on a high-salt diet. Physiological reports 1:e00128-e00128.

Collister JP, Nahey DB, Hartson R, Wiedmeyer CE, Banek CT, Osborn JW (2018) Lesion of the OVLT markedly attenuates chronic DOCA-salt hypertension in rats. American journal of physiology Regulatory, integrative and comparative physiology 315:R568-r575.

Colombari DS, Colombari E, Freiria-Oliveira AH, Antunes VR, Yao ST, Hindmarch C, Ferguson AV, Fry M, Murphy D, Paton JF (2011) Switching control of sympathetic activity from forebrain to hindbrain in chronic dehydration. The Journal of physiology 589:4457-4471.

Coote JH (2007) Landmarks in understanding the central nervous control of the cardiovascular system. Experimental physiology 92:3-18.

Coote JH, Downman CB (1966) Central pathways of some autonomic reflex discharges. The Journal of physiology 183:714-729.

Cornec-Le Gall E, Torres VE, Harris PC (2018) Genetic complexity of autosomal dominant polycystic kidney and liver diseases. Journal of the American Society of Nephrology : JASN 29:13-23.

Costa M, Majewski H (1988) Facilitation of noradrenaline release from sympathetic nerves through activation of ACTH receptors, beta-adrenoceptors and angiotensin II receptors. British journal of pharmacology 95:993-1001.

Cowley AW, Jr. (2000) Control of the renal medullary circulation by vasopressin V1 and V2 receptors in the rat. Experimental physiology 85 Spec No:223s-231s.

Cowley AW, Jr., Barber BJ (1983) Vasopressin vascular and reflex effects--a theoretical analysis. Progress in brain research 60:415-424.

Cowley AW, Jr., Monos E, Guyton AC (1974) Interaction of vasopressin and the baroreceptor reflex system in the regulation of arterial blood pressure in the dog. Circulation research 34:505-514.

Crofton JT, Ota M, Share L (1993) Role of vasopressin, the renin-angiotensin system and sex in Dahl salt-sensitive hypertension. Journal of hypertension 11:1031-1038.

Crofton JT, Dustan H, Share L, Brooks DP (1986) Vasopressin secretion in normotensive black and white men and women on normal and low sodium diets. The Journal of endocrinology 108:191-199.

Crofton JT, Share L, Shade RE, Allen C, Tarnowski D (1978) Vasopressin in the rat with spontaneous hypertension. The American journal of physiology 235:H361-366.

Crowley SD, Gurley SB, Herrera MJ, Ruiz P, Griffiths R, Kumar AP, Kim HS, Smithies O, Le TH, Coffman TM (2006) Angiotensin II causes hypertension and cardiac hypertrophy through its receptors in the kidney. Proceedings of the National Academy of Sciences of the United States of America 103:17985-17990.

Cullinan WE, Wolfe TJ (2000) Chronic stress regulates levels of mRNA transcripts encoding beta subunits of the GABA(A) receptor in the rat stress axis. Brain research 887:118-124.

Cunningham ET, Jr., Sawchenko PE (1988) Anatomical specificity of noradrenergic inputs to the paraventricular and supraoptic nuclei of the rat hypothalamus. The Journal of comparative neurology 274:60-76.

Cunningham ET, Jr., Bohn MC, Sawchenko PE (1990) Organization of adrenergic inputs to the paraventricular and supraoptic nuclei of the hypothalamus in the rat. The Journal of comparative neurology 292:651-667.

Cunningham JT, Nissen R, Renaud LP (1992a) Catecholamine depletion of the diagonal band reduces baroreflex inhibition of supraoptic neurons. The American journal of physiology 263:R363-367.

Cunningham JT, Nissen R, Renaud LP (1992b) Ibotenate lesions of the diagonal band of broca attenuate baroreceptor sensitivity of rat supraoptic vasopressin neurons. Journal of neuroendocrinology 4:303-309.

da Silva AQ, Fontes MA, Kanagy NL (2011) Chronic infusion of angiotensin receptor antagonists in the hypothalamic paraventricular nucleus prevents hypertension in a rat model of sleep apnea. Brain research 1368:231-238.

Daftary SS, Boudaba C, Tasker JG (2000) Noradrenergic regulation of parvocellular neurons in the rat hypothalamic paraventricular nucleus. Neuroscience 96:743-751.

Daftary SS, Boudaba C, Szabo K, Tasker JG (1998) Noradrenergic excitation of magnocellular neurons in the rat hypothalamic paraventricular nucleus via

intranuclear glutamatergic circuits. The Journal of neuroscience : the official journal of the Society for Neuroscience 18:10619-10628.

Dalgaard OZ (1957) Bilateral polycystic disease of the kidneys; a follow-up of two hundred and eighty-four patients and their families. Acta medica Scandinavica Supplementum 328:1-255.

Dampney RA (2016) Central neural control of the cardiovascular system: current perspectives. Advances in physiology education 40:283-296.

Dampney RA, Horiuchi J, McDowall LM (2008) Hypothalamic mechanisms coordinating cardiorespiratory function during exercise and defensive behaviour. Autonomic neuroscience : basic & clinical 142:3-10.

Dampney RA, Goodchild AK, Robertson LG, Montgomery W (1982) Role of ventrolateral medulla in vasomotor regulation: a correlative anatomical and physiological study. Brain research 249:223-235.

Dampney RA, Czachurski J, Dembowsky K, Goodchild AK, Seller H (1987) Afferent connections and spinal projections of the pressor region in the rostral ventrolateral medulla of the cat. Journal of the autonomic nervous system 20:73-86.

Dampney RA, Coleman MJ, Fontes MA, Hirooka Y, Horiuchi J, Li YW, Polson JW, Potts PD, Tagawa T (2002) Central mechanisms underlying short- and long-term regulation of the cardiovascular system. Clinical and experimental pharmacology & physiology 29:261-268.

Dampney RAL (2017) Resetting of the baroreflex control of sympathetic vasomotor activity during natural behaviors: description and conceptual model of central mechanisms. Frontiers in neuroscience 11:461.

Danielsen H, Pedersen EB, Nielsen AH, Herlevsen P, Kornerup HJ, Posborg V (1986a) Expansion of extracellular volume in early polycystic kidney disease. Acta medica Scandinavica 219:399-405.

Danielsen H, Nielsen AH, Pedersen EB, Herlevsen P, Kornerup HJ, Posborg V (1986b) Exaggerated natriuresis in adult polycystic kidney disease. Acta medica Scandinavica 219:59-66.

Day TA, Renaud LP (1984) Electrophysiological evidence that noradrenergic afferents selectively facilitate the activity of supraoptic vasopressin neurons. Brain research 303:233-240.

de Beus E, Bots ML, van Zuilen AD, Wetzels JF, Blankestijn PJ, Group MS (2015) Prevalence of apparent therapy-resistant hypertension and its effect on outcome in patients with chronic kidney disease. Hypertension (Dallas, Tex : 1979) 66:998-1005.

de Chaumont F, Dallongeville S, Chenouard N, Herve N, Pop S, Provoost T, Meas-Yedid V, Pankajakshan P, Lecomte T, Le Montagner Y, Lagache T, Dufour A, Olivo-Marin JC (2012) Icy: an open bioimage informatics platform for extended reproducible research. Nature methods 9:690-696.

De Hert S (2012) Physiology of hemodynamic homeostasis. Best practice & research Clinical anaesthesiology 26:409-419.

de Jager RL, Casteleijn NF, de Beus E, Bots ML, Vonken EE, Gansevoort RT, Blankestijn PJ (2017) Catheter-based renal denervation as therapy for chronic severe kidney-related pain. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association.

de Kloet AD, Liu M, Rodríguez V, Krause EG, Sumners C (2015) Role of neurons and glia in the CNS actions of the renin-angiotensin system in cardiovascular control. American journal of physiology Regulatory, integrative and comparative physiology 309:R444-R458.

de Kloet AD, Pitra S, Wang L, Hiller H, Pioquinto DJ, Smith JA, Sumners C, Stern JE, Krause EG (2016a) Angiotensin type-2 receptors influence the activity of vasopressin neurons in the paraventricular nucleus of the hypothalamus in male mice. Endocrinology 157:3167-3180.

de Kloet AD, Wang L, Ludin JA, Smith JA, Pioquinto DJ, Hiller H, Steckelings UM, Scheuer DA, Sumners C, Krause EG (2016b) Reporter mouse strain provides a novel look at angiotensin type-2 receptor distribution in the central nervous system. Brain structure & function 221:891-912.

de Kloet AD, Wang L, Pitra S, Hiller H, Smith JA, Tan Y, Nguyen D, Cahill KM, Sumners C, Stern JE, Krause EG (2017) A unique "angiotensin-sensitive" neuronal population coordinates neuroendocrine, cardiovascular, and behavioral responses to stress. The Journal of neuroscience : the official journal of the Society for Neuroscience 37:3478-3490.

Decavel C, Van den Pol AN (1990) GABA: a dominant neurotransmitter in the hypothalamus. The Journal of comparative neurology 302:1019-1037.

Deering J, Coote JH (2000) Paraventricular neurones elicit a volume expansion-like change of activity in sympathetic nerves to the heart and kidney in the rabbit. Experimental physiology 85:177-186.

Dell KM, Matheson M, Hartung EA, Warady BA, Furth SL, Chronic Kidney Disease in Children S (2016) Kidney disease progression in autosomal recessive polycystic kidney disease. The Journal of pediatrics 171:196-201.e191.

Dellmann HD (1998) Structure of the subfornical organ: a review. Microscopy research and technique 41:85-97.

Dembowsky K, Czachurski J, Seller H (1985) An intracellular study of the synaptic input to sympathetic preganglionic neurones of the third thoracic segment of the cat. Journal of the autonomic nervous system 13:201-244.

Dempsey B, Le S, Turner A, Bokiniec P, Ramadas R, Bjaalie JG, Menuet C, Neve R, Allen AM, Goodchild AK, McMullan S (2017a) Mapping and analysis of the connectome of sympathetic premotor neurons in the rostral ventrolateral medulla of the rat using a volumetric brain atlas. Frontiers in Neural Circuits 11.

Dempsey JC, Phelps IG, Bachmann-Gagescu R, Glass IA, Tully HM, Doherty D (2017b) Mortality in Joubert syndrome. American journal of medical genetics Part A 173:1237-1242.

DePuy SD, Stornetta RL, Bochorishvili G, Deisseroth K, Witten I, Coates M, Guyenet PG (2013) Glutamatergic neurotransmission between the C1 neurons and the parasympathetic preganglionic neurons of the dorsal motor nucleus of the vagus. The Journal of neuroscience : the official journal of the Society for Neuroscience 33:1486-1497.

Dergacheva O, Yamanaka A, Schwartz AR, Polotsky VY, Mendelowitz D (2017) Optogenetic identification of hypothalamic orexin neuron projections to paraventricular spinally projecting neurons. American journal of physiology Heart and circulatory physiology 312:H808-h817.

Devuyst O, Chapman AB, Gansevoort RT, Higashihara E, Perrone RD, Torres VE, Blais JD, Zhou W, Ouyang J, Czerwiec FS (2017) Urine osmolality, response to tolvaptan, and outcome in autosomal dominant polycystic kidney disease: results from the TEMPO 3:4 trial. Journal of the American Society of Nephrology : JASN 28:1592-1602.

DiBona GF (2005) Physiology in perspective: the wisdom of the body. neural control of the kidney. American journal of physiology Regulatory, integrative and comparative physiology 289:R633-641.

Diedrich A, Jordan J, Tank J, Shannon JR, Robertson R, Luft FC, Robertson D, Biaggioni I (2003) The sympathetic nervous system in hypertension: assessment by blood pressure variability and ganglionic blockade. Journal of hypertension 21:1677-1686.

Ding A, Kalaignanasundaram P, Ricardo SD, Abdelkader A, Witting PK, Broughton BR, Kim HB, Wyse BF, Phillips JK, Evans RG (2012) Chronic treatment with tempol does not significantly ameliorate renal tissue hypoxia or disease progression in a rodent model of polycystic kidney disease. Clinical and experimental pharmacology & physiology 39:917-929.

Donadio V, Kallio M, Karlsson T, Nordin M, Wallin BG (2002) Inhibition of human muscle sympathetic activity by sensory stimulation. The Journal of physiology 544:285-292.

Doulton TW, Saggar-Malik AK, He FJ, Carney C, Markandu ND, Sagnella GA, MacGregor GA (2006) The effect of sodium and angiotensin-converting enzyme inhibition on the classic circulating renin-angiotensin system in autosomal-dominant polycystic kidney disease patients. Journal of hypertension 24:939-945.

Drayer JI, Weber MA, Nakamura DK (1985) Automated ambulatory blood pressure monitoring: a study in age-matched normotensive and hypertensive men. American heart journal 109:1334-1338.

Duan PG, Kawano H, Masuko S (2008) Collateral projections from the subfornical organ to the median preoptic nucleus and paraventricular hypothalamic nucleus in the rat. Brain research 1198:68-72.

Dufloth DL, Morris M, Michelini LC (1997) Modulation of exercise tachycardia by vasopressin in the nucleus tractus solitarii. The American journal of physiology 273:R1271-1282.

Dugaich AP, Oliveira-Sales EB, Abreu NP, Boim MA, Bergamaschi CT, Campos RR (2011) Role of the rostral ventrolateral medulla in the arterial hypertension in chronic renal failure. International journal of hypertension 2010:219358.

Dunn FL, Brennan TJ, Nelson AE, Robertson GL (1973) The role of blood osmolality and volume in regulating vasopressin secretion in the rat. The Journal of clinical investigation 52:3212-3219.

Dutschmann M, Herbert H (2006) The Kolliker-Fuse nucleus gates the postinspiratory phase of the respiratory cycle to control inspiratory off-switch and upper airway resistance in rat. The European journal of neuroscience 24:1071-1084.

Dutton A, Dyball RE (1979) Phasic firing enhances vasopressin release from the rat neurohypophysis. The Journal of physiology 290:433-440.

Earle ML, Pittman QJ (1995) Involvement of the PVN and BST in 1K1C hypertension in the rat. Brain research 669:41-47.

Eccles MR, Stayner CA (2014) Polycystic kidney disease – where gene dosage counts. F1000Prime Reports 6:24.

Ecder T, Schrier RW (2001) Hypertension in autosomal-dominant polycystic kidney disease: early occurrence and unique aspects. Journal of the American Society of Nephrology : JASN 12:194-200.

Edwards RM, Trizna W, Kinter LB (1989) Renal microvascular effects of vasopressin and vasopressin antagonists. The American journal of physiology 256:F274-278.

El-Damanawi R, Lee M, Harris T, Mader LB, Bond S, Pavey H, Sandford RN, Wilkinson IB, Burrows A, Woznowski P, Ben-Shlomo Y, Karet Frankl FE, Hiemstra TF (2018) Randomised controlled trial of high versus ad libitum water intake in patients with autosomal dominant polycystic kidney disease: rationale and design of the DRINK feasibility trial. BMJ open 8:e022859.

El-Mas MM, Fan M, Abdel-Rahman AA (2013) Role of rostral ventrolateral medullary ERK/JNK/p38 MAPK signaling in the pressor effects of ethanol and its oxidative product acetaldehyde. Alcoholism, clinical and experimental research 37:1827-1837.

Esler M, Jennings G, Lambert G (1989) Noradrenaline release and the pathophysiology of primary human hypertension. American journal of hypertension 2:140s-146s.

Fagius J, Berne C (1994) Increase in muscle nerve sympathetic activity in humans after food intake. Clinical science (London, England : 1979) 86:159-167.

Farmer DGS, Pracejus N, Dempsey B, Turner A, Bokiniec P, Paton JFR, Pickering AE, Burguet J, Andrey P, Goodchild AK, McAllen RM, McMullan S (2019) On the presence and functional significance of sympathetic premotor neurons with collateralized spinal axons in the rat. The Journal of physiology 597:3407-3423.

Farrell TG, Paul V, Cripps TR, Malik M, Bennett ED, Ward D, Camm AJ (1991) Baroreflex sensitivity and electrophysiological correlates in patients after acute myocardial infarction. Circulation 83:945-952.

Feetham CH, O'Brien F, Barrett-Jolley R (2018) Ion channels in the paraventricular hypothalamic nucleus (PVN); emerging diversity and functional roles. Frontiers in physiology 9:760.

Ferguson AV, Kasting NW (1986) Electrical stimulation in subfornical organ increases plasma vasopressin concentrations in the conscious rat. The American journal of physiology 251:R425-428.

Ferguson AV, Renaud LP (1986) Systemic angiotensin acts at subfornical organ to facilitate activity of neurohypophysial neurons. The American journal of physiology 251:R712-717.

Ferguson AV, Bains JS (1997) Actions of angiotensin in the subfornical organ and area postrema: implications for long term control of autonomic output. Clinical and experimental pharmacology & physiology 24:96-101.

Ferguson AV, Day TA, Renaud LP (1984) Subfornical organ efferents influence the excitability of neurohypophyseal and tuberoinfundibular paraventricular nucleus neurons in the rat. Neuroendocrinology 39:423-428.

Ferreira-Neto HC, Yao ST, Antunes VR (2013) Purinergic and glutamatergic interactions in the hypothalamic paraventricular nucleus modulate sympathetic outflow. Purinergic signalling 9:337-349.

Ferreira-Neto HC, Biancardi VC, Stern JE (2017) A reduction in SK channels contributes to increased activity of hypothalamic magnocellular neurons during heart failure. The Journal of physiology 595:6429-6442.

Fick-Brosnahan GM, Belz MM, McFann KK, Johnson AM, Schrier RW (2002) Relationship between renal volume growth and renal function in autosomal dominant polycystic kidney disease: a longitudinal study. American journal of kidney diseases : the official journal of the National Kidney Foundation 39:1127-1134.

Fick GM, Johnson AM, Hammond WS, Gabow PA (1995) Causes of death in autosomal dominant polycystic kidney disease. Journal of the American Society of Nephrology : JASN 5:2048-2056.

Fick GM, Duley IT, Johnson AM, Strain JD, Manco-Johnson ML, Gabow PA (1994) The spectrum of autosomal dominant polycystic kidney disease in children. Journal of the American Society of Nephrology : JASN 4:1654-1660.

Fleming TM, Scott V, Naskar K, Joe N, Brown CH, Stern JE (2011) State-dependent changes in astrocyte regulation of extrasynaptic NMDA receptor signalling in neurosecretory neurons. The Journal of physiology 589:3929-3941.

Flor AFL, de Brito Alves JL, Franca-Silva MS, Balarini CM, Elias LLK, Ruginsk SG, Antunes-Rodrigues J, Braga VA, Cruz JC (2018) Glial cells are involved in ANG-II-induced vasopressin release and sodium intake in awake rats. Frontiers in physiology 9:430.

Fonnum F, Karlsen RL, Malthe-Sorenssen D, Skrede KK, Walaas I (1979) Localization of neurotransmitters, particularly glutamate, in hippocampus, septum, nucleus accumbens and superior colliculus. Progress in brain research 51:167-191.

Fonseca JM, Bastos AP, Amaral AG, Sousa MF, Souza LE, Malheiros DM, Piontek K, Irigoyen MC, Watnick TJ, Onuchic LF (2014) Renal cyst growth is the main determinant for hypertension and concentrating deficit in Pkd1-deficient mice. Kidney international 85:1137-1150.

Forrester SJ, Booz GW, Sigmund CD, Coffman TM, Kawai T, Rizzo V, Scalia R, Eguchi S (2018) Angiotensin II signal transduction: an update on mechanisms of physiology and pathophysiology. Physiological reviews 98:1627-1738.

Forsling ML, Martin MJ, Sturdy JC, Burton AM (1973) Observations on the release and clearance of neurophysin and the neurohypophysial hormones in the rat. The Journal of endocrinology 57:307-315.

Franchini KG, Cowley AW, Jr. (1996) Sensitivity of the renal medullary circulation to plasma vasopressin. The American journal of physiology 271:R647-653.

Freeman KL, Brooks VL (2007) AT(1) and glutamatergic receptors in paraventricular nucleus support blood pressure during water deprivation. American journal of physiology Regulatory, integrative and comparative physiology 292:R1675-1682.

Friis UG, Madsen K, Stubbe J, Hansen PB, Svenningsen P, Bie P, Skott O, Jensen BL (2013) Regulation of renin secretion by renal juxtaglomerular cells. Pflugers Archiv : European journal of physiology 465:25-37.

Frithiof R, Xing T, McKinley MJ, May CN, Ramchandra R (2014) Intracarotid hypertonic sodium chloride differentially modulates sympathetic nerve activity to the heart and kidney. American journal of physiology Regulatory, integrative and comparative physiology 306:R567-575.

Furlong TM, McDowall LM, Horiuchi J, Polson JW, Dampney RA (2014) The effect of air puff stress on c-Fos expression in rat hypothalamus and brainstem: central circuitry mediating sympathoexcitation and baroreflex resetting. The European journal of neuroscience 39:1429-1438.

Gabor A, Leenen FH (2012) Cardiovascular effects of angiotensin II and glutamate in the PVN of Dahl salt-sensitive rats. Brain research 1447:28-37.

Gabor A, Leenen FH (2013) Central mineralocorticoid receptors and the role of angiotensin II and glutamate in the paraventricular nucleus of rats with angiotensin II-induced hypertension. Hypertension (Dallas, Tex : 1979) 61:1083-1090.

Gabow PA, Ikle DW, Holmes JH (1984) Polycystic kidney disease: prospective analysis of nonazotemic patients and family members. Annals of internal medicine 101:238-247.

Gabow PA, Johnson AM, Kaehny WD, Kimberling WJ, Lezotte DC, Duley IT, Jones RH (1992) Factors affecting the progression of renal disease in autosomal-dominant polycystic kidney disease. Kidney international 41:1311-1319.

Gabow PA, Chapman AB, Johnson AM, Tangel DJ, Duley IT, Kaehny WD, Manco-Johnson M, Schrier RW (1990) Renal structure and hypertension in autosomal dominant polycystic kidney disease. Kidney international 38:1177-1180.

Ganten D, Hayduk K, Brecht HM, Boucher R, Genest J (1970) Evidence of renin release or production in splanchnic territory. Nature 226:551-552.

Gao J, Zhang H, Le KD, Chao J, Gao L (2011) Activation of central angiotensin type 2 receptors suppresses norepinephrine excretion and blood pressure in conscious rats. American journal of hypertension 24:724-730.

Gao J, Zhang F, Sun HJ, Liu TY, Ding L, Kang YM, Zhu GQ, Zhou YB (2014) Transneuronal tracing of central autonomic regions involved in cardiac sympathetic afferent reflex in rats. Journal of the neurological sciences 342:45-51.

Garcia-Luna C, Soberanes-Chavez P, de Gortari P (2017) Impaired hypothalamic cocaine- and amphetamine-regulated transcript expression in lateral hypothalamic area and paraventricular nuclei of dehydration-induced anorexic rats. Journal of neuroendocrinology 29.

Gattone VH, 2nd, Wang X, Harris PC, Torres VE (2003) Inhibition of renal cystic disease development and progression by a vasopressin V2 receptor antagonist. Nature medicine 9:1323-1326.

Gattone VH, 2nd, Siqueira TM, Jr., Powell CR, Trambaugh CM, Lingeman JE, Shalhav AL (2008) Contribution of renal innervation to hypertension in rat autosomal dominant polycystic kidney disease. Experimental biology and medicine (Maywood, NJ) 233:952-957.

Geelen G, Keil LC, Kravik SE, Wade CE, Thrasher TN, Barnes PR, Pyka G, Nesvig C, Greenleaf JE (1984) Inhibition of plasma vasopressin after drinking in dehydrated humans. The American journal of physiology 247:R968-971.

Geerling JC, Mettenleiter TC, Loewy AD (2003) Orexin neurons project to diverse sympathetic outflow systems. Neuroscience 122:541-550.

Geerling JC, Shin JW, Chimenti PC, Loewy AD (2010) Paraventricular hypothalamic nucleus: axonal projections to the brainstem. The Journal of comparative neurology 518:1460-1499.

Geraldes V, Goncalves-Rosa N, Liu B, Paton JF, Rocha I (2014) Chronic depression of hypothalamic paraventricular neuronal activity produces sustained hypotension in hypertensive rats. Experimental physiology 99:89-100.

Gizowski C, Zaelzer C, Bourque CW (2016) Clock-driven vasopressin neurotransmission mediates anticipatory thirst prior to sleep. Nature 537:685-688.

Glass MJ, Wang G, Coleman CG, Chan J, Ogorodnik E, Van Kempen TA, Milner TA, Butler SD (2015) NMDA receptor plasticity in the hypothalamic paraventricular nucleus contributes to the elevated blood pressure produced by angiotensin II. The Journal of neuroscience : the official journal of the Society for Neuroscience 35:9558-9567.

Goa KL, Wagstaff AJ (1996) Losartan potassium: a review of its pharmacology, clinical efficacy and tolerability in the management of hypertension. Drugs 51:820-845.

Goldstein DS, Levinson PD, Zimlichman R, Pitterman A, Stull R, Keiser HR (1985) Clonidine suppression testing in essential hypertension. Annals of internal medicine 102:42-49.

Gomez RE, Cannata MA, Milner TA, Anwar M, Reis DJ, Ruggiero DA (1993) Vasopressinergic mechanisms in the nucleus reticularis lateralis in blood pressure control. Brain research 604:90-105.

Gonzalez-Villalobos RA, Janjoulia T, Fletcher NK, Giani JF, Nguyen MT, Riquier-Brison AD, Seth DM, Fuchs S, Eladari D, Picard N, Bachmann S, Delpire E, Peti-Peterdi J, Navar LG, Bernstein KE, McDonough AA (2013) The absence of intrarenal ACE protects against hypertension. The Journal of clinical investigation 123:2011-2023.

Gonzalez AD, Wang G, Waters EM, Gonzales KL, Speth RC, Van Kempen TA, Marques-Lopes J, Young CN, Butler SD, Davisson RL, Iadecola C, Pickel VM, Pierce JP, Milner TA (2012) Distribution of angiotensin type 1a receptor-containing cells in the brains of bacterial artificial chromosome transgenic mice. Neuroscience 226:489-509.

Gordon FJ, Leone C (1991) Non-NMDA receptors in the nucleus of the tractus solitarius play the predominant role in mediating aortic baroreceptor reflexes. Brain research 568:319-322.

Goto A, Ikeda T, Tobian L, Iwai J, Johnson MA (1981) Brain lesions in the paraventricular nuclei and catecholaminergic neurons minimize salt hypertension in Dahl salt-sensitive rats. Clinical science (London, England : 1979) 61 Suppl 7:53s-55s.

Goto A, Ganguli M, Tobian L, Johnson MA, Iwai J (1982) Effect of an anteroventral third ventricle lesion on NaCl hypertension in Dahl salt-sensitive rats. The American journal of physiology 243:H614-618.

Goto M, Hoxha N, Osman R, Dell KM (2010) The renin-angiotensin system and hypertension in autosomal recessive polycystic kidney disease. Pediatric nephrology (Berlin, Germany) 25:2449-2457.

Graffe CC, Bech JN, Lauridsen TG, Pedersen EB (2012) Urinary excretion of AQP2 and ENaC in autosomal dominant polycystic kidney disease during basal conditions and after a hypertonic saline infusion. American journal of physiology Renal physiology 302:F917-927.

Grantham JJ, Chapman AB, Torres VE (2006a) Volume progression in autosomal dominant polycystic kidney disease: the major factor determining clinical outcomes. Clinical journal of the American Society of Nephrology : CJASN 1:148-157.

Grantham JJ, Torres VE, Chapman AB, Guay-Woodford LM, Bae KT, King BF, Jr., Wetzel LH, Baumgarten DA, Kenney PJ, Harris PC, Klahr S, Bennett WM, Hirschman GN, Meyers CM, Zhang X, Zhu F, Miller JP (2006b) Volume progression in polycystic kidney disease. The New England journal of medicine 354:2122-2130.

Grassi G, Pisano A, Bolignano D, Seravalle G, D'Arrigo G, Quarti-Trevano F, Mallamaci F, Zoccali C, Mancia G (2018) Sympathetic nerve traffic activation in essential hypertension and its correlates: systematic reviews and meta-analyses. Hypertension (Dallas, Tex : 1979) 72:483-491.

Greenwood JP, Stoker JB, Mary DA (1999) Single-unit sympathetic discharge : quantitative assessment in human hypertensive disease. Circulation 100:1305-1310.

Grindstaff RJ, Grindstaff RR, Sullivan MJ, Cunningham JT (2000a) Role of the locus ceruleus in baroreceptor regulation of supraoptic vasopressin neurons in the rat. American journal of physiology Regulatory, integrative and comparative physiology 279:R306-319.

Grindstaff RR, Cunningham JT (2001) Lesion of the perinuclear zone attenuates cardiac sensitivity of vasopressinergic supraoptic neurons. American journal of physiology Regulatory, integrative and comparative physiology 280:R630-638.

Grindstaff RR, Grindstaff RJ, Cunningham JT (2000b) Effects of right atrial distension on the activity of magnocellular neurons in the supraoptic nucleus. American journal of physiology Regulatory, integrative and comparative physiology 278:R1605-1615.

Groth RD, Dunbar RL, Mermelstein PG (2003) Calcineurin regulation of neuronal plasticity. Biochemical and biophysical research communications 311:1159-1171.

Gu GB, Simerly RB (1997) Projections of the sexually dimorphic anteroventral periventricular nucleus in the female rat. The Journal of comparative neurology 384:142-164.

Guan S, Fox J, Mitchell KD, Navar LG (1992) Angiotensin and angiotensin converting enzyme tissue levels in two-kidney, one clip hypertensive rats. Hypertension (Dallas, Tex : 1979) 20:763-767.

Guay-Woodford LM, Desmond RA (2003) Autosomal recessive polycystic kidney disease: the clinical experience in North America. Pediatrics 111:1072-1080.

Guertzenstein PG, Silver A (1974) Fall in blood pressure produced from discrete regions of the ventral surface of the medulla by glycine and lesions. The Journal of physiology 242:489-503.

Guillon G, Grazzini E, Andrez M, Breton C, Trueba M, Serradeil-LeGal C, Boccara G, Derick S, Chouinard L, Gallo-Payet N (1998) Vasopressin : a potent autocrine/paracrine regulator of mammal adrenal functions. Endocrine research 24:703-710.

Guimaraes S, Moura D (2001) Vascular adrenoceptors: an update. Pharmacological reviews 53:319-356.

Gustavo B, Wallace DP (2013) Novel role of ouabain as a cystogenic factor in autosomal dominant polycystic kidney disease. American journal of physiology Renal physiology 305:F797-F812.

Gutman MB, Ciriello J, Mogenson GJ (1988) Effects of plasma angiotensin II and hypernatremia on subfornical organ neurons. The American journal of physiology 254:R746-754.

Guyenet PG (2006) The sympathetic control of blood pressure. Nature Reviews Neuroscience 7:335-346.

Guyenet PG, Filtz TM, Donaldson SR (1987) Role of excitatory amino acids in rat vagal and sympathetic baroreflexes. Brain research 407:272-284.

Guyton AC (1989) Dominant role of the kidneys and accessory role of whole-body autoregulation in the pathogenesis of hypertension. American journal of hypertension 2:575-585.

Haam J, Halmos KC, Di S, Tasker JG (2014) Nutritional state-dependent ghrelin activation of vasopressin neurons via retrograde trans-neuronal-glial stimulation of excitatory

GABA circuits. The Journal of neuroscience : the official journal of the Society for Neuroscience 34:6201-6213.

Hainsworth R (2014) Cardiovascular control from cardiac and pulmonary vascular receptors. Experimental physiology 99:312-319.

Hallbeck M, Blomqvist A (1999) Spinal cord-projecting vasopressinergic neurons in the rat paraventricular hypothalamus. The Journal of comparative neurology 411:201-211.

Han Y, Shi Z, Zhang F, Yu Y, Zhong MK, Gao XY, Wang W, Zhu GQ (2007) Reactive oxygen species in the paraventricular nucleus mediate the cardiac sympathetic afferent reflex in chronic heart failure rats. European journal of heart failure 9:967-973.

Han Y, Fan ZD, Yuan N, Xie GQ, Gao J, De W, Gao XY, Zhu GQ (2011) Superoxide anions in the paraventricular nucleus mediate the enhanced cardiac sympathetic afferent reflex and sympathetic activity in renovascular hypertensive rats. Journal of applied physiology (Bethesda, Md : 1985) 110:646-652.

Hanaoka K, Guggino WB (2000) cAMP regulates cell proliferation and cyst formation in autosomal polycystic kidney disease cells. Journal of the American Society of Nephrology : JASN 11:1179-1187.

Hanaoka K, Qian F, Boletta A, Bhunia AK, Piontek K, Tsiokas L, Sukhatme VP, Guggino WB, Germino GG (2000) Co-assembly of polycystin-1 and -2 produces unique cation-permeable currents. Nature 408:990-994.

Handa M, Kondo K, Suzuki H, Saruta T (1984) Dexamethasone hypertension in rats: role of prostaglandins and pressor sensitivity to norepinephrine. Hypertension (Dallas, Tex : 1979) 6:236-241.

Hansson L, Karlander LE, Lundgren W, Peterson LE (1974) Hypertension in polycystic kidney disease. Scandinavian journal of urology and nephrology 8:203-205.

Harding JW, Felix D (1987) Angiotensin-sensitive neurons in the rat paraventricular nucleus: relative potencies of angiotensin II and angiotensin III. Brain research 410:130-134.

Harlan SM, Morgan DA, Agassandian K, Guo DF, Cassell MD, Sigmund CD, Mark AL, Rahmouni K (2011) Ablation of the leptin receptor in the hypothalamic arcuate nucleus abrogates leptin-induced sympathetic activation. Circulation research 108:808-812.

Harris MC (1979) Effects of chemoreceptor and baroreceptor stimulation on the discharge of hypothalamic supraoptic neurones in rats. The Journal of endocrinology 82:115-125.

Harris PC, Rossetti S (2010) Determinants of renal disease variability in ADPKD. Advances in chronic kidney disease 17:131-139.

Harrison J, Hildreth CM, Callahan SM, Goodchild AK, Phillips JK (2010) Cardiovascular autonomic dysfunction in a novel rodent model of polycystic kidney disease. Autonomic neuroscience 152:60-66.

Haselton JR, Vari RC (1998) Neuronal cell bodies in paraventricular nucleus affect renal hemodynamics and excretion via the renal nerves. The American journal of physiology 275:R1334-1342.

Haselton JR, Goering J, Patel KP (1994) Parvocellular neurons of the paraventricular nucleus are involved in the reduction in renal nerve discharge during isotonic volume expansion. Journal of the autonomic nervous system 50:1-11.

Hasser EM, Bishop VS (1990) Reflex effect of vasopressin after blockade of V1 receptors in the area postrema. Circulation research 67:265-271.

Hastings MH, Maywood ES, Brancaccio M (2018) Generation of circadian rhythms in the suprachiasmatic nucleus. Nature Reviews Neuroscience 19:453-469.

Hateboer N, v Dijk MA, Bogdanova N, Coto E, Saggar-Malik AK, San Millan JL, Torra R, Breuning M, Ravine D (1999) Comparison of phenotypes of polycystic kidney disease types 1 and 2. European PKD1-PKD2 Study Group. Lancet (London, England) 353:103-107.

Head GA, Adams MA (1992) Characterization of the baroreceptor heart rate reflex during development in spontaneously hypertensive rats. Clinical and experimental pharmacology & physiology 19:587-597.

Hendel MD, Collister JP (2005) Contribution of the subfornical organ to angiotensin II-induced hypertension. American journal of physiology Heart and circulatory physiology 288:H680-685.

Heringer-Walther S, Batista EN, Walther T, Khosla MC, Santos RA, Campagnole-Santos MJ (2001) Baroreflex improvement in shr after ace inhibition involves angiotensin-(1-7). Hypertension (Dallas, Tex : 1979) 37:1309-1314.

Herman JP, Eyigor O, Ziegler DR, Jennes L (2000) Expression of ionotropic glutamate receptor subunit mRNAs in the hypothalamic paraventricular nucleus of the rat. The Journal of comparative neurology 422:352-362.

Hernández VS, Vázquez-Juárez E, Márquez MM, Jáuregui-Huerta F, Barrio RA, Zhang L (2015) Extra-neurohypophyseal axonal projections from individual vasopressin-

containing magnocellular neurons in rat hypothalamus. Frontiers in neuroanatomy 9:130-130.

Hess WR (1954) Diencephalon, autonomic and extrapyramidal functions: Grune & Stratton.

Higa KT, Mori E, Viana FF, Morris M, Michelini LC (2002) Baroreflex control of heart rate by oxytocin in the solitary-vagal complex. American journal of physiology Regulatory, integrative and comparative physiology 282:R537-545.

Hildebrandt F (2010) Genetic kidney diseases. Lancet (London, England) 375:1287-1295.

Hildebrandt F, Zhou W (2007) Nephronophthisis-associated ciliopathies. Journal of the American Society of Nephrology : JASN 18:1855-1871.

Hildebrandt F, Attanasio M, Otto E (2009) Nephronophthisis: disease mechanisms of a ciliopathy. Journal of the American Society of Nephrology : JASN 20:23-35.

Hildebrandt F, Waldherr R, Kutt R, Brandis M (1992) The nephronophthisis complex: clinical and genetic aspects. The Clinical investigator 70:802-808.

Hildreth C, Goodchild A, Phillips J (2013a) Insight into autonomic nervous system control of heart rate in the rat using analysis of heart rate variability and baroreflex sensitivity. In: Stimulation and Inhibition of Neurons (Pilowsky PM, Farnham MMJ, Fong AY, eds), pp 203-223: Humana Press.

Hildreth CM, Goodchild AK (2010) Role of ionotropic GABA, glutamate and glycine receptors in the tonic and reflex control of cardiac vagal outflow in the rat. BMC neuroscience 11:128-128.

Hildreth CM, Kandukuri DS, Goodchild AK, Phillips JK (2013b) Temporal development of baroreceptor dysfunction in a rodent model of chronic kidney disease. Clinical and experimental pharmacology & physiology 40:458-465.

Hirase H, Iwai Y, Takata N, Shinohara Y, Mishima T (2014) Volume transmission signalling via astrocytes. Philos Trans R Soc Lond B Biol Sci 369:20130604.

Hirooka Y, Polson JW, Potts PD, Dampney RA (1997) Hypoxia-induced Fos expression in neurons projecting to the pressor region in the rostral ventrolateral medulla. Neuroscience 80:1209-1224.

Hiyama TY, Yoshida M, Matsumoto M, Suzuki R, Matsuda T, Watanabe E, Noda M (2013) Endothelin-3 expression in the subfornical organ enhances the sensitivity of Na(x), the brain sodium-level sensor, to suppress salt intake. Cell metabolism 17:507-519.

Ho TA, Godefroid N, Gruzon D, Haymann JP, Marechal C, Wang X, Serra A, Pirson Y, Devuyst O (2012) Autosomal dominant polycystic kidney disease is associated with

central and nephrogenic defects in osmoregulation. Kidney international 82:1121-1129.

Hohle S, Spitznagel H, Rascher W, Culman J, Unger T (1995) Angiotensin AT1 receptor-mediated vasopressin release and drinking are potentiated by an AT2 receptor antagonist. European journal of pharmacology 275:277-282.

Hohnloser SH, Klingenheben T, van de Loo A, Hablawetz E, Just H, Schwartz PJ (1994) Reflex versus tonic vagal activity as a prognostic parameter in patients with sustained ventricular tachycardia or ventricular fibrillation. Circulation 89:1068-1073.

Holbein WW, Toney GM (2013) Sympathetic network drive during water deprivation does not increase respiratory or cardiac rhythmic sympathetic nerve activity. Journal of applied physiology (Bethesda, Md : 1985) 114:1689-1696.

Holbein WW, Toney GM (2015) Activation of the hypothalamic paraventricular nucleus by forebrain hypertonicity selectively increases tonic vasomotor sympathetic nerve activity. American journal of physiology Regulatory, integrative and comparative physiology 308:R351-R359.

Holbein WW, Bardgett ME, Toney GM (2014) Blood pressure is maintained during dehydration by hypothalamic paraventricular nucleus-driven tonic sympathetic nerve activity. The Journal of physiology 592:3783-3799.

Honda K, Negoro H, Dyball RE, Higuchi T, Takano S (1990) The osmoreceptor complex in the rat: evidence for interactions between the supraoptic and other diencephalic nuclei. The Journal of physiology 431:225-241.

Hooper NM, Turner AJ (1987) Isolation of two differentially glycosylated forms of peptidyl-dipeptidase A (angiotensin converting enzyme) from pig brain: a re-evaluation of their role in neuropeptide metabolism. The Biochemical journal 241:625-633.

Hopp K, Wang X, Ye H, Irazabal MV, Harris PC, Torres VE (2015) Effects of hydration in rats and mice with polycystic kidney disease. American journal of physiology Renal physiology 308:F261-F266.

Hosoya Y, Sugiura Y, Okado N, Loewy AD, Kohno K (1991) Descending input from the hypothalamic paraventricular nucleus to sympathetic preganglionic neurons in the rat. Experimental brain research 85:10-20.

Huang J, Chowhdury SI, Weiss ML (2002) Distribution of sympathetic preganglionic neurons innervating the kidney in the rat: PRV transneuronal tracing and serial reconstruction. Autonomic neuroscience : basic & clinical 95:57-70.

Huang W, Sved AF, Stricker EM (2000) Water ingestion provides an early signal inhibiting osmotically stimulated vasopressin secretion in rats. American journal of physiology Regulatory, integrative and comparative physiology 279:R756-760.

Huangfu D, Hwang LJ, Riley TA, Guyenet PG (1992) Splanchnic nerve response to A5 area stimulation in rats. The American journal of physiology 263:R437-446.

Hwang KR, Chan SH, Chan JY (1998) Noradrenergic neurotransmission at PVN in locus ceruleus-induced baroreflex suppression in rats. The American journal of physiology 274:H1284-1292.

Ibrahim NH, Thandapilly SJ, Jia Y, Netticadan T, Aukema H (2016) Soy protein alleviates hypertension and fish oil improves diastolic heart function in the Han:SPRD-Cy rat model of cystic kidney disease. Lipids 51:635-642.

Imboden H, Harding JW, Abhold RH, Ganten D, Felix D (1987) Improved immunohistochemical staining of angiotensin II in rat brain using affinity purified antibodies. Brain research 426:225-234.

Intebi AD, Flaxman MS, Ganong WF, Deschepper CF (1990) Angiotensinogen production by rat astroglial cells in vitro and in vivo. Neuroscience 34:545-554.

Iovino M, Steardo L (1984) Vasopressin release to central and peripheral angiotensin II in rats with lesions of the subfornical organ. Brain research 322:365-368.

Isidori AM, Graziadio C, Paragliola RM, Cozzolino A, Ambrogio AG, Colao A, Corsello SM, Pivonello R (2015) The hypertension of Cushing's syndrome: controversies in the pathophysiology and focus on cardiovascular complications. Journal of hypertension 33:44-60.

Itaya Y, Suzuki H, Matsukawa S, Kondo K, Saruta T (1986) Central renin-angiotensin system and the pathogenesis of DOCA-salt hypertension in rats. The American journal of physiology 251:H261-268.

Ito S, Komatsu K, Tsukamoto K, Sved AF (2000) Excitatory amino acids in the rostral ventrolateral medulla support blood pressure in spontaneously hypertensive rats. Hypertension (Dallas, Tex : 1979) 35:413-417.

Ito S, Komatsu K, Tsukamoto K, Sved AF (2001) Tonic excitatory input to the rostral ventrolateral medulla in Dahl salt-sensitive rats. Hypertension (Dallas, Tex : 1979) 37:687-691.

Ivanov A, Purves D (1989) Ongoing electrical activity of superior cervical ganglion cells in mammals of different size. The Journal of comparative neurology 284:398-404.

Ivy DD, Shaffer EM, Johnson AM, Kimberling WJ, Dobin A, Gabow PA (1995) Cardiovascular abnormalities in children with autosomal dominant polycystic kidney disease. Journal of the American Society of Nephrology : JASN 5:2032-2036.

Jackson EK, Herzer WA, Vyas SJ, Kost CK, Jr. (1999) Angiotensin II-induced renal vasoconstriction in genetic hypertension. The Journal of pharmacology and experimental therapeutics 291:329-334.

Jackson K, Silva HM, Zhang W, Michelini LC, Stern JE (2005) Exercise training differentially affects intrinsic excitability of autonomic and neuroendocrine neurons in the hypothalamic paraventricular nucleus. Journal of neurophysiology 94:3211-3220.

Jafar TH, Stark PC, Schmid CH, Strandgaard S, Kamper AL, Maschio G, Becker G, Perrone RD, Levey AS (2005) The effect of angiotensin-converting-enzyme inhibitors on progression of advanced polycystic kidney disease. Kidney international 67:265-271.

Janig W, Habler HJ (2003) Neurophysiological analysis of target-related sympathetic pathways--from animal to human: similarities and differences. Acta physiologica Scandinavica 177:255-274.

Jansen AS, Nguyen XV, Karpitskiy V, Mettenleiter TC, Loewy AD (1995) Central command neurons of the sympathetic nervous system: basis of the fight-or-flight response. Science (New York, NY) 270:644-646.

Jeewandara TM, Ameer OZ, Boyd R, Wyse BF, Underwood CF, Phillips JK (2015) Protective cardiorenal effects of spironolactone in a rodent model of polycystic kidney disease. Clinical and experimental pharmacology & physiology 42:353-360.

Jewell PA, Verney Ernest B (1957) An experimental attempt to determine the site of the neurohypophysial and osmoreceptors in the dog. Philosophical Transactions of the Royal Society of London Series B, Biological Sciences 240:197-324.

Jhamandas JH, Lind RW, Renaud LP (1989) Angiotensin II may mediate excitatory neurotransmission from the subfornical organ to the hypothalamic supraoptic nucleus: an anatomical and electrophysiological study in the rat. Brain research 487:52-61.

Jiang Z, Rajamanickam S, Justice NJ (2018) Local corticotropin-releasing factor signaling in the hypothalamic paraventricular nucleus. The Journal of neuroscience : the official journal of the Society for Neuroscience 38:1874-1890.

Jin CB, Rockhold RW (1989) Effects of paraventricular hypothalamic microinfusions of kainic acid on cardiovascular and renal excretory function in conscious rats. The Journal of pharmacology and experimental therapeutics 251:969-975.

Joe N, Scott V, Brown CH (2014) Glial regulation of extrasynaptic NMDA receptor-mediated excitation of supraoptic nucleus neurones during dehydration. Journal of neuroendocrinology 26:35-42.

Johansson M, Gao SA, Friberg P, Annerstedt M, Carlstrom J, Ivarsson T, Jensen G, Ljungman S, Mathillas O, Nielsen FD, Strombom U (2007) Baroreflex effectiveness index and baroreflex sensitivity predict all-cause mortality and sudden death in hypertensive patients with chronic renal failure. Journal of hypertension 25:163-168.

Johansson M, Gao SA, Friberg P, Annerstedt M, Bergstrom G, Carlstrom J, Ivarsson T, Jensen G, Ljungman S, Mathillas O, Nielsen FD, Strombom U (2005) Reduced baroreflex effectiveness index in hypertensive patients with chronic renal failure. American journal of hypertension 18:995-1000; discussion 1016.

John SG, Sigrist MK, McIntyre CW (2008) Impaired baroreflex sensitivity predicts mortality in chronic kidney disease. In: Computers in Cardiology, 2008, pp 745-748. Bologna: Computers in Cardiology.

Johnson AK, Xue B (2018) Central nervous system neuroplasticity and the sensitization of hypertension. Nature reviews Nephrology 14:750-766.

Johnson AM, Gabow PA (1997) Identification of patients with autosomal dominant polycystic kidney disease at highest risk for end-stage renal disease. Journal of the American Society of Nephrology : JASN 8:1560-1567.

Jones BE, Moore RY (1977) Ascending projections of the locus coeruleus in the rat. II. Autoradiographic study. Brain research 127:25-53.

Kaariainen H, Koskimies O, Norio R (1988) Dominant and recessive polycystic kidney disease in children: evaluation of clinical features and laboratory data. Pediatric nephrology (Berlin, Germany) 2:296-302.

Kalsbeek A, La Fleur S, Van Heijningen C, Buijs RM (2004) Suprachiasmatic GABAergic inputs to the paraventricular nucleus control plasma glucose concentrations in the rat via sympathetic innervation of the liver. The Journal of neuroscience : the official journal of the Society for Neuroscience 24:7604-7613.

Kaneko T, Fujiyama F, Hioki H (2002) Immunohistochemical localization of candidates for vesicular glutamate transporters in the rat brain. The Journal of comparative neurology 444:39-62.

Kannan H, Hayashida Y, Yamashita H (1989) Increase in sympathetic outflow by paraventricular nucleus stimulation in awake rats. The American journal of physiology 256:R1325-1330.

Kaplan BS, Fay J, Shah V, Dillon MJ, Barratt TM (1989) Autosomal recessive polycystic kidney disease. Pediatric nephrology (Berlin, Germany) 3:43-49.

Kato H, Iwai N, Inui H, Kimoto K, Uchiyama Y, Inagami T (1993) Regulation of vascular angiotensin release. Hypertension (Dallas, Tex : 1979) 21:446-454.

Kaur M, Chandran D, Lal C, Bhowmik D, Jaryal AK, Deepak KK, Agarwal SK (2013) Renal transplantation normalizes baroreflex sensitivity through improvement in central arterial stiffness. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 28:2645-2655.

Kawano H, Masuko S (2001) Tyrosine hydroxylase-immunoreactive projections from the caudal ventrolateral medulla to the subfornical organ in the rat. Brain research 903:154-161.

Kawano H, Masuko S (2010) Region-specific projections from the subfornical organ to the paraventricular hypothalamic nucleus in the rat. Neuroscience 169:1227-1234.

Kayaba Y, Nakamura A, Kasuya Y, Ohuchi T, Yanagisawa M, Komuro I, Fukuda Y, Kuwaki T (2003) Attenuated defense response and low basal blood pressure in orexin knockout mice. American journal of physiology Regulatory, integrative and comparative physiology 285:R581-593.

Keith DS, Torres VE, Johnson CM, Holley KE (1994) Effect of sodium chloride, enalapril, and losartan on the development of polycystic kidney disease in Han:SPRD rats. American journal of kidney diseases : the official journal of the National Kidney Foundation 24:491-498.

Kelleher CL, McFann KK, Johnson AM, Schrier RW (2004) Characteristics of hypertension in young adults with autosomal dominant polycystic kidney disease compared with the general U.S. population. American journal of hypertension 17:1029-1034.

Kennefick TM, Al-Nimri MA, Oyama TT, Thompson MM, Kelly FJ, Chapman JG, Anderson S (1999) Hypertension and renal injury in experimental polycystic kidney disease. Kidney international 56:2181-2190.

Kenney MJ, Claassen DE, Fels RJ, Saindon CS (1999) Cold stress alters characteristics of sympathetic nerve discharge bursts. Journal of applied physiology (Bethesda, Md : 1985) 87:732-742.

Kenney MJ, Weiss ML, Patel KP, Wang Y, Fels RJ (2001) Paraventricular nucleus bicuculline alters frequency components of sympathetic nerve discharge bursts. American journal of physiology Heart and circulatory physiology 281:H1233-1241.

Kent BB, Drane JW, Blumenstein B, Manning JW (1972) A mathematical model to assess changes in the baroreceptor reflex. Cardiology 57:295-310.

Khanmoradi M, Nasimi A (2016) Angiotensin II in the paraventricular nucleus stimulates sympathetic outflow to the cardiovascular system and make vasopressin release in rat. Neuroscience letters 632:98-103.

Khanmoradi M, Nasimi A (2017) Endogenous angiotensin II in the paraventricular nucleus regulates arterial pressure during hypotension in rat, a single-unit study. Neuroscience research 114:35-42.

Kim I, Fu Y, Hui K, Moeckel G, Mai W, Li C, Liang D, Zhao P, Ma J, Chen XZ, George AL, Jr., Coffey RJ, Feng ZP, Wu G (2008) Fibrocystin/polyductin modulates renal tubular formation by regulating polycystin-2 expression and function. Journal of the American Society of Nephrology : JASN 19:455-468.

Kim JS, Kim WB, Kim YB, Lee Y, Kim YS, Shen FY, Lee SW, Park D, Choi HJ, Hur J, Park JJ, Han HC, Colwell CS, Cho YW, Kim YI (2011) Chronic hyperosmotic stress converts GABAergic inhibition into excitation in vasopressin and oxytocin neurons in the rat. The Journal of neuroscience : the official journal of the Society for Neuroscience 31:13312-13322.

Kim YB, Kim YS, Kim WB, Shen FY, Lee SW, Chung HJ, Kim JS, Han HC, Colwell CS, Kim YI (2013) GABAergic excitation of vasopressin neurons: possible mechanism underlying sodium-dependent hypertension. Circulation research 113:1296-1307.

King BF, Reed JE, Bergstralh EJ, Sheedy PF, 2nd, Torres VE (2000) Quantification and longitudinal trends of kidney, renal cyst, and renal parenchyma volumes in autosomal dominant polycystic kidney disease. Journal of the American Society of Nephrology : JASN 11:1505-1511.

Kinsman B, Cowles J, Lay J, Simmonds SS, Browning KN, Stocker SD (2014) Osmoregulatory thirst in mice lacking the transient receptor potential vanilloid type 1 (TRPV1) and/or type 4 (TRPV4) receptor. American journal of physiology Regulatory, integrative and comparative physiology 307:R1092-1100.

Kinsman BJ, Browning KN, Stocker SD (2017a) NaCl and osmolarity produce different responses in organum vasculosum of the lamina terminalis neurons, sympathetic nerve activity and blood pressure. The Journal of physiology 595:6187-6201.

Kinsman BJ, Simmonds SS, Browning KN, Stocker SD (2017b) Organum vasculosum of the lamina terminalis detects NaCl to elevate sympathetic nerve activity and blood pressure. Hypertension (Dallas, Tex : 1979) 69:163-170.

Klein IH, Ligtenberg G, Oey PL, Koomans HA, Blankestijn PJ (2001) Sympathetic activity is increased in polycystic kidney disease and is associated with hypertension. Journal of the American Society of Nephrology : JASN 12:2427-2433.

Klein IH, Ligtenberg G, Oey PL, Koomans HA, Blankestijn PJ (2003a) Enalapril and losartan reduce sympathetic hyperactivity in patients with chronic renal failure. Journal of the American Society of Nephrology : JASN 14:425-430.

Klein IH, Ligtenberg G, Neumann J, Oey PL, Koomans HA, Blankestijn PJ (2003b) Sympathetic nerve activity is inappropriately increased in chronic renal disease. Journal of the American Society of Nephrology : JASN 14:3239-3244.

Knepel W, Nutto D, Meyer DK (1982) Effect of transection of subfornical organ efferent projections on vasopressin release induced by angiotensin or isoprenaline in the rat. Brain research 248:180-184.

Knobloch HS, Charlet A, Hoffmann LC, Eliava M, Khrulev S, Cetin AH, Osten P, Schwarz MK, Seeburg PH, Stoop R, Grinevich V (2012) Evoked axonal oxytocin release in the central amygdala attenuates fear response. Neuron 73:553-566.

Knott TK, Marrero HG, Fenton RA, Custer EE, Dobson JG, Jr., Lemos JR (2007) Endogenous adenosine inhibits CNS terminal Ca(2+) currents and exocytosis. Journal of cellular physiology 210:309-314.

Koba S, Hanai E, Kumada N, Kataoka N, Nakamura K, Watanabe T (2018) Sympathoexcitation by hypothalamic paraventricular nucleus neurons projecting to the rostral ventrolateral medulla. The Journal of physiology 596:4581-4595.

Kobori H, Nangaku M, Navar LG, Nishiyama A (2007) The intrarenal renin-angiotensin system: from physiology to the pathobiology of hypertension and kidney disease. Pharmacological reviews 59:251-287.

Kobuch S, Fazalbhoy A, Brown R, Macefield VG, Henderson LA (2018) Muscle sympathetic nerve activity-coupled changes in brain activity during sustained muscle pain. Brain and behavior 8:e00888-e00888.

Kocyigit I, Yilmaz MI, Unal A, Ozturk F, Eroglu E, Yazici C, Orscelik O, Sipahioglu MH, Tokgoz B, Oymak O (2013) A link between the intrarenal renin angiotensin system and hypertension in autosomal dominant polycystic kidney disease. American journal of nephrology 38:218-225.

Kondo N, Arima H, Banno R, Kuwahara S, Sato I, Oiso Y (2004) Osmoregulation of vasopressin release and gene transcription under acute and chronic hypovolemia in

rats. American Journal of Physiology-Endocrinology and Metabolism 286:E337-E346.

Konishi S, Tsunoo A, Otsuka M (1981) Enkephalin as a transmitter for presynaptic inhibition in sympathetic ganglia. Nature 294:80-82.

Koren MJ, Devereux RB, Casale PN, Savage DD, Laragh JH (1991) Relation of left ventricular mass and geometry to morbidity and mortality in uncomplicated essential hypertension. Annals of internal medicine 114:345-352.

Korim WS, Elsaafien K, Basser JR, Setiadi A, May CN, Yao ST (2018) In renovascular hypertension, TNF-alpha type-1 receptors in the area postrema mediate increases in cardiac and renal sympathetic nerve activity and blood pressure. Cardiovascular research.

Korpal AK, Han SY, Schwenke DO, Brown CH (2017) A switch from GABA inhibition to excitation of vasopressin neurons exacerbates the development angiotensin II-dependent hypertension. Journal of neuroendocrinology.

Koshimizu TA, Nakamura K, Egashira N, Hiroyama M, Nonoguchi H, Tanoue A (2012) Vasopressin V1a and V1b receptors: from molecules to physiological systems. Physiological reviews 92:1813-1864.

Koshiya N, Huangfu D, Guyenet PG (1993) Ventrolateral medulla and sympathetic chemoreflex in the rat. Brain research 609:174-184.

Kotas ME, Medzhitov R (2015) Homeostasis, inflammation, and disease susceptibility. Cell 160:816-827.

Krashes MJ, Koda S, Ye C, Rogan SC, Adams AC, Cusher DS, Maratos-Flier E, Roth BL, Lowell BB (2011) Rapid, reversible activation of AgRP neurons drives feeding behavior in mice. The Journal of clinical investigation 121:1424-1428.

Krause EG, Melhorn SJ, Davis JF, Scott KA, Ma LY, de Kloet AD, Benoit SC, Woods SC, Sakai RR (2008) Angiotensin type 1 receptors in the subfornical organ mediate the drinking and hypothalamic-pituitary-adrenal response to systemic isoproterenol. Endocrinology 149:6416-6424.

Krause EG, Alleyne AR, Cahill KM, Smeltzer MD, Bruce EB, Tan Y, Harden SW, Frazier CJ, de Kloet AD (2018) Identifying ‘angiotensin sensitive’ neurons in the lamina terminalis that coordinate endocrine, cardiovascular and behavioral responses mediating body fluid homeostasis. The FASEB Journal 32:598.597-598.597.

Krieger EM (1989) Arterial baroreceptor resetting in hypertension (the J. W. McCubbin memorial lecture). Clinical and experimental pharmacology & physiology Supplement 15:3-17.

Krisch B, Leonhardt H, Oksche A (1987) Compartments in the organum vasculosum laminae terminalis of the rat and their delineation against the outer cerebrospinal fluid-containing space. Cell and tissue research 250:331-347.

Kunkler PE, Hwang BH (1995) Lower GABAA receptor binding in the amygdala and hypothalamus of spontaneously hypertensive rats. Brain research bulletin 36:57-61.

Kurtz TW, Griffin KA, Bidani AK, Davisson RL, Hall JE (2005) Recommendations for blood pressure measurement in humans and experimental animals: part 2: blood pressure measurement in experimental animals: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. Arteriosclerosis, thrombosis, and vascular biology 25:e22-33.

La Rovere MT, Bigger JT, Jr., Marcus FI, Mortara A, Schwartz PJ (1998) Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) Investigators. Lancet (London, England) 351:478-484.

Lacerda JE, Campos RR, Araujo GC, Andreatta-Van Leyen S, Lopes OU, Guertzenstein PG (2003) Cardiovascular responses to microinjections of GABA or anesthetics into the rostral ventrolateral medulla of conscious and anesthetized rats. Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas 36:1269-1277.

Lacy P, Carr SJ, O'Brien D, Fentum B, Williams B, Paul SK, Robinson TG (2006) Reduced glomerular filtration rate in pre-dialysis non-diabetic chronic kidney disease patients is associated with impaired baroreceptor sensitivity and reduced vascular compliance. Clinical Science 110:101-108.

Lager DJ, Qian Q, Bengal RJ, Ishibashi M, Torres VE (2001) The pck rat: a new model that resembles human autosomal dominant polycystic kidney and liver disease. Kidney international 59:126-136.

Lal C, Kaur M, Jaryal AK, Deepak KK, Bhowmik D, Agarwal SK (2017) Reduced Baroreflex Sensitivity, Decreased Heart Rate Variability with Increased Arterial Stiffness in Predialysis. Indian journal of nephrology 27:446-451.

Langlet F, Mullier A, Bouret SG, Prevot V, Dehouck B (2013) Tanycyte-like cells form a blood-cerebrospinal fluid barrier in the circumventricular organs of the mouse brain. The Journal of comparative neurology 521:3389-3405.

Langley JN (1893) The Arrangement of the Sympathetic Nervous System, based chiefly on Observations upon Pilo-motor Nerves. The Journal of physiology 15:176-248.121.

Laredo J, Hamilton BP, Hamlyn JM (1994) Ouabain is secreted by bovine adrenocortical cells. Endocrinology 135:794-797.

Larsen PJ, Mikkelsen JD (1995) Functional identification of central afferent projections conveying information of acute "stress" to the hypothalamic paraventricular nucleus. The Journal of neuroscience : the official journal of the Society for Neuroscience 15:2609-2627.

Larsen PJ, Moller M, Mikkelsen JD (1991) Efferent projections from the periventricular and medial parvicellular subnuclei of the hypothalamic paraventricular nucleus to circumventricular organs of the rat: a Phaseolus vulgaris-leucoagglutinin (PHA-L) tracing study. The Journal of comparative neurology 306:462-479.

Latchford KJ, Ferguson AV (2004) ANG II-induced excitation of paraventricular nucleus magnocellular neurons: a role for glutamate interneurons. American journal of physiology Regulatory, integrative and comparative physiology 286:R894-902.

Latchford KJ, Ferguson AV (2005) Angiotensin depolarizes parvocellular neurons in paraventricular nucleus through modulation of putative nonselective cationic and potassium conductances. American journal of physiology Regulatory, integrative and comparative physiology 289:R52-58.

Lau EO, Lo CY, Yao Y, Mak AF, Jiang L, Huang Y, Yao X (2016) Aortic baroreceptors display higher mechanosensitivity than carotid baroreceptors. Frontiers in physiology 7:384.

Lawrence AJ, Jarrott B (1994) L-glutamate as a neurotransmitter at baroreceptor afferents: evidence from in vivo microdialysis. Neuroscience 58:585-591.

Lea JP, Jin SG, Roberts BR, Shuler MS, Marrero MB, Tumlin JA (2002) Angiotensin II stimulates calcineurin activity in proximal tubule epithelia through AT-1 receptor-mediated tyrosine phosphorylation of the PLC-gamma1 isoform. Journal of the American Society of Nephrology : JASN 13:1750-1756.

Leenen FH, Stricker EM (1974) Plasma renin activity and thirst following hypovolemia or caval ligation in rats. The American journal of physiology 226:1238-1242.

Leib DE, Zimmerman CA, Poormoghaddam A, Huey EL, Ahn JS, Lin YC, Tan CL, Chen Y, Knight ZA (2017) The forebrain thirst circuit drives drinking through negative reinforcement. Neuron 96:1272-1281.e1274.

Leier CV, Baker PB, Kilman JW, Wooley CF (1984) Cardiovascular abnormalities associated with adult polycystic kidney disease. Annals of internal medicine 100:683-688.

Leite LH, Zheng H, Coimbra CC, Patel KP (2012) Contribution of the paraventricular nucleus in autonomic adjustments to heat stress. Experimental biology and medicine (Maywood, NJ) 237:570-577.

Lemos FO, Ehrlich BE (2018) Polycystin and calcium signaling in cell death and survival. Cell calcium 69:37-45.

Leng G, Brown CH, Russell JA (1999) Physiological pathways regulating the activity of magnocellular neurosecretory cells. Progress in neurobiology 57:625-655.

Leng G, Blackburn RE, Dyball RE, Russell JA (1989) Role of anterior peri-third ventricular structures in the regulation of supraoptic neuronal activity and neurohypophysical hormone secretion in the rat. Journal of neuroendocrinology 1:35-46.

Leng G, Brown CH, Bull PM, Brown D, Scullion S, Currie J, Blackburn-Munro RE, Feng J, Onaka T, Verbalis JG, Russell JA, Ludwig M (2001) Responses of magnocellular neurons to osmotic stimulation involves coactivation of excitatory and inhibitory input: an experimental and theoretical analysis. The Journal of neuroscience : the official journal of the Society for Neuroscience 21:6967-6977.

Lenkei Z, Corvol P, Llorens-Cortes C (1995) Comparative expression of vasopressin and angiotensin type-1 receptor mRNA in rat hypothalamic nuclei: a double in situ hybridization study. Brain research Molecular brain research 34:135-142.

Leone C, Gordon FJ (1989) Is L-glutamate a neurotransmitter of baroreceptor information in the nucleus of the tractus solitarius? The Journal of pharmacology and experimental therapeutics 250:953-962.

Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP (1990) Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. The New England journal of medicine 322:1561-1566.

Li AJ, Wang Q, Elsarelli MM, Brown RL, Ritter S (2015a) Hindbrain catecholamine neurons activate orexin neurons during systemic glucoprivation in male rats. Endocrinology 156:2807-2820.

Li D-P, Pan H-L (2007a) Glutamatergic inputs in the hypothalamic paraventricular nucleus maintain sympathetic vasomotor tone in hypertension. Hypertension (Dallas, Tex : 1979) 49:916-925.

Li DP, Pan HL (2005) Angiotensin II attenuates synaptic GABA release and excites paraventricular-rostral ventrolateral medulla output neurons. The Journal of pharmacology and experimental therapeutics 313:1035-1045.

Li DP, Pan HL (2006) Plasticity of GABAergic control of hypothalamic presympathetic neurons in hypertension. American journal of physiology Heart and circulatory physiology 290:H1110-1119.

Li DP, Pan HL (2007b) Glutamatergic inputs in the hypothalamic paraventricular nucleus maintain sympathetic vasomotor tone in hypertension. Hypertension (Dallas, Tex : 1979) 49:916-925.

Li DP, Pan HL (2017) Glutamatergic regulation of hypothalamic presympathetic neurons in hypertension. Current hypertension reports 19:78.

Li DP, Chen SR, Pan HL (2003a) Angiotensin II stimulates spinally projecting paraventricular neurons through presynaptic disinhibition. The Journal of neuroscience : the official journal of the Society for Neuroscience 23:5041-5049.

Li DP, Zhou JJ, Pan HL (2015b) Endogenous casein kinase-1 modulates NMDA receptor activity of hypothalamic presympathetic neurons and sympathetic outflow in hypertension. The Journal of physiology 593:4439-4452.

Li DP, Chen SR, Finnegan TF, Pan HL (2004) Signalling pathway of nitric oxide in synaptic GABA release in the rat paraventricular nucleus. The Journal of physiology 554:100-110.

Li DP, Yang Q, Pan HM, Pan HL (2008) Pre- and postsynaptic plasticity underlying augmented glutamatergic inputs to hypothalamic presympathetic neurons in spontaneously hypertensive rats. The Journal of physiology 586:1637-1647.

Li DP, Zhou JJ, Zhang J, Pan HL (2017) CaMKII regulates synaptic NMDA receptor activity of hypothalamic presympathetic neurons and sympathetic outflow in hypertension. The Journal of neuroscience : the official journal of the Society for Neuroscience 37:10690-10699.

Li M, Wen C, Fraser T, Whitworth JA (1999) Adrenocorticotrophin-induced hypertension: effects of mineralocorticoid and glucocorticoid receptor antagonism. Journal of hypertension 17:419-426.

Li W, Peng H, Mehaffey EP, Kimball CD, Grobe JL, van Gool JM, Sullivan MN, Earley S, Danser AH, Ichihara A, Feng Y (2014) Neuron-specific (pro)renin receptor knockout prevents the development of salt-sensitive hypertension. Hypertension (Dallas, Tex : 1979) 63:316-323.

Li Y-F, Cornish KG, Patel KP (2003b) Alteration of NMDA NR1 receptors within the paraventricular nucleus of hypothalamus in rats with heart failure. Circulation research 93:990-997.

Li Y, Wright JM, Qian F, Germino GG, Guggino WB (2005) Polycystin 2 interacts with type I inositol 1,4,5-trisphosphate receptor to modulate intracellular Ca2+ signaling. The Journal of biological chemistry 280:41298-41306.

Li YF, Mayhan WG, Patel KP (2001) NMDA-mediated increase in renal sympathetic nerve discharge within the PVN: role of nitric oxide. American journal of physiology Heart and circulatory physiology 281:H2328-2336.

Li YF, Cornish KG, Patel KP (2003c) Alteration of NMDA NR1 receptors within the paraventricular nucleus of hypothalamus in rats with heart failure. Circulation research 93:990-997.

Li YF, Jackson KL, Stern JE, Rabeler B, Patel KP (2006) Interaction between glutamate and GABA systems in the integration of sympathetic outflow by the paraventricular nucleus of the hypothalamus. American journal of physiology Heart and circulatory physiology 291:H2847-2856.

Li Z, Ferguson AV (1993a) Subfornical organ efferents to paraventricular nucleus utilize angiotensin as a neurotransmitter. The American journal of physiology 265:R302-309.

Li Z, Ferguson AV (1993b) Angiotensin II responsiveness of rat paraventricular and subfornical organ neurons in vitro. Neuroscience 55:197-207.

Li Z, Bains JS, Ferguson AV (1993) Functional evidence that the angiotensin antagonist losartan crosses the blood-brain barrier in the rat. Brain research bulletin 30:33-39.

Ligtenberg G, Blankestijn PJ, Oey PL, Klein IHH, Dijkhorst-Oei L-T, Boomsma F, Wieneke GH, van Huffelen AC, Koomans HA (1999) Reduction of sympathetic hyperactivity by enalapril in patients with chronic renal failure. The New England journal of medicine 340:1321-1328.

Lim K, Burke SL, Head GA (2013) Obesity-related hypertension and the role of insulin and leptin in high-fat-fed rabbits. Hypertension (Dallas, Tex : 1979) 61:628-634.

Lind RW (1986) Bi-directional, chemically specified neural connections between the subfornical organ and the midbrain raphe system. Brain research 384:250-261.

Lind RW, Van Hoesen GW, Johnson AK (1982) An HRP study of the connections of the subfornical organ of the rat. The Journal of comparative neurology 210:265-277.

Lind RW, Swanson LW, Ganten D (1984) Angiotensin II immunoreactive pathways in the central nervous system of the rat: evidence for a projection from the subfornical organ to the paraventricular nucleus of the hypothalamus. Clinical and experimental hypertension Part A, Theory and practice 6:1915-1920.

Lindpaintner K, Jin M, Wilhelm MJ, Suzuki F, Linz W, Schoelkens BA, Ganten D (1988) Intracardiac generation of angiotensin and its physiologic role. Circulation 77:I18-23.

Ling GY, Cao WH, Onodera M, Ju KH, Kurihara H, Kurihara Y, Yazaki Y, Kumada M, Fukuda Y, Kuwaki T (1998) Renal sympathetic nerve activity in mice: comparison between mice and rats and between normal and endothelin-1 deficient mice. Brain research 808:238-249.

Lippoldt A, Bunnemann B, Iwai N, Metzger R, Inagami T, Fuxe K, Ganten D (1993) Cellular localization of angiotensin type 1 receptor and angiotensinogen mRNAs in the subfornical organ of the rat brain. Neuroscience letters 150:153-158.

Lipski J, Kanjhan R, Kruszewska B, Rong W (1996) Properties of presympathetic neurones in the rostral ventrolateral medulla in the rat: an intracellular study "in vivo'. The Journal of physiology 490 (Pt 3):729-744.

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402-408.

Llewellyn-Smith IJ, Verberne AJM (2011) Central Regulation of Autonomic Functions: Oxford University Press.

Llewellyn T, Zheng H, Liu X, Xu B, Patel KP (2012) Median preoptic nucleus and subfornical organ drive renal sympathetic nerve activity via a glutamatergic mechanism within the paraventricular nucleus. American journal of physiology Regulatory, integrative and comparative physiology 302:R424-432.

Loewy AD, Burton H (1978) Nuclei of the solitary tract: efferent projections to the lower brain stem and spinal cord of the cat. The Journal of comparative neurology 181:421-449.

Loghman-Adham M, Soto CE, Inagami T, Cassis L (2004) The intrarenal renin-angiotensin system in autosomal dominant polycystic kidney disease. American journal of physiology Renal physiology 287:F775-788.

Loghman-Adham M, Soto CE, Inagami T, Sotelo-Avila C (2005) Expression of components of the renin-angiotensin system in autosomal recessive polycystic kidney disease. The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society 53:979-988.

Lorenz JN, Loreaux EL, Dostanic-Larson I, Lasko V, Schnetzer JR, Paul RJ, Lingrel JB (2008) ACTH-induced hypertension is dependent on the ouabain-binding site of the alpha2-Na+-K+-ATPase subunit. American journal of physiology Heart and circulatory physiology 295:H273-280.

Ludens JH, Clark MA, Robinson FG, DuCharme DW (1992) Rat adrenal cortex is a source of a circulating ouabainlike compound. Hypertension (Dallas, Tex : 1979) 19:721-724.

Ludwig M, Leng G (2006) Dendritic peptide release and peptide-dependent behaviours. Nature Reviews Neuroscience 7:126-136.

Ludwig M, Williams K, Callahan MF, Morris M (1996) Salt loading abolishes osmotically stimulated vasopressin release within the supraoptic nucleus. Neuroscience letters 215:1-4.

Ludwig M, Horn T, Callahan MF, Grosche A, Morris M, Landgraf R (1994) Osmotic stimulation of the supraoptic nucleus: central and peripheral vasopressin release and blood pressure. The American journal of physiology 266:E351-356.

Lumiaho A, Ikaheimo R, Miettinen R, Niemitukia L, Laitinen T, Rantala A, Lampainen E, Laakso M, Hartikainen J (2001) Mitral valve prolapse and mitral regurgitation are common in patients with polycystic kidney disease type 1. American journal of kidney diseases : the official journal of the National Kidney Foundation 38:1208-1216.

Macefield VG, Wallin BG, Vallbo AB (1994) The discharge behaviour of single vasoconstrictor motoneurones in human muscle nerves. The Journal of physiology 481 (Pt 3):799-809.

Macova M, Pavel J, Saavedra JM (2009) A peripherally administered, centrally acting angiotensin II AT2 antagonist selectively increases brain AT1 receptors and decreases brain tyrosine hydroxylase transcription, pituitary vasopressin and ACTH. Brain research 1250:130-140.

Madden CJ, Sved AF (2003) Cardiovascular regulation after destruction of the C1 cell group of the rostral ventrolateral medulla in rats. American journal of physiology Heart and circulatory physiology 285:H2734-2748.

Madden CJ, Stocker SD, Sved AF (2006) Attenuation of homeostatic responses to hypotension and glucoprivation after destruction of catecholaminergic rostral ventrolateral medulla neurons. American journal of physiology Regulatory, integrative and comparative physiology 291:R751-759.

Maiorov DN, Malpas SC, Head GA (2000) Influence of pontine A5 region on renal sympathetic nerve activity in conscious rabbits. American journal of physiology Regulatory, integrative and comparative physiology 278:R311-319.

Maiorov DN, Wilton ER, Badoer E, Petrie D, Head GA, Malpas SC (1999) Sympathetic response to stimulation of the pontine A5 region in conscious rabbits. Brain research 815:227-236.

Malpas SC (2010) Sympathetic nervous system overactivity and its role in the development of cardiovascular disease. Physiological reviews 90:513-557.

Malpas SC, Coote JH (1994) Role of vasopressin in sympathetic response to paraventricular nucleus stimulation in anesthetized rats. The American journal of physiology 266:R228-236.

Mandelblat-Cerf Y, Kim A, Burgess CR, Subramanian S, Tannous BA, Lowell BB, Andermann ML (2017) Bidirectional anticipation of future osmotic challenges by vasopressin neurons. Neuron 93:57-65.

Mangiapane ML, Simpson JB (1980) Subfornical organ lesions reduce the pressor effect of systemic angiotensin II. Neuroendocrinology 31:380-384.

Mangiapane ML, Thrasher TN, Keil LC, Simpson JB, Ganong WF (1983) Deficits in drinking and vasopressin secretion after lesions of the nucleus medianus. Neuroendocrinology 37:73-77.

Mangiapane ML, Thrasher TN, Keil LC, Simpson JB, Ganong WF (1984) Role for the subfornical organ in vasopressin release. Brain research bulletin 13:43-47.

Mangoo-Karim R, Uchic M, Lechene C, Grantham JJ (1989) Renal epithelial cyst formation and enlargement in vitro: dependence on cAMP. Proceedings of the National Academy of Sciences of the United States of America 86:6007-6011.

Mangos GJ, Turner SW, Fraser TB, Whitworth JA (2000) The role of corticosterone in corticotrophin (ACTH)-induced hypertension in the rat. Journal of hypertension 18:1849-1855.

Marina N, Ang R, Machhada A, Kasymov V, Karagiannis A, Hosford PS, Mosienko V, Teschemacher AG, Vihko P, Paton JF, Kasparov S, Gourine AV (2015) Brainstem

hypoxia contributes to the development of hypertension in the spontaneously hypertensive rat. Hypertension (Dallas, Tex : 1979) 65:775-783.

Marrero HG, Lemos JR (2010) Ionic conditions modulate stimulus-induced capacitance changes in isolated neurohypophysial terminals of the rat. The Journal of physiology 588:287-300.

Martin DS, Haywood JR (1998) Reduced GABA inhibition of sympathetic function in renal-wrapped hypertensive rats. The American journal of physiology 275:R1523-1529.

Martin DS, Segura T, Haywood JR (1991) Cardiovascular responses to bicuculline in the paraventricular nucleus of the rat. Hypertension (Dallas, Tex : 1979) 18:48-55.

Martin DS, Rodrigo MC, Egland MC, Barnes LU (1997) Disinhibition of the hypothalamic paraventricular nucleus increases mean circulatory filling pressure in conscious rats. Brain research 756:106-113.

Martinez-Vea A, Valero FA, Bardaji A, Gutierrez C, Broch M, Garcia C, Richart C, Oliver JA (2000) Left ventricular hypertrophy in hypertensive patients with autosomal dominant polycystic kidney disease: influence of blood pressure and humoral and neurohormonal factors. American journal of nephrology 20:193-200.

Martins-Pinge MC, Mueller PJ, Foley CM, Heesch CM, Hasser EM (2012) Regulation of arterial pressure by the paraventricular nucleus in conscious rats: interactions among glutamate, GABA, and nitric oxide. Frontiers in physiology 3:490.

Masuda N, Ootsuka Y, Terui N (1992) Neurons in the caudal ventrolateral medulla mediate the somato-sympathetic inhibitory reflex response via GABA receptors in the rostral ventrolateral medulla. Journal of the autonomic nervous system 40:91-98.

Matsuda T, Hiyama TY, Niimura F, Matsusaka T, Fukamizu A, Kobayashi K, Kobayashi K, Noda M (2017) Distinct neural mechanisms for the control of thirst and salt appetite in the subfornical organ. Nature neuroscience 20:230-241.

Matsuguchi H, Schmid PG, Van Orden D, Mark AL (1981) Does vasopressin contribute to salt-induced hypertension in the Dahl strain? Hypertension (Dallas, Tex : 1979) 3:174-181.

Matsukawa T, Miyamoto T (2011) Angiotensin II-stimulated secretion of arginine vasopressin is inhibited by atrial natriuretic peptide in humans. American journal of physiology Regulatory, integrative and comparative physiology 300:R624-629.

Matsukawa T, Gotoh E, Minamisawa K, Kihara M, Ueda S, Shionoiri H, Ishii M (1991) Effects of intravenous infusions of angiotensin II on muscle sympathetic nerve activity in humans. The American journal of physiology 261:R690-696.

Matsuoka T, Wisner DH (1997) Hemodynamic and metabolic effects of vasopressin blockade in endotoxin shock. Surgery 121:162-173.

McAllen RM, Malpas SC (1997) Sympathetic burst activity: characteristics and significance. Clinical and experimental pharmacology & physiology 24:791-799.

McBryde FD, Abdala AP, Hendy EB, Pijacka W, Marvar P, Moraes DJ, Sobotka PA, Paton JF (2013) The carotid body as a putative therapeutic target for the treatment of neurogenic hypertension. Nature communications 4:2395.

McCooke J, Appels R, Barrero R, Ding A, Ozimek-Kulik J, Bellgard M, Morahan G, Phillips J (2012) A novel mutation causing nephronophthisis in the Lewis polycystic kidney rat localises to a conserved RCC1 domain in Nek8. BMC Genomics 13:1-17.

McDowall LM, Horiuchi J, Killinger S, Dampney RA (2006) Modulation of the baroreceptor reflex by the dorsomedial hypothalamic nucleus and perifornical area. American journal of physiology Regulatory, integrative and comparative physiology 290:R1020-1026.

McKinley MJ, Denton DA, Weisinger RS (1978) Sensors for antidiuresis and thirst--osmoreceptors or CSF sodium detectors? Brain research 141:89-103.

McKinley MJ, Badoer E, Oldfield BJ (1992) Intravenous angiotensin II induces Fos-immunoreactivity in circumventricular organs of the lamina terminalis. Brain research 594:295-300.

McKinley MJ, Allen AM, Burns P, Colvill LM, Oldfield BJ (1998) Interaction of circulating hormones with the brain: the roles of the subfornical organ and the organum vasculosum of the lamina terminalis. Clinical and experimental pharmacology & physiology Supplement 25:S61-67.

McKinley MJ, Yao ST, Uschakov A, McAllen RM, Rundgren M, Martelli D (2015) The median preoptic nucleus: front and centre for the regulation of body fluid, sodium, temperature, sleep and cardiovascular homeostasis. Acta physiologica (Oxford, England) 214:8-32.

McKinley MJ, Allen AM, May CN, McAllen RM, Oldfield BJ, Sly D, Mendelsohn FA (2001) Neural pathways from the lamina terminalis influencing cardiovascular and body fluid homeostasis. Clinical and experimental pharmacology & physiology 28:990-992.

McKinley MJ, Congiu M, Denton DA, Park RG, Penschow J, Simpson JB, Tarjan E, Weisinger RS, Wright RD (1984) The anterior wall of the third cerebral ventricle and homeostatic responses to dehydration. Journal de physiologie 79:421-427.

McKinley MJ, Mathai ML, McAllen RM, McClear RC, Miselis RR, Pennington GL, Vivas L, Wade JD, Oldfield BJ (2004) Vasopressin secretion: osmotic and hormonal regulation by the lamina terminalis. Journal of neuroendocrinology 16:340-347.

McLachlan EM (2003) Transmission of signals through sympathetic ganglia--modulation, integration or simply distribution? Acta physiologica Scandinavica 177:227-235.

McLachlan EM, Davies PJ, Habler HJ, Jamieson J (1997) On-going and reflex synaptic events in rat superior cervical ganglion cells. The Journal of physiology 501 (Pt 1):165-181.

Mehta PK, Griendling KK (2007) Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. American journal of physiology Cell physiology 292:C82-97.

Mehta S, Zhang J (2014) Using a genetically encoded FRET-based reporter to visualize calcineurin phosphatase activity in living cells. Methods in molecular biology (Clifton, NJ) 1071:139-149.

Meijer E, Bakker SJ, van der Jagt EJ, Navis G, de Jong PE, Struck J, Gansevoort RT (2011) Copeptin, a surrogate marker of vasopressin, is associated with disease severity in autosomal dominant polycystic kidney disease. Clinical journal of the American Society of Nephrology : CJASN 6:361-368.

Mekahli D, Sammels E, Luyten T, Welkenhuyzen K, van den Heuvel LP, Levtchenko EN, Gijsbers R, Bultynck G, Parys JB, De Smedt H, Missiaen L (2012) Polycystin-1 and polycystin-2 are both required to amplify inositol-trisphosphate-induced Ca2+ release. Cell calcium 51:452-458.

Meldrum BS (2000) Glutamate as a neurotransmitter in the brain: review of physiology and pathology. The Journal of nutrition 130:1007s-1015s.

Mendelsohn FA, Quirion R, Saavedra JM, Aguilera G, Catt KJ (1984) Autoradiographic localization of angiotensin II receptors in rat brain. Proceedings of the National Academy of Sciences of the United States of America 81:1575-1579.

Mendonca MM, Santana JS, da Cruz KR, Ianzer D, Ghedini PC, Nalivaiko E, Fontes MAP, Ferreira RN, Pedrino GR, Colugnati DB, Xavier CH (2018) Involvement of GABAergic and adrenergic neurotransmissions on paraventricular nucleus of hypothalamus in the control of cardiac function. Frontiers in physiology 9:670.

Menezes RC, Fontes MA (2007) Cardiovascular effects produced by activation of GABA receptors in the rostral ventrolateral medulla of conscious rats. Neuroscience 144:336-343.

Menon V, Rudym D, Chandra P, Miskulin D, Perrone R, Sarnak M (2011) Inflammation, oxidative stress, and insulin resistance in polycystic kidney disease. Clinical journal of the American Society of Nephrology : CJASN 6:7-13.

Methven S, MacGregor MS, Traynor JP, O'Reilly DSJ, Deighan CJ (2010) Assessing proteinuria in chronic kidney disease: protein-creatinine ratio versus albumin-creatinine ratio. Nephrology dialysis transplantation 25:2991-2996.

Michalski A, Grzeszczak W (1996) The effect of hypervolemia on electrolyte level and and level of volume regulating hormones in patients with autosomal dominant polycystic kidney disease. Polskie Archiwum Medycyny Wewnetrznej 96:329-343.

Michelini LC, Bonagamba LG (1988) Baroreceptor reflex modulation by vasopressin microinjected into the nucleus tractus solitarii of conscious rats. Hypertension (Dallas, Tex : 1979) 11:I75-79.

Michelini LC, Stern JE (2009) Exercise-induced neuronal plasticity in central autonomic networks: role in cardiovascular control. Experimental physiology 94:947-960.

Miki K, Oda M, Kamijyo N, Kawahara K, Yoshimoto M (2004) Lumbar sympathetic nerve activity and hindquarter blood flow during REM sleep in rats. The Journal of physiology 557:261-271.

Millan MA, Jacobowitz DM, Aguilera G, Catt KJ (1991) Differential distribution of AT1 and AT2 angiotensin II receptor subtypes in the rat brain during development. Proceedings of the National Academy of Sciences of the United States of America 88:11440-11444.

Miller E, Zodet M (2003) The medical expenditure panel survey (MEPS). Research Findings #25: Trends in the Pharmaceutical Treatment of Hypertension, 1997 to 2003. In: (Services DoHH, ed).

Miller RL, Loewy AD (2013) ENaC γ-expressing astrocytes in the circumventricular organs, white matter, and ventral medullary surface: sites for Na+ regulation by glial cells. Journal of chemical neuroanatomy 53:72-80.

Miller RL, Wang MH, Gray PA, Salkoff LB, Loewy AD (2013) ENaC-expressing neurons in the sensory circumventricular organs become c-Fos activated following systemic sodium changes. American journal of physiology Regulatory, integrative and comparative physiology 305:R1141-1152.

Miselis RR (1981) The efferent projections of the subfornical organ of the rat: a circumventricular organ within a neural network subserving water balance. Brain research 230:1-23.

Miyajima E, Bunag RD (1985) Dietary salt loading produces baroreflex impairment and mild hypertension in rats. The American journal of physiology 249:H278-284.

Miyakawa T, Mizushima A, Hirose K, Yamazawa T, Bezprozvanny I, Kurosaki T, Iino M (2001) Ca(2+)-sensor region of IP(3) receptor controls intracellular Ca(2+) signaling. The EMBO journal 20:1674-1680.

Miyakubo H, Hayashi Y, Tanaka J (2002) Enhanced response of subfornical organ neurons projecting to the hypothalamic paraventricular nucleus to angiotensin II in spontaneously hypertensive rats. Autonomic neuroscience : basic & clinical 95:131-136.

Miyata S, Tsujioka H, Itoh M, Matsunaga W, Kuramoto H, Kiyohara T (2001) Time course of Fos and Fras expression in the hypothalamic supraoptic neurons during chronic osmotic stimulation. Brain research Molecular brain research 90:39-47.

Miyawaki T, Goodchild AK, Pilowsky PM (2002) Evidence for a tonic GABA-ergic inhibition of excitatory respiratory-related afferents to presympathetic neurons in the rostral ventrolateral medulla. Brain research 924:56-62.

Mohring J, Mohring B, Petri M, Haack D (1977) Vasopressor role of ADH in the pathogenesis of malignant DOC hypertension. The American journal of physiology 232:F260-269.

Mohring J, Kintz J, Schoun J, McNeill JR (1981) Pressor responsiveness and cardiovascular reflex activity in spontaneously hypertensive and normotensive rates during vasopressin infusion. Journal of cardiovascular pharmacology 3:948-957.

Mohring J, Glanzer K, Maciel JA, Jr., Dusing R, Kramer HJ, Arbogast R, Koch-Weser J (1980) Greatly enhanced pressor response to antidiuretic hormone in patients with impaired cardiovascular reflexes due to idiopathic orthostatic hypotension. Journal of cardiovascular pharmacology 2:367-376.

Monge Garcia MI, Jian Z, Settels JJ, Hunley C, Cecconi M, Hatib F, Pinsky MR (2018) Performance comparison of ventricular and arterial dP/dtmax for assessing left ventricular systolic function during different experimental loading and contractile conditions. Critical care (London, England) 22:325.

Montani JP, Liard JF, Schoun J, Mohring J (1980) Hemodynamic effects of exogenous and endogenous vasopressin at low plasma concentrations in conscious dogs. Circulation research 47:346-355.

Montano N, Furlan R, Guzzetti S, McAllen RM, Julien C (2009) Analysis of sympathetic neural discharge in rats and humans. Philosophical transactions Series A, Mathematical, physical, and engineering sciences 367:1265-1282.

Moore RY (1977) Organum vasculosum lamina terminalis: innervation by serotonin neurons of the midbrain raphe. Neuroscience letters 5:297-302.

Moore RY, Halaris AE, Jones BE (1978) Serotonin neurons of the midbrain raphe: ascending projections. The Journal of comparative neurology 180:417-438.

Moreira TS, Takakura AC, Colombari E, Guyenet PG (2006) Central chemoreceptors and sympathetic vasomotor outflow. The Journal of physiology 577:369-386.

Morgenthaler NG, Struck J, Alonso C, Bergmann A (2006) Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin. Clinical chemistry 52:112-119.

Morgenthaler NG, Struck J, Jochberger S, Dunser MW (2008) Copeptin: clinical use of a new biomarker. Trends in endocrinology and metabolism: TEM 19:43-49.

Morita S, Miyata S (2012) Different vascular permeability between the sensory and secretory circumventricular organs of adult mouse brain. Cell and tissue research 349:589-603.

Morita S, Furube E, Mannari T, Okuda H, Tatsumi K, Wanaka A, Miyata S (2016) Heterogeneous vascular permeability and alternative diffusion barrier in sensory circumventricular organs of adult mouse brain. Cell and tissue research 363:497-511.

Morrison SF (1999) RVLM and raphe differentially regulate sympathetic outflows to splanchnic and brown adipose tissue. The American journal of physiology 276:R962-973.

Morrison SF (2001) Differential control of sympathetic outflow. American journal of physiology Regulatory, integrative and comparative physiology 281:R683-698.

Morrison SF, Cao WH (2000) Different adrenal sympathetic preganglionic neurons regulate epinephrine and norepinephrine secretion. American journal of physiology Regulatory, integrative and comparative physiology 279:R1763-1775.

Mourao AA, Moreira MC, Melo AB, Lopes PR, Rebelo AC, Rosa DA, Freiria-Oliveira AH, Colombari E, Pedrino GR (2016) Does the median preoptic nucleus contribute to sympathetic hyperactivity in spontaneously hypertensive rats? Autonomic neuroscience : basic & clinical 195:29-33.

Munch PA, Andresen MC, Brown AM (1983) Rapid resetting of aortic baroreceptors in vitro. The American journal of physiology 244:H672-680.

Mustafa RA, Yu ASL (2018) Burden of proof for tolvaptan in ADPKD: did REPRISE provide the answer? Clinical journal of the American Society of Nephrology : CJASN 13:1107-1109.

Nagakura A, Hiyama TY, Noda M (2010) Na(x)-deficient mice show normal vasopressin response to dehydration. Neuroscience letters 472:161-165.

Nagao S, Nishii K, Katsuyama M, Kurahashi H, Marunouchi T, Takahashi H, Wallace DP (2006) Increased water intake decreases progression of polycystic kidney disease in the PCK rat. Journal of the American Society of Nephrology : JASN 17:2220-2227.

Nakajima A, Lu Y, Kawano H, Horie S, Muto S (2015) Association of arginine vasopressin surrogate marker urinary copeptin with severity of autosomal dominant polycystic kidney disease (ADPKD). Clinical and experimental nephrology 19:1199-1205.

Nakamura K, Matsumura K, Kobayashi S, Kaneko T (2005) Sympathetic premotor neurons mediating thermoregulatory functions. Neuroscience research 51:1-8.

Nakata T, Takeda K, Itho H, Hirata M, Kawasaki S, Hayashi J, Oguro M, Sasaki S, Nakagawa M (1989) Paraventricular nucleus lesions attenuate the development of hypertension in DOCA/salt-treated rats. American journal of hypertension 2:625-630.

Nash DA, Jr (1977) Hypertension in polycystic kidney disease without renal failure. Archives of Internal Medicine 137:1571-1575.

Nauli SM, Alenghat FJ, Luo Y, Williams E, Vassilev P, Li X, Elia AE, Lu W, Brown EM, Quinn SJ, Ingber DE, Zhou J (2003) Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. Nature genetics 33:129-137.

Neff RA, Mihalevich M, Mendelowitz D (1998) Stimulation of NTS activates NMDA and non-NMDA receptors in rat cardiac vagal neurons in the nucleus ambiguus. Brain research 792:277-282.

Nehme B, Henry M, Mouginot D, Drolet G (2012) The expression pattern of the Na(+) sensor, Na(X) in the hydromineral homeostatic network: A comparative study between the rat and mouse. Frontiers in neuroanatomy 6:26.

Neumann J, Ligtenberg G, Oey L, Koomans HA, Blankestijn PJ (2004) Moxonidine normalizes sympathetic hyperactivity in patients with eprosartan-treated chronic renal failure. Journal of the American Society of Nephrology : JASN 15:2902-2907.

Ng K, Hildreth CM, Avolio AP, Phillips JK (2011a) Angiotensin-converting enzyme inhibitor limits pulse-wave velocity and aortic calcification in a rat model of cystic renal disease. American journal of physiology Renal physiology 301:F959-F966.

Ng K, Hildreth CM, Phillips JK, Avolio AP (2011b) Aortic stiffness is associated with vascular calcification and remodeling in a chronic kidney disease rat model. American journal of physiology Renal physiology 300:F1431-F1436.

Nguyen G (2007) The (pro)renin receptor: a new kid in town. Seminars in nephrology 27:519-523.

Nigro EA, Castelli M, Boletta A (2015) Role of the polycystins in cell migration, polarity, and tissue morphogenesis. Cells 4:687-705.

Nilsson H, Ljung B, Sjoblom N, Wallin BG (1985) The influence of the sympathetic impulse pattern on contractile responses of rat mesenteric arteries and veins. Acta physiologica Scandinavica 123:303-309.

Nishihara M, Takesue K, Hirooka Y (2017) Renal denervation enhances GABA-ergic input into the PVN leading to blood pressure lowering in chronic kidney disease. Autonomic neuroscience : basic & clinical 204:88-97.

Nishimura M, Ohtsuka K, Nanbu A, Takahashi H, Yoshimura M (1998) Benzamil blockade of brain Na+ channels averts Na(+)-induced hypertension in rats. The American journal of physiology 274:R635-644.

Nissen R, Renaud LP (1994) GABA receptor mediation of median preoptic nucleus-evoked inhibition of supraoptic neurosecretory neurones in rat. The Journal of physiology 479 (Pt 2):207-216.

Nissen R, Cunningham JT, Renaud LP (1993) Lateral hypothalamic lesions alter baroreceptor-evoked inhibition of rat supraoptic vasopressin neurones. The Journal of physiology 470:751-766.

Nomura K, Hiyama TY, Sakuta H, Matsuda T, Lin CH, Kobayashi K, Kobayashi K, Kuwaki T, Takahashi K, Matsui S, Noda M (2019) [Na(+)] increases in body fluids sensed by central Nax induce sympathetically mediated blood pressure elevations via H(+)-dependent activation of ASIC1a. Neuron 101:60-75.e66.

Northcott CA, Watts S, Chen Y, Morris M, Chen A, Haywood JR (2010) Adenoviral inhibition of AT1a receptors in the paraventricular nucleus inhibits acute increases in mean arterial blood pressure in the rat. American journal of physiology Regulatory, integrative and comparative physiology 299:R1202-1211.

Nosaka S, Yamamoto T, Yasunaga K (1979) Localization of vagal cardioinhibitory preganglionic neurons with rat brain stem. The Journal of comparative neurology 186:79-92.

Nowak KL, Farmer H, Cadnapaphornchai MA, Gitomer B, Chonchol M (2017) Vascular dysfunction in children and young adults with autosomal dominant polycystic kidney disease. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 32:342-347.

O'Donaughy TL, Qi Y, Brooks VL (2006) Central action of increased osmolality to support blood pressure in deoxycorticosterone acetate-salt rats. Hypertension (Dallas, Tex : 1979) 48:658-663.

O'Meara CC, Hoffman M, Sweeney WE, Jr., Tsaih S-W, Xiao B, Jacob HJ, Avner ED, Moreno C (2012) Role of genetic modifiers in an orthologous rat model of ARPKD. Physiological genomics 44:741-753.

Ohta H, Talman WT (1994) Both NMDA and non-NMDA receptors in the NTS participate in the baroreceptor reflex in rats. The American journal of physiology 267:R1065-1070.

Okubo Y, Kakizawa S, Hirose K, Iino M (2001) Visualization of IP(3) dynamics reveals a novel AMPA receptor-triggered IP(3) production pathway mediated by voltage-dependent Ca(2+) influx in Purkinje cells. Neuron 32:113-122.

Oldfield BJ, Miselis RR, McKinley MJ (1991a) Median preoptic nucleus projections to vasopressin-containing neurones of the supraoptic nucleus in sheep. A light and electron microscopic study. Brain research 542:193-200.

Oldfield BJ, Hards DK, McKinley MJ (1991b) Projections from the subfornical organ to the supraoptic nucleus in the rat: ultrastructural identification of an interposed synapse in the median preoptic nucleus using a combination of neuronal tracers. Brain research 558:13-19.

Oldfield BJ, Hards DK, McKinley MJ (1992) Neurons in the median preoptic nucleus of the rat with collateral branches to the subfornical organ and supraoptic nucleus. Brain research 586:86-90.

Oldfield BJ, Badoer E, Hards DK, McKinley MJ (1994) Fos production in retrogradely labelled neurons of the lamina terminalis following intravenous infusion of either hypertonic saline or angiotensin II. Neuroscience 60:255-262.

Oldfield BJ, Bicknell RJ, McAllen RM, Weisinger RS, McKinley MJ (1991c) Intravenous hypertonic saline induces Fos immunoreactivity in neurons throughout the lamina terminalis. Brain research 561:151-156.

Oldfield BJ, Davern PJ, Giles ME, Allen AM, Badoer E, McKinley MJ (2001) Efferent neural projections of angiotensin receptor (AT1) expressing neurones in the

hypothalamic paraventricular nucleus of the rat. Journal of neuroendocrinology 13:139-146.

Oliet SH, Bourque CW (1992) Properties of supraoptic magnocellular neurones isolated from the adult rat. The Journal of physiology 455:291-306.

Omran H (2010) NPHP proteins: gatekeepers of the ciliary compartment. The Journal of cell biology 190:715-717.

Omran H, Fernandez C, Jung M, Häffner K, Fargier B, Villaquiran A, Waldherr R, Gretz N, Brandis M, Rüschendorf F, Reis A, Hildebrandt F (2000) Identification of a new gene locus for adolescent nephronophthisis, on chromosome 3q22 in a large Venezuelan pedigree. American journal of human genetics 66:118-127.

Osborn JW (2005) Hypothesis: set-points and long-term control of arterial pressure. A theoretical argument for a long-term arterial pressure control system in the brain rather than the kidney. Clinical and experimental pharmacology & physiology 32:384-393.

Osborn JW, Hendel MD, Collister JP, Ariza-Guzman PA, Fink GD (2012) The role of the subfornical organ in angiotensin II-salt hypertension in the rat. Experimental physiology 97:80-88.

Osborn JW, Jr., Skelton MM, Cowley AW, Jr. (1987) Hemodynamic effects of vasopressin compared with angiotensin II in conscious rats. The American journal of physiology 252:H628-637.

Ott D, Murgott J, Rafalzik S, Wuchert F, Schmalenbeck B, Roth J, Gerstberger R (2010) Neurons and glial cells of the rat organum vasculosum laminae terminalis directly respond to lipopolysaccharide and pyrogenic cytokines. Brain research 1363:93-106.

Otto EA, Trapp ML, Schultheiss UT, Helou J, Quarmby LM, Hildebrandt F (2008) NEK8 mutations affect ciliary and centrosomal localization and may cause nephronophthisis. Journal of the American Society of Nephrology : JASN 19:587-592.

Otto EA et al. (2003) Mutations in INVS encoding inversin cause nephronophthisis type 2, linking renal cystic disease to the function of primary cilia and left-right axis determination. Nature genetics 34:413-420.

Ow CP, Abdelkader A, Hilliard LM, Phillips JK, Evans RG (2014) Determinants of renal tissue hypoxia in a rat model of polycystic kidney disease. American journal of physiology Regulatory, integrative and comparative physiology 307:R1207-1215.

Page MC, Cassaglia PA, Brooks VL (2011) GABA in the paraventricular nucleus tonically suppresses baroreflex function: alterations during pregnancy. American journal of physiology Regulatory, integrative and comparative physiology 300:R1452-1458.

Pang CC (2001) Autonomic control of the venous system in health and disease: effects of drugs. Pharmacology & therapeutics 90:179-230.

Paredes RG (2003) Medial preoptic area/anterior hypothalamus and sexual motivation. Scandinavian journal of psychology 44:203-212.

Pasantes-Morales H, Tuz K (2006) Volume changes in neurons: hyperexcitability and neuronal death. Contributions to nephrology 152:221-240.

Patel KP, Schmid PG (1988) Role of median preoptic area in vasopressin-mediated bradycardia. The American journal of physiology 254:H1172-1178.

Patel KP, Mayhan WG, Bidasee KR, Zheng H (2011) Enhanced angiotensin II-mediated central sympathoexcitation in streptozotocin-induced diabetes: role of superoxide anion. American journal of physiology Regulatory, integrative and comparative physiology 300:R311-320.

Paul M, Poyan Mehr A, Kreutz R (2006) Physiology of local renin-angiotensin systems. Physiological reviews 86:747-803.

Paul M, Wagner D, Metzger R, Ganten D, Lang RE, Suzuki F, Murakami K, Burbach JH, Ludwig G (1988) Quantification of renin mRNA in various mouse tissues by a novel solution hybridization assay. Journal of hypertension 6:247-252.

Paxinos G, Watson C (2013) The rat brain in stereotaxic coordinates, 7th edition. Waltham, MA: Elsevier Science.

Peaston RT, Weinkove C (2004) Measurement of catecholamines and their metabolites. Annals of clinical biochemistry 41:17-38.

Pedersen NP, Ferrari L, Venner A, Wang JL, Abbott SBG, Vujovic N, Arrigoni E, Saper CB, Fuller PM (2017) Supramammillary glutamate neurons are a key node of the arousal system. Nature communications 8:1405.

Pei Y, Watnick T (2010) Diagnosis and screening of autosomal dominant polycystic kidney disease. Advances in chronic kidney disease 17:140-152.

Peters JH, McDougall SJ, Kellett DO, Jordan D, Llewellyn-Smith IJ, Andresen MC (2008) Oxytocin enhances cranial visceral afferent synaptic transmission to the solitary tract nucleus. The Journal of neuroscience : the official journal of the Society for Neuroscience 28:11731-11740.

Petrov T, Howarth AG, Krukoff TL, Stevenson BR (1994) Distribution of the tight junction-associated protein ZO-1 in circumventricular organs of the CNS. Brain research Molecular brain research 21:235-246.

Phillips JK, Goodchild AK, Dubey R, Sesiashvili E, Takeda M, Chalmers J, Pilowsky PM, Lipski J (2001) Differential expression of catecholamine biosynthetic enzymes in the rat ventrolateral medulla. The Journal of comparative neurology 432:20-34.

Phillips JK, Hopwood D, Loxley RA, Ghatora K, Coombes JD, Tan YS, Harrison JL, McKitrick DJ, Holobotvskyy V, Arnolda LF, Rangan GK (2007) Temporal relationship between renal cyst development, hypertension and cardiac hypertrophy in a new rat model of autosomal recessive polycystic kidney disease. Kidney & blood pressure research 30:129-144.

Phillips PA, Abrahams JM, Kelly JM, Mooser V, Trinder D, Johnston CI (1990) Localization of vasopressin binding sites in rat tissues using specific V1 and V2 selective ligands. Endocrinology 126:1478-1484.

Pickel VM, Chan J, Ganten D (1986) Dual peroxidase and colloidal gold-labeling study of angiotensin converting enzyme and angiotensin-like immunoreactivity in the rat subfornical organ. The Journal of neuroscience : the official journal of the Society for Neuroscience 6:2457-2469.

Pickering TG (1988) The influence of daily activity on ambulatory blood pressure. American heart journal 116:1141-1145.

Pietila-Effati PM, Salmela AK, Koistinen MJ (2018) Intravascular Renal Denervation in Renal Dialysis Patients with Uncontrolled Hypertension: A Case Series of Four Patients. The American journal of case reports 19:985-991.

Pilowsky PM, Goodchild AK (2002) Baroreceptor reflex pathways and neurotransmitters: 10 years on. Journal of hypertension 20:1675-1688.

Pirnik Z, Mravec B, Kiss A (2004) Fos protein expression in mouse hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei upon osmotic stimulus: colocalization with vasopressin, oxytocin, and tyrosine hydroxylase. Neurochemistry international 45:597-607.

Pirson Y, Chauveau D, Torres V (2002) Management of cerebral aneurysms in autosomal dominant polycystic kidney disease. Journal of the American Society of Nephrology : JASN 13:269-276.

Polonia JJ, Paiva MQ, Guimaraes S (1985) Pharmacological characterization of postsynaptic alpha-adrenoceptor subtypes in five different dog arteries in-vitro. The Journal of pharmacy and pharmacology 37:205-208.

Porath B et al. (2016) Mutations in GANAB, Encoding the Glucosidase IIalpha Subunit, Cause Autosomal-Dominant Polycystic Kidney and Liver Disease. American journal of human genetics 98:1193-1207.

Porter JP (1988) Electrical stimulation of paraventricular nucleus increases plasma renin activity. The American journal of physiology 254:R325-330.

Potts JT, Paton JF, Mitchell JH, Garry MG, Kline G, Anguelov PT, Lee SM (2003) Contraction-sensitive skeletal muscle afferents inhibit arterial baroreceptor signalling in the nucleus of the solitary tract: role of intrinsic GABA interneurons. Neuroscience 119:201-214.

Praetorius HA, Leipziger J (2013) Primary cilium-dependent sensing of urinary flow and paracrine purinergic signaling. Seminars in cell & developmental biology 24:3-10.

Purves D, Rubin E, Snider WD, Lichtman J (1986) Relation of animal size to convergence, divergence, and neuronal number in peripheral sympathetic pathways. The Journal of neuroscience : the official journal of the Society for Neuroscience 6:158-163.

Pyner S (2014) The paraventricular nucleus and heart failure. Experimental physiology 99:332-339.

Pyner S, Coote JH (1999) Identification of an efferent projection from the paraventricular nucleus of the hypothalamus terminating close to spinally projecting rostral ventrolateral medullary neurons. Neuroscience 88:949-957.

Pyner S, Coote JH (2000) Identification of branching paraventricular neurons of the hypothalamus that project to the rostroventrolateral medulla and spinal cord. Neuroscience 100:549-556.

Qadri F, Waldmann T, Wolf A, Hohle S, Rascher W, Unger T (1998) Differential contribution of angiotensinergic and cholinergic receptors in the hypothalamic paraventricular nucleus to osmotically induced AVP release. The Journal of pharmacology and experimental therapeutics 285:1012-1018.

Qian Q, Li M, Cai Y, Ward CJ, Somlo S, Harris PC, Torres VE (2003) Analysis of the polycystins in aortic vascular smooth muscle cells. Journal of the American Society of Nephrology : JASN 14:2280-2287.

Qiao X, Zhou JJ, Li DP, Pan HL (2017) Src kinases regulate glutamatergic input to hypothalamic presympathetic neurons and sympathetic outflow in hypertension. Hypertension (Dallas, Tex : 1979) 69:154-162.

Qiu M, Li J, Tan L, Zhang M, Zhou G, Zeng T, Li A (2018) Targeted ablation of distal cerebrospinal fluid-contacting nucleus alleviates renal fibrosis in chronic kidney disease. Frontiers in physiology 9:1640.

Quek KJ, Ameer OZ, Phillips JK (2018) AT1 Receptor Antagonism Improves Structural, Functional, and Biomechanical Properties in Resistance Arteries in a Rodent Chronic Kidney Disease Model. American journal of hypertension 31:696-705.

Quek KJ, Boyd R, Ameer OZ, Zangerl B, Butlin M, Murphy TV, Avolio AP, Phillips JK (2016) Progressive vascular remodelling, endothelial dysfunction and stiffness in mesenteric resistance arteries in a rodent model of chronic kidney disease. Vascular pharmacology 81:42-52.

Rahbari-Oskoui F, Williams O, Chapman A (2014) Mechanisms and management of hypertension in autosomal dominant polycystic kidney disease. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 29:2194-2201.

Rahman E, Niaz FA, Al-Suwaida A, Nahrir S, Bashir M, Rahman H, Hammad D (2009) Analysis of causes of mortality in patients with autosomal dominant polycystic kidney disease: a single center study. Saudi journal of kidney diseases and transplantation : an official publication of the Saudi Center for Organ Transplantation, Saudi Arabia 20:806-810.

Rall JE, Odel HM (1949) Congenital polycystic disease of the kidney; review of the literature and data on 207 cases. The American journal of the medical sciences 218:399-407.

Ramchandra R, Barrett CJ (2015) Regulation of the renal sympathetic nerves in heart failure. Frontiers in physiology 6:238.

Ramchandra R, Hood SG, Frithiof R, May CN (2009) Discharge properties of cardiac and renal sympathetic nerves and their impaired responses to changes in blood volume in heart failure. American journal of physiology Regulatory, integrative and comparative physiology 297:R665-674.

Ramchandra R, Hood SG, Frithiof R, McKinley MJ, May CN (2013) The role of the paraventricular nucleus of the hypothalamus in the regulation of cardiac and renal sympathetic nerve activity in conscious normal and heart failure sheep. The Journal of physiology 591:93-107.

Ramkumar N, Stuart D, Rees S, Hoek AV, Sigmund CD, Kohan DE (2014) Collecting duct-specific knockout of renin attenuates angiotensin II-induced hypertension. American journal of physiology Renal physiology 307:F931-938.

Ramunni A, Saracino A, Esposito T, Saliani MT, Coratelli P (2004) Renal vascular resistance and renin-angiotensin system in the pathogenesis of early hypertension in autosomal dominant polycystic kidney disease. Hypertension research : official journal of the Japanese Society of Hypertension 27:221-225.

Randle JC, Bourque CW, Renaud LP (1986) Serial reconstruction of Lucifer yellow-labeled supraoptic nucleus neurons in perfused rat hypothalamic explants. Neuroscience 17:453-467.

Ranson RN, Motawei K, Pyner S, Coote JH (1998) The paraventricular nucleus of the hypothalamus sends efferents to the spinal cord of the rat that closely appose sympathetic preganglionic neurones projecting to the stellate ganglion. Experimental brain research 120:164-172.

Ranson SW (1937) Some functions of the hypothalamus: Harvey lecture, December 17, 1936. Bulletin of the New York Academy of Medicine 13:241-271.

Rasouli M (2016) Basic concepts and practical equations on osmolality: Biochemical approach. Clinical biochemistry 49:936-941.

Rathner JA, McAllen RM (1999) Differential control of sympathetic drive to the rat tail artery and kidney by medullary premotor cell groups. Brain research 834:196-199.

Raven PB, Fadel PJ, Smith SA (2002) The influence of central command on baroreflex resetting during exercise. Exercise and sport sciences reviews 30:39-44.

Reeders ST (1992) Multilocus polycystic disease. Nature genetics 1:235-237.

Reis WL, Biancardi VC, Zhou Y, Stern JE (2016) A functional coupling between carbon monoxide and nitric oxide contributes to increased vasopressin neuronal activity in heart failure rats. Endocrinology 157:2052-2066.

Riccio E, Esposito G, Franzone A, Imbriaco M, Santangelo M, Pisani A (2014) Renal sympathetic-nerve ablation for uncontrolled hypertension in a patient with single-kidney autosomal dominant polycystic kidney disease. Journal of clinical hypertension (Greenwich, Conn) 16:385-386.

Richard D, Bourque CW (1995) Synaptic control of rat supraoptic neurones during osmotic stimulation of the organum vasculosum lamina terminalis in vitro. The Journal of physiology 489 (Pt 2):567-577.

Ridao N, Luno J, Garcia de Vinuesa S, Gomez F, Tejedor A, Valderrabano F (2001) Prevalence of hypertension in renal disease. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 16 Suppl 1:70-73.

Rinschen MM, Schermer B, Benzing T (2014) Vasopressin-2 receptor signaling and autosomal dominant polycystic kidney disease: from bench to bedside and back again. Journal of the American Society of Nephrology : JASN 25:1140-1147.

Roberts MM, Robinson AG, Fitzsimmons MD, Grant F, Lee WS, Hoffman GE (1993) c-fos expression in vasopressin and oxytocin neurons reveals functional heterogeneity within magnocellular neurons. Neuroendocrinology 57:388-400.

Rockhold RW, Acuff CG, Clower BR (1990) Excitotoxic lesions of the paraventricular hypothalamus: metabolic and cardiac effects. Neuropharmacology 29:663-673.

Roland BL, Sawchenko PE (1993) Local origins of some GABAergic projections to the paraventricular and supraoptic nuclei of the hypothalamus in the rat. The Journal of comparative neurology 332:123-143.

Rose CR, Felix L, Zeug A, Dietrich D, Reiner A, Henneberger C (2017) Astroglial glutamate signaling and uptake in the hippocampus. Frontiers in molecular neuroscience 10:451.

Ross CA, Ruggiero DA, Reis DJ (1981) Projections to the spinal cord from neurons close to the ventral surface of the hindbrain in the rat. Neuroscience letters 21:143-148.

Ross CA, Ruggiero DA, Park DH, Joh TH, Sved AF, Fernandez-Pardal J, Saavedra JM, Reis DJ (1984) Tonic vasomotor control by the rostral ventrolateral medulla: effect of electrical or chemical stimulation of the area containing C1 adrenaline neurons on arterial pressure, heart rate, and plasma catecholamines and vasopressin. The Journal of neuroscience : the official journal of the Society for Neuroscience 4:474-494.

Rossi NF, Maliszewska-Scislo M, Chen H, Black SM, Sharma S, Ravikov R, Augustyniak RA (2010) Neuronal nitric oxide synthase within paraventricular nucleus: blood pressure and baroreflex in two-kidney, one-clip hypertensive rats. Experimental physiology 95:845-857.

Roy RK, Augustine RA, Brown CH, Schwenke DO (2018) Activation of oxytocin neurons in the paraventricular nucleus drives cardiac sympathetic nerve activation following myocardial infarction in rats. Communications biology 1:160.

Roy S, Dillon MJ, Trompeter RS, Barratt TM (1997) Autosomal recessive polycystic kidney disease: long-term outcome of neonatal survivors. Pediatric nephrology (Berlin, Germany) 11:302-306.

Rundgren M, Fyhrquist F (1978) A study of permanent adipsia induced by medial forebrain lesions. Acta physiologica Scandinavica 103:463-471.

Rundqvist B, Elam M, Bergmann-Sverrisdottir Y, Eisenhofer G, Friberg P (1997) Increased cardiac adrenergic drive precedes generalized sympathetic activation in human heart failure. Circulation 95:169-175.

Sabban EL, Schilt N, Serova LI, Masineni SN, Stier CT, Jr. (2009) Kinetics and persistence of cardiovascular and locomotor effects of immobilization stress and influence of ACTH treatment. Neuroendocrinology 89:98-108.

Sagar PS, Zhang J, Luciuk M, Mannix C, Wong ATY, Rangan GK (2019) Increased water intake reduces long-term renal and cardiovascular disease progression in experimental polycystic kidney disease. PloS one 14:e0209186.

Saigusa T, Dang Y, Bunni MA, Amria MY, Steele SL, Fitzgibbon WR, Bell PD (2015) Activation of the intrarenal renin-angiotensin-system in murine polycystic kidney disease. Physiological reports 3.

Saito H, Takahashi Y, Takahashi S (2017) Measurement of blood pressure to detect elusive kidney disease. Pediatrics international : official journal of the Japan Pediatric Society 59:638-639.

Salih M, Bovee DM, Roksnoer LCW, Casteleijn NF, Bakker SJL, Gansevoort RT, Zietse R, Danser AHJ, Hoorn EJ (2017) Urinary renin-angiotensin markers in polycystic kidney disease. American journal of physiology Renal physiology 313:F874-f881.

Salman IM (2016) Major autonomic neuroregulatory pathways underlying short- and long-term control of cardiovascular function. Current hypertension reports 18:18.

Salman IM, Hildreth CM, Phillips JK (2017) Chronic kidney disease impairs renal nerve and haemodynamic reflex responses to vagal afferent input through a central mechanism. Autonomic neuroscience : basic & clinical 204:65-73.

Salman IM, Hildreth CM, Ameer OZ, Phillips JK (2014) Differential contribution of afferent and central pathways to the development of baroreflex dysfunction in chronic kidney disease. Hypertension (Dallas, Tex : 1979) 63:804-810.

Salman IM, Phillips JK, Ameer OZ, Hildreth CM (2015a) Abnormal central control underlies impaired baroreflex control of heart rate and sympathetic nerve activity in female Lewis polycystic kidney rats. Journal of hypertension 33:1418-1428.

Salman IM, Sarma Kandukuri D, Harrison JL, Hildreth CM, Phillips JK (2015b) Direct conscious telemetry recordings demonstrate increased renal sympathetic nerve activity in rats with chronic kidney disease. Frontiers in physiology 6:218.

Salo LM, Nalivaiko E, Anderson CR, McAllen RM (2009) Control of cardiac rate, contractility, and atrioventricular conduction by medullary raphe neurons in anesthetized rats. American journal of physiology Heart and circulatory physiology 296:H318-324.

Sandgren JA, Linggonegoro DW, Zhang SY, Sapouckey SA, Claflin KE, Pearson NA, Leidinger MR, Pierce GL, Santillan MK, Gibson-Corley KN, Sigmund CD, Grobe JL (2018) Angiotensin AT1A receptors expressed in vasopressin-producing cells of the supraoptic nucleus contribute to osmotic control of vasopressin. American journal of physiology Regulatory, integrative and comparative physiology 314:R770-r780.

Sans-Atxer L, Torra R, Fernández-Llama P (2013) Hypertension in autosomal-dominant polycystic kidney disease (ADPKD). Clinical kidney journal 6:457-463.

Santos NC, Figueira-Coelho J, Martins-Silva J, Saldanha C (2003) Multidisciplinary utilization of dimethyl sulfoxide: pharmacological, cellular, and molecular aspects. Biochemical pharmacology 65:1035-1041.

Santos PC, Krieger JE, Pereira AC (2012) Renin-angiotensin system, hypertension, and chronic kidney disease: pharmacogenetic implications. Journal of pharmacological sciences 120:77-88.

Saper CB, Levisohn D (1983) Afferent connections of the median preoptic nucleus in the rat: anatomical evidence for a cardiovascular integrative mechanism in the anteroventral third ventricular (AV3V) region. Brain research 288:21-31.

Saper CB, Lowell BB (2014) The hypothalamus. Current biology : CB 24:R1111-1116.

Saper CB, Reis DJ, Joh T (1983) Medullary catecholamine inputs to the anteroventral third ventricular cardiovascular regulatory region in the rat. Neuroscience letters 42:285-291.

Saper CB, Loewy AD, Swanson LW, Cowan WM (1976) Direct hypothalamo-autonomic connections. Brain research 117:305-312.

Sapolsky RM, Romero LM, Munck AU (2000) How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine reviews 21:55-89.

Sattar MA, Latiff A, Gan EK (1985) Effect of captopril on converting enzyme activity in chemically sympathectomized, spontaneously hypertensive rats. Japanese journal of pharmacology 39:291-297.

Sawchenko PE, Swanson LW (1981) Central noradrenergic pathways for the integration of hypothalamic neuroendocrine and autonomic responses. Science (New York, NY) 214:685-687.

Sawchenko PE, Swanson LW (1982) The organization of noradrenergic pathways from the brainstem to the paraventricular and supraoptic nuclei in the rat. Brain research 257:275-325.

Sawchenko PE, Swanson LW (1983) The organization of forebrain afferents to the paraventricular and supraoptic nuclei of the rat. The Journal of comparative neurology 218:121-144.

Schacht F (1931) Hypertension in cases of cogenital polycystic kidney. Arch Intern Med 47:500-509.

Schafer K, Gretz N, Bader M, Oberbaumer I, Eckardt KU, Kriz W, Bachmann S (1994) Characterization of the Han:SPRD rat model for hereditary polycystic kidney disease. Kidney international 46:134-152.

Schiltz JC, Hoffman GE, Stricker EM, Sved AF (1997) Decreases in arterial pressure activate oxytocin neurons in conscious rats. The American journal of physiology 273:R1474-1483.

Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez JY, White DJ, Hartenstein V, Eliceiri K, Tomancak P, Cardona A (2012) Fiji: an open-source platform for biological-image analysis. Nature methods 9:676-682.

Schneider JE (2004) Energy balance and reproduction. Physiology & behavior 81:289-317.

Schramm LP, Strack AM, Platt KB, Loewy AD (1993) Peripheral and central pathways regulating the kidney: a study using pseudorabies virus. Brain research 616:251-262.

Schreihofer AM, Guyenet PG (2000) Sympathetic reflexes after depletion of bulbospinal catecholaminergic neurons with anti-DbetaH-saporin. American journal of physiology Regulatory, integrative and comparative physiology 279:R729-742.

Schreihofer AM, Guyenet PG (2003) Baro-activated neurons with pulse-modulated activity in the rat caudal ventrolateral medulla express GAD67 mRNA. Journal of neurophysiology 89:1265-1277.

Schreihofer AM, Stornetta RL, Guyenet PG (2000) Regulation of sympathetic tone and arterial pressure by rostral ventrolateral medulla after depletion of C1 cells in rat. The Journal of physiology 529 Pt 1:221-236.

Schrier R, McFann K, Johnson A, Chapman A, Edelstein C, Brosnahan G, Ecder T, Tison L (2002) Cardiac and renal effects of standard versus rigorous blood pressure control in autosomal-dominant polycystic kidney disease: results of a seven-year prospective randomized study. Journal of the American Society of Nephrology : JASN 13:1733-1739.

Schrier RW, Johnson AM, McFann K, Chapman AB (2003) The role of parental hypertension in the frequency and age of diagnosis of hypertension in offspring with autosomal-dominant polycystic kidney disease. Kidney international 64:1792-1799.

Schrier RW, Abebe KZ, Perrone RD, Torres VE, Braun WE, Steinman TI, Winklhofer FT, Brosnahan G, Czarnecki PG, Hogan MC, Miskulin DC, Rahbari-Oskoui FF, Grantham JJ, Harris PC, Flessner MF, Bae KT, Moore CG, Chapman AB (2014) Blood pressure in early autosomal dominant polycystic kidney disease. The New England journal of medicine 371:2255-2266.

Schwartz J, Reid IA (1981) Effect of vasopressin blockade on blood pressure regulation during hemorrhage in conscious dogs. Endocrinology 109:1778-1780.

Schwartz MD, Urbanski HF, Nunez AA, Smale L (2011) Projections of the suprachiasmatic nucleus and ventral subparaventricular zone in the Nile grass rat (Arvicanthis niloticus). Brain research 1367:146-161.

Schwensen KG, Burgess JS, Graf NS, Alexander SI, Harris DC, Phillips JK, Rangan GK (2011) Early cyst growth is associated with the increased nuclear expression of cyclin D1/Rb protein in an autosomal-recessive polycystic kidney disease rat model. Nephron Experimental nephrology 117:e93-103.

Scislo TJ, Augustyniak RA, O'Leary DS (1998) Differential arterial baroreflex regulation of renal, lumbar, and adrenal sympathetic nerve activity in the rat. The American journal of physiology 275:R995-r1002.

Scott V, Bishop VR, Leng G, Brown CH (2009a) Dehydration-induced modulation of kappa-opioid inhibition of vasopressin neurone activity. The Journal of physiology 587:5679-5689.

Scott V, Bishop VR, Leng G, Brown CH (2009b) Dehydration-induced modulation of kappa-opioid inhibition of vasopressin neurone activity. J Physiol 587:5679-5689.

Scrogin KE, Grygielko ET, Brooks VL (1999) Osmolality: a physiological long-term regulator of lumbar sympathetic nerve activity and arterial pressure. The American journal of physiology 276:R1579-1586.

Scrogin KE, McKeogh DF, Brooks VL (2002) Is osmolality a long-term regulator of renal sympathetic nerve activity in conscious water-deprived rats? American journal of physiology Regulatory, integrative and comparative physiology 282:R560-568.

Secomb TW (2016) Hemodynamics. Comprehensive Physiology 6:975-1003.

Sedman A, Bell P, Manco-Johnson M, Schrier R, Warady BA, Heard EO, Butler-Simon N, Gabow P (1987) Autosomal dominant polycystic kidney disease in childhood: a longitudinal study. Kidney international 31:1000-1005.

Seeman T, Dusek J, Vondrak K, Blahova K, Simkova E, Kreisinger J, Dvorak P, Kyncl M, Hribal Z, Janda J (2004) Renal concentrating capacity is linked to blood pressure in children with autosomal dominant polycystic kidney disease. Physiological research 53:629-634.

Seller H (1996) Carl Ludwig and the localization of the medullary vasomotor center: old and new concepts of the generation of sympathetic tone. Pflugers Archiv : European journal of physiology 432:R94-98.

Serova LI, Gueorguiev V, Cheng SY, Sabban EL (2008) Adrenocorticotropic hormone elevates gene expression for catecholamine biosynthesis in rat superior cervical ganglia and locus coeruleus by an adrenal independent mechanism. Neuroscience 153:1380-1389.

Setiadi A, Korim WS, Elsaafien K, Yao ST (2018) The role of the blood-brain barrier in hypertension. Experimental physiology 103:337-342.

Severs WB, Keil LC, Klase PA, Deen KC (1981) Urethane anesthesia in rats. Altered ability to regulate hydration. Pharmacology 22:209-226.

Shafer DS, Zhang Z (2013) Introductory Statistics.

Shafton AD, McAllen RM (2013) Location of cat brain stem neurons that drive sweating. American journal of physiology Regulatory, integrative and comparative physiology 304:R804-809.

Shafton AD, Ryan A, Badoer E (1998) Neurons in the hypothalamic paraventricular nucleus send collaterals to the spinal cord and to the rostral ventrolateral medulla in the rat. Brain research 801:239-243.

Shan Z, Cuadra AE, Sumners C, Raizada MK (2008) Characterization of a functional (pro)renin receptor in rat brain neurons. Experimental physiology 93:701-708.

Shan Z, Shi P, Cuadra AE, Dong Y, Lamont GJ, Li Q, Seth DM, Navar LG, Katovich MJ, Sumners C, Raizada MK (2010) Involvement of the brain (pro)renin receptor in cardiovascular homeostasis. Circulation research 107:934-938.

Sharif Naeini R, Witty MF, Seguela P, Bourque CW (2006) An N-terminal variant of Trpv1 channel is required for osmosensory transduction. Nature neuroscience 9:93-98.

Sharp FR, Sagar SM, Hicks K, Lowenstein D, Hisanaga K (1991) c-fos mRNA, Fos, and Fos-related antigen induction by hypertonic saline and stress. The Journal of neuroscience : the official journal of the Society for Neuroscience 11:2321-2331.

Sharshar T, Blanchard A, Paillard M, Raphael JC, Gajdos P, Annane D (2003) Circulating vasopressin levels in septic shock. Critical care medicine 31:1752-1758.

Shen HH (2017) Core Concept: Circumventing the blood-brain barrier. Proceedings of the National Academy of Sciences of the United States of America 114:11261-11263.

Shi L, Hu F, Morrissey P, Yao J, Xu Z (2003) Intravenous angiotensin induces brain c-fos expression and vasopressin release in the near-term ovine fetus. American journal of physiology Endocrinology and metabolism 285:E1216-1222.

Shi P, Diez-Freire C, Jun JY, Qi Y, Katovich MJ, Li Q, Sriramula S, Francis J, Sumners C, Raizada MK (2010) Brain microglial cytokines in neurogenic hypertension. Hypertension (Dallas, Tex : 1979) 56:297-303.

Shi Z, Li B, Brooks VL (2015a) Role of the paraventricular nucleus of the hypothalamus in the sympathoexcitatory effects of leptin. Hypertension (Dallas, Tex : 1979) 66:1034-1041.

Shi Z, Madden CJ, Brooks VL (2017) Arcuate neuropeptide Y inhibits sympathetic nerve activity via multiple neuropathways. The Journal of clinical investigation 127:2868-2880.

Shi Z, Cassaglia PA, Gotthardt LC, Brooks VL (2015b) Hypothalamic paraventricular and arcuate nuclei contribute to elevated sympathetic nerve activity in pregnant rats: roles of neuropeptide Y and α-melanocyte-stimulating hormone. Hypertension (Dallas, Tex : 1979) 66:1191-1198.

Shillingford JM, Piontek KB, Germino GG, Weimbs T (2010) Rapamycin ameliorates PKD resulting from conditional inactivation of Pkd1. Journal of the American Society of Nephrology : JASN 21:489-497.

Shoji M, Share L, Crofton JT (1989) Effect on vasopressin release of microinjection of angiotensin II into the paraventricular nucleus of conscious rats. Neuroendocrinology 50:327-333.

Siddiqi L, Prakken NH, Velthuis BK, Cramer MJ, Oey PL, Boer P, Bots ML, Blankestijn PJ (2010) Sympathetic activity in chronic kidney disease patients is related to left ventricular mass despite antihypertensive treatment. Nephrology, dialysis,

transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 25:3272-3277.

Simms RJ, Eley L, Sayer JA (2009) Nephronophthisis. European journal of human genetics : EJHG 17:406-416.

Simpson MA, Cross HE, Cross L, Helmuth M, Crosby AH (2009) Lethal cystic kidney disease in Amish neonates associated with homozygous nonsense mutation of NPHP3. American journal of kidney diseases : the official journal of the National Kidney Foundation 53:790-795.

Singru PS, Wittmann G, Farkas E, Zseli G, Fekete C, Lechan RM (2012) Refeeding-activated glutamatergic neurons in the hypothalamic paraventricular nucleus (PVN) mediate effects of melanocortin signaling in the nucleus tractus solitarius (NTS). Endocrinology 153:3804-3814.

Sink KM, Leng X, Williamson J, Kritchevsky SB, Yaffe K, Kuller L, Yasar S, Atkinson H, Robbins M, Psaty B, Goff DC, Jr. (2009) Angiotensin-converting enzyme inhibitors and cognitive decline in older adults with hypertension: results from the Cardiovascular Health Study. Archives of internal medicine 169:1195-1202.

Sjoblom-Widfeldt N, Nilsson H (1990) Sympathetic transmission in small mesenteric arteries from the rat: influence of impulse pattern. Acta physiologica Scandinavica 138:523-528.

Sleight P, Robinson JL, Brooks DE, Rees PM (1977) Characteristics of single carotid sinus baroreceptor fibers and whole nerve activity in the normotensive and the renal hypertensive dog. Circulation research 41:750-758.

Sly DJ, Colvill L, McKinley MJ, Oldfield BJ (1999) Identification of neural projections from the forebrain to the kidney, using the virus pseudorabies. Journal of the autonomic nervous system 77:73-82.

Smith BN, Armstrong WE (1990) Tuberal supraoptic neurons--I. Morphological and electrophysiological characteristics observed with intracellular recording and biocytin filling in vitro. Neuroscience 38:469-483.

Smith PM, Ferguson AV (1997) Vasopressin acts in the subfornical organ to decrease blood pressure. Neuroendocrinology 66:130-135.

Smith PM, Chambers AP, Price CJ, Ho W, Hopf C, Sharkey KA, Ferguson AV (2009) The subfornical organ: a central nervous system site for actions of circulating leptin. American journal of physiology Regulatory, integrative and comparative physiology 296:R512-520.

Sohara E, Luo Y, Zhang J, Manning DK, Beier DR, Zhou J (2008) Nek8 regulates the expression and localization of polycystin-1 and polycystin-2. Journal of the American Society of Nephrology : JASN 19:469-476.

Son SJ, Filosa JA, Potapenko ES, Biancardi VC, Zheng H, Patel KP, Tobin VA, Ludwig M, Stern JE (2013) Dendritic peptide release mediates interpopulation crosstalk between neurosecretory and preautonomic networks. Neuron 78:1036-1049.

Song K, Allen AM, Paxinos G, Mendelsohn FA (1992) Mapping of angiotensin II receptor subtype heterogeneity in rat brain. The Journal of comparative neurology 316:467-484.

Sophocleous A, Elmatzoglou I, Souvatzoglou A (2003) Circulating endogenous digitalis-like factor(s) (EDLF) in man is derived from the adrenals and its secretion is ACTH-dependent. Journal of endocrinological investigation 26:668-674.

Spinelli L, Golino P, Piscione F, Chiariello M, Focaccio A, Ambrosio G, Condorelli M (1987) Effects of oral salt load on arginine-vasopressin secretion in normal subjects. Annals of clinical and laboratory science 17:350-357.

Sposito NM, Gross PM (1987) Topography and morphometry of capillaries in the rat subfornical organ. Journal of Comparative Neurology 260:36-46.

Stachniak TJ, Trudel E, Bourque CW (2014) Cell-specific retrograde signals mediate antiparallel effects of angiotensin II on osmoreceptor afferents to vasopressin and oxytocin neurons. Cell reports 8:355-362.

Standish A, Enquist LW, Schwaber JS (1994) Innervation of the heart and its central medullary origin defined by viral tracing. Science (New York, NY) 263:232-234.

Stanek KA, Neil JJ, Sawyer WB, Loewy AD (1984) Changes in regional blood flow and cardiac output after L-glutamate stimulation of A5 cell group. The American journal of physiology 246:H44-51.

Stapelfeldt WH, Szurszewski JH (1989) The electrophysiological effects of neurotensin on neurones of guinea-pig prevertebral sympathetic ganglia. The Journal of physiology 411:301-323.

Stauss HM (2007) Identification of blood pressure control mechanisms by power spectral analysis. Clinical and experimental pharmacology & physiology 34:362-368.

Stein RD, Weaver LC, Yardley CP (1989) Ventrolateral medullary neurones: effects on magnitude and rhythm of discharge of mesenteric and renal nerves in cats. The Journal of physiology 408:571-586.

Stern JE (2015) Neuroendocrine-autonomic integration in the paraventricular nucleus: novel roles for dendritically released neuropeptides. Journal of neuroendocrinology 27:487-497.

Stern JE, Potapenko ES (2013) Enhanced NMDA receptor-mediated intracellular calcium signaling in magnocellular neurosecretory neurons in heart failure rats. American journal of physiology Regulatory, integrative and comparative physiology 305:R414-422.

Stern JE, Son S, Biancardi VC, Zheng H, Sharma N, Patel KP (2016) Astrocytes contribute to angiotensin II stimulation of hypothalamic neuronal activity and sympathetic outflow. Hypertension (Dallas, Tex : 1979) 68:1483-1493.

Stevens SL, Shaw TE, Dykhuizen E, Lessov NS, Hill JK, Wurst W, Stenzel-Poore MP (2003) Reduced cerebral injury in CRH-R1 deficient mice after focal ischemia: a potential link to microglia and atrocytes that express CRH-R1. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 23:1151-1159.

Stocker SD, Toney GM (2005) Median preoptic neurones projecting to the hypothalamic paraventricular nucleus respond to osmotic, circulating Ang II and baroreceptor input in the rat. The Journal of physiology 568:599-615.

Stocker SD, Toney GM (2007) Vagal afferent input alters the discharge of osmotic and ANG II-responsive median preoptic neurons projecting to the hypothalamic paraventricular nucleus. Brain research 1131:118-128.

Stocker SD, Gordon KW (2015) Glutamate receptors in the hypothalamic paraventricular nucleus contribute to insulin-induced sympathoexcitation. Journal of neurophysiology 113:1302-1309.

Stocker SD, Keith KJ, Toney GM (2004a) Acute inhibition of the hypothalamic paraventricular nucleus decreases renal sympathetic nerve activity and arterial blood pressure in water-deprived rats. American journal of physiology Regulatory, integrative and comparative physiology 286:R719-725.

Stocker SD, Cunningham JT, Toney GM (2004b) Water deprivation increases Fos immunoreactivity in PVN autonomic neurons with projections to the spinal cord and rostral ventrolateral medulla. American journal of physiology Regulatory, integrative and comparative physiology 287:R1172-1183.

Stocker SD, Hunwick KJ, Toney GM (2005) Hypothalamic paraventricular nucleus differentially supports lumbar and renal sympathetic outflow in water-deprived rats. The Journal of physiology 563:249-263.

Stocker SD, Simmons JR, Stornetta RL, Toney GM, Guyenet PG (2006) Water deprivation activates a glutamatergic projection from the hypothalamic paraventricular nucleus to the rostral ventrolateral medulla. The Journal of comparative neurology 494:673-685.

Stocker SD, Lang SM, Simmonds SS, Wenner MM, Farquhar WB (2015) Cerebrospinal fluid hypernatremia elevates sympathetic nerve activity and blood pressure via the rostral ventrolateral medulla. Hypertension (Dallas, Tex : 1979) 66:1184-1190.

Stokman MF, van der Zwaag B, van de Kar N, van Haelst MM, van Eerde AM, van der Heijden JW, Kroes HY, Ippel E, Schulp AJA, van Gassen KL, van Rooij I, Giles RH, Beales PL, Roepman R, Arts HH, Bongers E, Renkema KY, Knoers N, van Reeuwijk J, Lilien MR (2018) Clinical and genetic analyses of a Dutch cohort of 40 patients with a nephronophthisis-related ciliopathy. Pediatric nephrology (Berlin, Germany) 33:1701-1712.

Stornetta RL (2009) Neurochemistry of bulbospinal presympathetic neurons of the medulla oblongata. Journal of chemical neuroanatomy 38:222-230.

Stornetta RL, Hawelu-Johnson CL, Guyenet PG, Lynch KR (1988) Astrocytes synthesize angiotensinogen in brain. Science (New York, NY) 242:1444-1446.

Stornetta RL, Inglis MA, Viar KE, Guyenet PG (2016) Afferent and efferent connections of C1 cells with spinal cord or hypothalamic projections in mice. Brain structure & function 221:4027-4044.

Strack AM, Sawyer WB, Hughes JH, Platt KB, Loewy AD (1989) A general pattern of CNS innervation of the sympathetic outflow demonstrated by transneuronal pseudorabies viral infections. Brain research 491:156-162.

Stricker EM, Verbalis JG (1986) Interaction of osmotic and volume stimuli in regulation of neurohypophyseal secretion in rats. The American journal of physiology 250:R267-275.

Stuesse SL (1982) Origins of cardiac vagal preganglionic fibers: a retrograde transport study. Brain research 236:15-25.

Su ZZ, Leszczyniecka M, Kang DC, Sarkar D, Chao W, Volsky DJ, Fisher PB (2003) Insights into glutamate transport regulation in human astrocytes: cloning of the promoter for excitatory amino acid transporter 2 (EAAT2). Proceedings of the National Academy of Sciences of the United States of America 100:1955-1960.

Sun MK, Guyenet PG (1985) GABA-mediated baroreceptor inhibition of reticulospinal neurons. The American journal of physiology 249:R672-680.

Sunn N, McKinley MJ, Oldfield BJ (2003) Circulating angiotensin II activates neurones in circumventricular organs of the lamina terminalis that project to the bed nucleus of the stria terminalis. Journal of neuroendocrinology 15:725-731.

Sunn N, Egli M, Burazin TC, Burns P, Colvill L, Davern P, Denton DA, Oldfield BJ, Weisinger RS, Rauch M, Schmid HA, McKinley MJ (2002) Circulating relaxin acts on subfornical organ neurons to stimulate water drinking in the rat. Proceedings of the National Academy of Sciences of the United States of America 99:1701-1706.

Swanson LW, Kuypers HG (1980) The paraventricular nucleus of the hypothalamus: cytoarchitectonic subdivisions and organization of projections to the pituitary, dorsal vagal complex, and spinal cord as demonstrated by retrograde fluorescence double-labeling methods. The Journal of comparative neurology 194:555-570.

Sweeney WE, Gunay-Aygun M, Patil A, Avner ED (2014) Childhood polycystic kidney disease. In: Pediatric Nephrology (Avner ED, Harmon WE, Niaudet P, Yoshikawa N, Emma F, Goldstein S, eds), pp 1-58. Berlin, Heidelberg: Springer Berlin Heidelberg.

Swenne CA (2013) Baroreflex sensitivity: mechanisms and measurement. Netherlands Heart Journal 21:58-60.

Szinnai G, Morgenthaler NG, Berneis K, Struck J, Muller B, Keller U, Christ-Crain M (2007) Changes in plasma copeptin, the c-terminal portion of arginine vasopressin during water deprivation and excess in healthy subjects. The Journal of clinical endocrinology and metabolism 92:3973-3978.

Tada Y, Wada K, Shimada K, Makino H, Liang EI, Murakami S, Kudo M, Kitazato KT, Nagahiro S, Hashimoto T (2014) Roles of hypertension in the rupture of intracranial aneurysms. Stroke 45:579-586.

Takeda K, Nakata T, Takesako T, Itoh H, Hirata M, Kawasaki S, Hayashi J, Oguro M, Sasaki S, Nakagawa M (1991) Sympathetic inhibition and attenuation of spontaneous hypertension by PVN lesions in rats. Brain research 543:296-300.

Tan CL, Knight ZA (2018) Regulation of body temperature by the nervous system. Neuron 98:31-48.

Tan J, Wang JM, Leenen FH (2005) Inhibition of brain angiotensin-converting enzyme by peripheral administration of trandolapril versus lisinopril in Wistar rats. American journal of hypertension 18:158-164.

Tanaka J, Saito H, Kaba H (1987) Subfornical organ and hypothalamic paraventricular nucleus connections with median preoptic nucleus neurons: an electrophysiological study in the rat. Experimental brain research 68:579-585.

Tanaka J, Kaba H, Saito H, Seto K (1986) Efferent pathways from the region of the subfornical organ to hypothalamic paraventricular nucleus: an electrophysiological study in the rat. Experimental brain research 62:509-514.

Taxini CL, Moreira TS, Takakura AC, Bicego KC, Gargaglioni LH, Zoccal DB (2017) Role of A5 noradrenergic neurons in the chemoreflex control of respiratory and sympathetic activities in unanesthetized conditions. Neuroscience 354:146-157.

Taylor AC, McCarthy JJ, Stocker SD (2008) Mice lacking the transient receptor vanilloid potential 1 channel display normal thirst responses and central Fos activation to hypernatremia. American journal of physiology Regulatory, integrative and comparative physiology 294:R1285-1293.

Telega G, Cronin D, Avner ED (2013) New approaches to the autosomal recessive polycystic kidney disease patient with dual kidney-liver complications. Pediatric transplantation 17:328-335.

Thomas WG, Pipolo L, Qian H (1999) Identification of a Ca2+/calmodulin-binding domain within the carboxyl-terminus of the angiotensin II (AT1A) receptor. FEBS letters 455:367-371.

Thrasher TN (2005) Baroreceptors, baroreceptor unloading, and the long-term control of blood pressure. American journal of physiology Regulatory, integrative and comparative physiology 288:R819-827.

Thrasher TN, Keil LC (1987) Regulation of drinking and vasopressin secretion: role of organum vasculosum laminae terminalis. The American journal of physiology 253:R108-120.

Thrasher TN, Keil LC, Ramsay DJ (1982) Lesions of the organum vasculosum of the lamina terminalis (OVLT) attenuate osmotically-induced drinking and vasopressin secretion in the dog. Endocrinology 110:1837-1839.

Tinucci T, Abrahao SB, Santello JL, Mion D, Jr. (2001) Mild chronic renal insufficiency induces sympathetic overactivity. Journal of human hypertension 15:401-406.

Tiruneh MA, Huang BS, Leenen FH (2013) Role of angiotensin II type 1 receptors in the subfornical organ in the pressor responses to central sodium in rats. Brain research 1527:79-86.

Tobey JC, Fry HK, Mizejewski CS, Fink GD, Weaver LC (1983) Differential sympathetic responses initiated by angiotensin and sodium chloride. The American journal of physiology 245:R60-68.

Tomiyama O, Shiigai T, Ideura T, Tomita K, Mito Y, Shinohara S, Takeuchi J (1980) Baroreflex sensitivity in renal failure. Clinical science (London, England : 1979) 58:21-27.

Toney GM, Stocker SD (2010) Hyperosmotic activation of CNS sympathetic drive: implications for cardiovascular disease. The Journal of physiology 588:3375-3384.

Torres VE (2008a) Vasopressin antagonists in polycystic kidney disease. Semin Nephrol 28:306-317.

Torres VE (2008b) Vasopressin antagonists in polycystic kidney disease. Seminars in nephrology 28:306-317.

Torres VE, Harris PC (2009) Autosomal dominant polycystic kidney disease: the last 3 years. Kidney international 76:149-168.

Torres VE, Wilson DM, Burnett JC, Jr., Johnson CM, Offord KP (1991) Effect of inhibition of converting enzyme on renal hemodynamics and sodium management in polycystic kidney disease. Mayo Clinic proceedings 66:1010-1017.

Torres VE, Wang X, Qian Q, Somlo S, Harris PC, Gattone VH, 2nd (2004) Effective treatment of an orthologous model of autosomal dominant polycystic kidney disease. Nature medicine 10:363-364.

Torres VE, Donovan KA, Scicli G, Holley KE, Thibodeau SN, Carretero OA, Inagami T, McAteer JA, Johnson CM (1992) Synthesis of renin by tubulocystic epithelium in autosomal-dominant polycystic kidney disease. Kidney international 42:364-373.

Torres VE, Chapman AB, Devuyst O, Gansevoort RT, Perrone RD, Dandurand A, Ouyang J, Czerwiec FS, Blais JD (2018) Multicenter, open-label, extension trial to evaluate the long-term efficacy and safety of early versus delayed treatment with tolvaptan in autosomal dominant polycystic kidney disease: the TEMPO 4:4 Trial. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 33:477-489.

Torres VE, Chapman AB, Devuyst O, Gansevoort RT, Grantham JJ, Higashihara E, Perrone RD, Krasa HB, Ouyang J, Czerwiec FS (2012) Tolvaptan in patients with autosomal dominant polycystic kidney disease. The New England journal of medicine 367:2407-2418.

Torres VE, Chapman AB, Devuyst O, Gansevoort RT, Perrone RD, Koch G, Ouyang J, McQuade RD, Blais JD, Czerwiec FS, Sergeyeva O (2017) Tolvaptan in later-stage autosomal dominant polycystic kidney disease. The New England journal of medicine 377:1930-1942.

Touyz RM, Deng LY, Li JS, Schiffrin EL (1996) Differential effects of vasopressin and endothelin-1 on vascular contractile and calcium responses in pressurized small arteries from spontaneously hypertensive rats. Journal of hypertension 14:983-991.

Tribollet E, Dreifuss JJ (1981) Localization of neurones projecting to the hypothalamic paraventricular nucleus area of the rat: a horseradish peroxidase study. Neuroscience 6:1315-1328.

Trolliet MR, Phillips MI (1992) The effect of chronic bilateral nephrectomy on plasma and brain angiotensin. Journal of hypertension 10:29-36.

Tsuda T, Takefuji M, Wettschureck N, Kotani K, Morimoto R, Okumura T, Kaur H, Eguchi S, Sakaguchi T, Ishihama S, Kikuchi R, Unno K, Matsushita K, Ishikawa S, Offermanns S, Murohara T (2017) Corticotropin releasing hormone receptor 2 exacerbates chronic cardiac dysfunction. The Journal of experimental medicine 214:1877-1888.

Tsushima H, Mori M, Matsuda T (1994) Microinjections of angiotensin II into the supraoptic and paraventricular nuclei produce potent antidiureses by vasopressin release mediated through adrenergic and angiotensin receptors. Japanese journal of pharmacology 66:241-246.

Tucker AB, Stocker SD (2016) Hypernatremia-induced vasopressin secretion is not altered in TRPV1-/- rats. American journal of physiology Regulatory, integrative and comparative physiology 311:R451-456.

Tufan F, Uslu B, Cekrezi B, Uysal M, Alpay N, Turkmen K, Disci R, Ozbey NC, Ecder T (2010) Assessment of adrenal functions in patients with autosomal dominant polycystic kidney disease. Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association 118:741-746.

Turner A, Kumar N, Farnham M, Lung M, Pilowsky P, McMullan S (2013) Rostroventrolateral medulla neurons with commissural projections provide input to sympathetic premotor neurons: anatomical and functional evidence. The European journal of neuroscience 38:2504-2515.

Turner MR, Pallone TL (1997) Vasopressin constricts outer medullary descending vasa recta isolated from rat kidneys. The American journal of physiology 272:F147-151.

Tweedle CD, Hatton GI (1984) Synapse formation and disappearance in adult rat supraoptic nucleus during different hydration states. Brain research 309:373-376.

Ullian ME (1999) The role of corticosteriods in the regulation of vascular tone. Cardiovascular research 41:55-64.

Ulrich-Lai YM, Engeland WC (2002) Adrenal splanchnic innervation modulates adrenal cortical responses to dehydration stress in rats. Neuroendocrinology 76:79-92.

Ulrich-Lai YM, Jones KR, Ziegler DR, Cullinan WE, Herman JP (2011a) Forebrain origins of glutamatergic innervation to the rat paraventricular nucleus of the hypothalamus: differential inputs to the anterior versus posterior subregions. The Journal of comparative neurology 519:1301-1319.

Ulrich-Lai YM, Jones KR, Ziegler DR, Cullinan WE, Herman JP (2011b) Forebrain origins of glutamatergic innervation to the rat paraventricular nucleus of the hypothalamus: differential inputs to the anterior versus posterior subregions. The Journal of comparative neurology 519:1301-1319.

Underhill SM, Wheeler DS, Amara SG (2015) Differential regulation of two isoforms of the glial glutamate transporter EAAT2 by DLG1 and CaMKII. The Journal of neuroscience : the official journal of the Society for Neuroscience 35:5260-5270.

Underwood CF, Boyd R, Phillips JK, Hildreth CM (2018) Increased excitatory regulation of the hypothalamic paraventricular nucleus and circulating vasopressin results in the hypertension observed in polycystic kidney disease. Journal of hypertension.

Underwood CF, Hildreth CM, Wyse BF, Boyd R, Goodchild AK, Phillips JK (2017) Uraemia: an unrecognized driver of central neurohumoral dysfunction in chronic kidney disease? Acta physiologica (Oxford, England) 219:305-323.

Uschakov A, McGinty D, Szymusiak R, McKinley MJ (2009) Functional correlates of activity in neurons projecting from the lamina terminalis to the ventrolateral periaqueductal gray. The European journal of neuroscience 30:2347-2355.

USRDS (2016) United States Renal Data System annual data report: Epidemiology of kidney disease in the United States. In: (National Institutes of Health NIoDaDaKD, ed). Bethesda, MD.

Valenta LJ, Elias AN, Eisenberg H (1986) ACTH stimulation of adrenal epinephrine and norepinephrine release. Hormone research 23:16-20.

Valero FA, Martinez-Vea A, Bardaji A, Gutierrez C, Garcia C, Richart C, Oliver JA (1999) Ambulatory blood pressure and left ventricular mass in normotensive patients with autosomal dominant polycystic kidney disease. Journal of the American Society of Nephrology : JASN 10:1020-1026.

Valtin H (1982) The brattleboro rat. Annals of the New York Academy of Sciences 394:1-828.

Van Bockstaele EJ, Pieribone VA, Aston-Jones G (1989) Diverse afferents converge on the nucleus paragigantocellularis in the rat ventrolateral medulla: retrograde and anterograde tracing studies. The Journal of comparative neurology 290:561-584.

van Bodegom M, Homberg JR, Henckens M (2017) Modulation of the hypothalamic-pituitary-adrenal axis by early life stress exposure. Frontiers in cellular neuroscience 11:87.

van den Pol AN (1991) Glutamate and aspartate immunoreactivity in hypothalamic presynaptic axons. The Journal of neuroscience : the official journal of the Society for Neuroscience 11:2087-2101.

van den Pol AN, Wuarin JP, Dudek FE (1990) Glutamate, the dominant excitatory transmitter in neuroendocrine regulation. Science (New York, NY) 250:1276-1278.

van Gastel MDA, Torres VE (2017) Polycystic kidney disease and the vasopressin pathway. Annals of nutrition & metabolism 70 Suppl 1:43-50.

Vann SD, Nelson AJ (2015) The mammillary bodies and memory: more than a hippocampal relay. Progress in brain research 219:163-185.

Veitenheimer B, Osborn JW (2011) Role of spinal V1a receptors in regulation of arterial pressure during acute and chronic osmotic stress. American journal of physiology Regulatory, integrative and comparative physiology 300:R460-469.

Veitenheimer B, Osborn JW (2013) Effects of intrathecal kynurenate on arterial pressure during chronic osmotic stress in conscious rats. American journal of physiology Heart and circulatory physiology 304:H303-310.

Veitenheimer BJ, Engeland WC, Guzman PA, Fink GD, Osborn JW (2012) Effect of global and regional sympathetic blockade on arterial pressure during water deprivation in conscious rats. American journal of physiology Heart and circulatory physiology 303:H1022-1034.

Veltmar A, Culman J, Qadri F, Rascher W, Unger T (1992) Involvement of adrenergic and angiotensinergic receptors in the paraventricular nucleus in the angiotensin II-induced

vasopressin release. The Journal of pharmacology and experimental therapeutics 263:1253-1260.

Verbalis JG (2007) How does the brain sense osmolality? Journal of the American Society of Nephrology : JASN 18:3056-3059.

Verney EB (1947) The antidiuretic hormone and the factors which determine its release. Proceedings of the Royal Society of London Series B, Biological sciences 135:25-106.

Vieira AA, Nahey DB, Collister JP (2010) Role of the organum vasculosum of the lamina terminalis for the chronic cardiovascular effects produced by endogenous and exogenous ANG II in conscious rats. American journal of physiology Regulatory, integrative and comparative physiology 299:R1564-1571.

Voisin DL, Chapman C, Poulain DA, Herbison AE (1994) Extracellular GABA concentrations in rat supraoptic nucleus during lactation and following haemodynamic changes: an in vivo microdialysis study. Neuroscience 63:547-558.

Vollard F, Fahr K (1914) Die Brightische Neirenkrankheit. Berlin, Julius Springer.

von Lutterotti N, Catanzaro DF, Sealey JE, Laragh JH (1994) Renin is not synthesized by cardiac and extrarenal vascular tissues. A review of experimental evidence. Circulation 89:458-470.

Waldherr R, Lennert T, Weber HP, Fodisch HJ, Scharer K (1982) The nephronophthisis complex. A clinicopathologic study in children. Virchows Archiv A, Pathological anatomy and histology 394:235-254.

Wang D, Iversen J, Strandgaard S (1999) Contractility and endothelium-dependent relaxation of resistance vessels in polycystic kidney disease rats. Journal of vascular research 36:502-509.

Wang D, Iversen J, Wilcox CS, Strandgaard S (2003) Endothelial dysfunction and reduced nitric oxide in resistance arteries in autosomal-dominant polycystic kidney disease. Kidney international 64:1381-1388.

Wang F, Flanagan J, Su N, Wang LC, Bui S, Nielson A, Wu X, Vo HT, Ma XJ, Luo Y (2012) RNAscope: a novel in situ RNA analysis platform for formalin-fixed, paraffin-embedded tissues. The Journal of molecular diagnostics : JMD 14:22-29.

Wang G, Coleman CG, Chan J, Faraco G, Marques-Lopes J, Milner TA, Guruju MR, Anrather J, Davisson RL, Iadecola C, Pickel VM (2013) Angiotensin II slow-pressor hypertension enhances NMDA currents and NOX2-dependent superoxide production

in hypothalamic paraventricular neurons. American journal of physiology Regulatory, integrative and comparative physiology 304:R1096-1106.

Wang HW, Huang BS, White RA, Chen A, Ahmad M, Leenen FH (2016a) Mineralocorticoid and angiotensin II type 1 receptors in the subfornical organ mediate angiotensin II - induced hypothalamic reactive oxygen species and hypertension. Neuroscience 329:112-121.

Wang L, Hiller H, Smith JA, de Kloet AD, Krause EG (2016b) Angiotensin type 1a receptors in the paraventricular nucleus of the hypothalamus control cardiovascular reactivity and anxiety-like behavior in male mice. Physiological genomics 48:667-676.

Wang LA, Nguyen DH, Mifflin SW (2018) Corticotropin-releasing hormone projections from the paraventricular nucleus of the hypothalamus to the nucleus of the solitary tract increases blood pressure. Journal of neurophysiology.

Wang S, Zhang J, Nauli SM, Li X, Starremans PG, Luo Y, Roberts KA, Zhou J (2007) Fibrocystin/polyductin, found in the same protein complex with polycystin-2, regulates calcium responses in kidney epithelia. Molecular and cellular biology 27:3241-3252.

Wang X, Gattone V, 2nd, Harris PC, Torres VE (2005) Effectiveness of vasopressin V2 receptor antagonists OPC-31260 and OPC-41061 on polycystic kidney disease development in the PCK rat. Journal of the American Society of Nephrology : JASN 16:846-851.

Wang X, Wu Y, Ward CJ, Harris PC, Torres VE (2008) Vasopressin directly regulates cyst growth in polycystic kidney disease. Journal of the American Society of Nephrology : JASN 19:102-108.

Watanabe E, Hiyama TY, Shimizu H, Kodama R, Hayashi N, Miyata S, Yanagawa Y, Obata K, Noda M (2006) Sodium-level-sensitive sodium channel Na(x) is expressed in glial laminate processes in the sensory circumventricular organs. American journal of physiology Regulatory, integrative and comparative physiology 290:R568-576.

Watson ML, Macnicol AM, Allan PL, Wright AF (1992) Effects of angiotensin converting enzyme inhibition in adult polycystic kidney disease. Kidney international 41:206-210.

Wehrwein EA, Orer HS, Barman SM (2016) Overview of the anatomy, physiology, and pharmacology of the autonomic nervous system. Comprehensive Physiology 6:1239-1278.

Wei SG, Yu Y, Zhang ZH, Felder RB (2015) Proinflammatory cytokines upregulate sympathoexcitatory mechanisms in the subfornical organ of the rat. Hypertension (Dallas, Tex : 1979) 65:1126-1133.

Wei SG, Yu Y, Zhang ZH, Weiss RM, Felder RB (2008) Mitogen-activated protein kinases mediate upregulation of hypothalamic angiotensin II type 1 receptors in heart failure rats. Hypertension (Dallas, Tex : 1979) 52:679-686.

Wei SG, Zhang ZH, Beltz TG, Yu Y, Johnson AK, Felder RB (2013) Subfornical organ mediates sympathetic and hemodynamic responses to blood-borne proinflammatory cytokines. Hypertension (Dallas, Tex : 1979) 62:118-125.

Weinberger MH (1996) Salt sensitivity of blood pressure in humans. Hypertension (Dallas, Tex : 1979) 27:481-490.

Weiss ML, Hatton GI (1990) Collateral input to the paraventricular and supraoptic nuclei in rat. I. Afferents from the subfornical organ and the anteroventral third ventricle region. Brain research bulletin 24:231-238.

Welsh DG, Segal SS (1998) Endothelial and smooth muscle cell conduction in arterioles controlling blood flow. The American journal of physiology 274:H178-186.

Wenker IC, Abe C, Viar KE, Stornetta DS, Stornetta RL, Guyenet PG (2017) Blood pressure regulation by the rostral ventrolateral medulla in conscious rats: effects of hypoxia, hypercapnia, baroreceptor denervation and anesthesia. The Journal of neuroscience : the official journal of the Society for Neuroscience 37:4565-4583.

Westerhaus MJ, Loewy AD (1999) Sympathetic-related neurons in the preoptic region of the rat identified by viral transneuronal labeling. The Journal of comparative neurology 414:361-378.

White JD, Krause JE, McKelvy JF (1984) In vivo biosynthesis and transport of oxytocin, vasopressin, and neurophysins to posterior pituitary and nucleus of the solitary tract. The Journal of neuroscience : the official journal of the Society for Neuroscience 4:1262-1270.

Whitworth JA, Saines D, Thatcher R, Butkus A, Scoggins BA (1983) Blood pressure and metabolic effects of ACTH in normotensive and hypertensive man. Clinical and experimental hypertension Part A, Theory and practice 5:501-522.

Whitworth JA, Hewitson TD, Ming L, Wilson RS, Scoggins BA, Wright RD, Kincaid-Smith P (1990) Adrenocorticotrophin-induced hypertension in the rat: haemodynamic, metabolic and morphological characteristics. Journal of hypertension 8:27-36.

Wilbert-Lampen U, Straube F, Trapp A, Deutschmann A, Plasse A, Steinbeck G (2006) Effects of corticotropin-releasing hormone (CRH) on monocyte function, mediated by CRH-receptor subtype R1 and R2: a potential link between mood disorders and endothelial dysfunction? Journal of cardiovascular pharmacology 47:110-116.

Willey CJ, Blais JD, Hall AK, Krasa HB, Makin AJ, Czerwiec FS (2017) Prevalence of autosomal dominant polycystic kidney disease in the European Union. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 32:1356-1363.

Womack MD, Morris R, Gent TC, Barrett-Jolley R (2007) Substance P targets sympathetic control neurons in the paraventricular nucleus. Circulation research 100:1650-1658.

Wong ATY et al. (2018) Randomised controlled trial to determine the efficacy and safety of prescribed water intake to prevent kidney failure due to autosomal dominant polycystic kidney disease (PREVENT-ADPKD). BMJ open 8:e018794.

Wong LF, Polson JW, Murphy D, Paton JF, Kasparov S (2002) Genetic and pharmacological dissection of pathways involved in the angiotensin II-mediated depression of baroreflex function. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 16:1595-1601.

Wu G, Markowitz GS, Li L, D'Agati VD, Factor SM, Geng L, Tibara S, Tuchman J, Cai Y, Park JH, van Adelsberg J, Hou H, Jr., Kucherlapati R, Edelmann W, Somlo S (2000) Cardiac defects and renal failure in mice with targeted mutations in Pkd2. Nature genetics 24:75-78.

Wüthrich RP, Kistler AD, Rodriguez D, Kapoor S, Mei C (2017) Blood pressure control for polycystic kidney disease. In: Polycystic Kidney Disease (X L, ed): Codon Publications.

Xie AX, Petravicz J, McCarthy KD (2015) Molecular approaches for manipulating astrocytic signaling in vivo. Frontiers in cellular neuroscience 9:144.

Xiong XQ, Chen WW, Han Y, Zhou YB, Zhang F, Gao XY, Zhu GQ (2012) Enhanced adipose afferent reflex contributes to sympathetic activation in diet-induced obesity hypertension. Hypertension (Dallas, Tex : 1979) 60:1280-1286.

Xu B, Zheng H, Patel KP (2012) Enhanced activation of RVLM-projecting PVN neurons in rats with chronic heart failure. American journal of physiology Heart and circulatory physiology 302:H1700-H1711.

Xue B, Zhang Z, Johnson RF, Johnson AK (2012) Sensitization of slow pressor angiotensin II (Ang II)-initiated hypertension: induction of sensitization by prior Ang II treatment. Hypertension (Dallas, Tex : 1979) 59:459-466.

Yamada Y, Yamamura Y, Chihara T, Onogawa T, Nakamura S, Yamashita T, Mori T, Tominaga M, Yabuuchi Y (1994) OPC-21268, a vasopressin V1 antagonist, produces hypotension in spontaneously hypertensive rats. Hypertension (Dallas, Tex : 1979) 23:200-204.

Yamaguchi K, Morimoto A, Murakami N (1993) Organum vasculosum laminae terminalis (OVLT) in rabbit and rat: topographic studies. The Journal of comparative neurology 330:352-362.

Yamamura Y, Ogawa H, Chihara T, Kondo K, Onogawa T, Nakamura S, Mori T, Tominaga M, Yabuuchi Y (1991) OPC-21268, an orally effective, nonpeptide vasopressin V1 receptor antagonist. Science (New York, NY) 252:572-574.

Yamashita H, Koizumi K (1979) Influence of carotid and aortic baroreceptors on neurosecretory neurons in supraoptic nuclei. Brain research 170:259-277.

Yang T, Xu C (2017) Physiology and pathophysiology of the intrarenal renin-angiotensin system: an update. Journal of the American Society of Nephrology : JASN 28:1040-1049.

Yang Z, Coote JH (2007) Paraventricular nucleus influence on renal sympathetic activity in vasopressin gene-deleted rats. Experimental physiology 92:109-117.

Yang Z, Wheatley M, Coote JH (2002) Neuropeptides, amines and amino acids as mediators of the sympathetic effects of paraventricular nucleus activation in the rat. Experimental physiology 87:663-674.

Yao ST, May CN (2013) Intra-carotid angiotensin II activates tyrosine hydroxylase-expressing rostral ventrolateral medulla neurons following blood-brain barrier disruption in rats. Neuroscience 245:148-156.

Yao Y, Hildreth CM, Farnham MM, Saha M, Sun QJ, Pilowsky PM, Phillips JK (2015) The effect of losartan on differential reflex control of sympathetic nerve activity in chronic kidney disease. Journal of hypertension 33:1249-1260.

Ye ZY, Li DP, Pan HL (2013) Regulation of Hypothalamic Presympathetic Neurons and Sympathetic Outflow by Group II Metabotropic Glutamate Receptors in Spontaneously Hypertensive Rats. Hypertension (Dallas, Tex : 1979) 62:255-262.

Ye ZY, Li DP, Li L, Pan HL (2011) Protein kinase CK2 increases glutamatergic input in the hypothalamus and sympathetic vasomotor tone in hypertension. The Journal of neuroscience : the official journal of the Society for Neuroscience 31:8271-8279.

Ye ZY, Li L, Li DP, Pan HL (2012a) Casein kinase 2-mediated synaptic GluN2A up-regulation increases N-methyl-D-aspartate receptor activity and excitability of hypothalamic neurons in hypertension. The Journal of biological chemistry 287:17438-17446.

Ye ZY, Li DP, Byun HS, Li L, Pan HL (2012b) NKCC1 upregulation disrupts chloride homeostasis in the hypothalamus and increases neuronal activity-sympathetic drive in hypertension. The Journal of neuroscience : the official journal of the Society for Neuroscience 32:8560-8568.

Yiannikouris F, Gupte M, Putnam K, Thatcher S, Charnigo R, Rateri DL, Daugherty A, Cassis LA (2012) Adipocyte deficiency of angiotensinogen prevents obesity-induced hypertension in male mice. Hypertension (Dallas, Tex : 1979) 60:1524-1530.

Yimin Y, Hildreth CM, Sheran L, Boyd R, Kouchaki Z, Butlin M, Avolio AP, Pilowsky PM, Phillips JK (2017) Increased arterial stiffness does not respond to renal denervation in an animal model of secondary hypertension. Conference proceedings : Annual International Conference of the IEEE Engineering in Medicine and Biology Society IEEE Engineering in Medicine and Biology Society Annual Conference 2017:258-261.

Yoder BK, Hou X, Guay-Woodford LM (2002) The polycystic kidney disease proteins, polycystin-1, polycystin-2, polaris, and cystin, are co-localized in renal cilia. Journal of the American Society of Nephrology : JASN 13:2508-2516.

Yoshihara D, Kugita M, Sasaki M, Horie S, Nakanishi K, Abe T, Aukema HM, Yamaguchi T, Nagao S (2013) Telmisartan ameliorates fibrocystic liver disease in an orthologous rat model of human autosomal recessive polycystic kidney disease. PloS one 8:e81480-e81480.

Young CN, Morgan DA, Butler SD, Mark AL, Davisson RL (2013) The brain subfornical organ mediates leptin-induced increases in renal sympathetic activity but not its metabolic effects. Hypertension (Dallas, Tex : 1979) 61:737-744.

Yu JJ, Zhang Y, Wang Y, Wen ZY, Liu XH, Qin J, Yang JL (2013) Inhibition of calcineurin in the prefrontal cortex induced depressive-like behavior through mTOR signaling pathway. Psychopharmacology 225:361-372.

Yuan CM, Manunta P, Hamlyn JM, Chen S, Bohen E, Yeun J, Haddy FJ, Pamnani MB (1993) Long-term ouabain administration produces hypertension in rats. Hypertension (Dallas, Tex : 1979) 22:178-187.

Zafar I, Tao Y, Falk S, McFann K, Schrier RW, Edelstein CL (2007) Effect of statin and angiotensin-converting enzyme inhibition on structural and hemodynamic alterations in autosomal dominant polycystic kidney disease model. American journal of physiology Renal physiology 293:F854-859.

Zagon A, Smith AD (1993) Monosynaptic projections from the rostral ventrolateral medulla oblongata to identified sympathetic preganglionic neurons. Neuroscience 54:729-743.

Zaretsky DV, Zaretskaia MV, Samuels BC, Cluxton LK, DiMicco JA (2003) Microinjection of muscimol into raphe pallidus suppresses tachycardia associated with air stress in conscious rats. The Journal of physiology 546:243-250.

Zeltner R, Poliak R, Stiasny B, Schmieder RE, Schulze BD (2008) Renal and cardiac effects of antihypertensive treatment with ramipril vs metoprolol in autosomal dominant polycystic kidney disease. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 23:573-579.

Zerres K, Rudnik-Schoneborn S, Deget F, Holtkamp U, Brodehl J, Geisert J, Scharer K (1996) Autosomal recessive polycystic kidney disease in 115 children: clinical presentation, course and influence of gender. Arbeitsgemeinschaft fur Padiatrische, Nephrologie. Acta paediatrica (Oslo, Norway : 1992) 85:437-445.

Zhang BB, Jin H, Bing YH, Zhang XY, Chu CP, Li YZ, Qiu DL (2019) A nitric oxide-dependent presynaptic LTP at glutamatergic synapses of the PVN magnocellular neurosecretory cells in vitro in rats. Frontiers in cellular neuroscience 13:283.

Zhang L, Hernandez VS (2013) Synaptic innervation to rat hippocampus by vasopressin-immuno-positive fibres from the hypothalamic supraoptic and paraventricular nuclei. Neuroscience 228:139-162.

Zhang L, Xiong XQ, Fan ZD, Gan XB, Gao XY, Zhu GQ (2012) Involvement of enhanced cardiac sympathetic afferent reflex in sympathetic activation in early stage of diabetes. Journal of applied physiology (Bethesda, Md : 1985) 113:47-55.

Zhang X, Abdel-Rahman AR, Wooles WR (1992) Vasopressin receptors in the area postrema differentially modulate baroreceptor responses in rats. European journal of pharmacology 222:81-91.

Zhang X, Hense HW, Riegger GA, Schunkert H (1999) Association of arginine vasopressin and arterial blood pressure in a population-based sample. Journal of hypertension 17:319-324.

Zhao Z, Wang L, Gao W, Hu F, Zhang J, Ren Y, Lin R, Feng Q, Cheng M, Ju D, Chi Q, Wang D, Song S, Luo M, Zhan C (2017) A Central Catecholaminergic Circuit Controls Blood Glucose Levels during Stress. Neuron 95:138-152.e135.

Zheng H, Li YF, Wang W, Patel KP (2009) Enhanced angiotensin-mediated excitation of renal sympathetic nerve activity within the paraventricular nucleus of anesthetized rats with heart failure. American journal of physiology Regulatory, integrative and comparative physiology 297:R1364-R1374.

Zhou JJ, Shao JY, Ma H, Pan HL, Li DP (2019) Enhancement of hypothalamic corticotrophin releasing hormone signal contributes to elevated sympathetic outflow in spontaneous hypertension rat. FASEB 33:744.747-744.747.

Zhu GQ, Patel KP, Zucker IH, Wang W (2002) Microinjection of ANG II into paraventricular nucleus enhances cardiac sympathetic afferent reflex in rats. American journal of physiology Heart and circulatory physiology 282:H2039-2045.

Zhu GQ, Xu Y, Zhou LM, Li YH, Fan LM, Wang W, Gao XY, Chen Q (2009) Enhanced cardiac sympathetic afferent reflex involved in sympathetic overactivity in renovascular hypertensive rats. Experimental physiology 94:785-794.

Ziegler DR, Cullinan WE, Herman JP (2005) Organization and regulation of paraventricular nucleus glutamate signaling systems: N-methyl-D-aspartate receptors. The Journal of comparative neurology 484:43-56.

Zimmerman CA, Leib DE, Knight ZA (2017) Neural circuits underlying thirst and fluid homeostasis. Nature Reviews Neuroscience 18:459-469.

Zimmerman CA, Huey EL, Ahn JS, Beutler LR, Tan CL, Kosar S, Bai L, Chen Y, Corpuz TV, Madisen L, Zeng H, Knight ZA (2019) A gut-to-brain signal of fluid osmolarity controls thirst satiation. Nature 568:98-102.

Zimmerman MC, Lazartigues E, Lang JA, Sinnayah P, Ahmad IM, Spitz DR, Davisson RL (2002) Superoxide mediates the actions of angiotensin II in the central nervous system. Circulation research 91:1038-1045.

Zittema D, Boertien WE, van Beek AP, Dullaart RP, Franssen CF, de Jong PE, Meijer E, Gansevoort RT (2012) Vasopressin, copeptin, and renal concentrating capacity in patients with autosomal dominant polycystic kidney disease without renal

impairment. Clinical journal of the American Society of Nephrology : CJASN 7:906-913.

Zittema D, Versteeg IB, Gansevoort RT, van Goor H, de Heer E, Veraar KAM, Peters DJM, Meijer E (2016) Dose-titrated vasopressin V2 receptor antagonist improves renoprotection in a mouse model for autosomal dominant polycystic kidney disease. American journal of nephrology 44:194-203.

Zittema D, van den Berg E, Meijer E, Boertien WE, Muller Kobold AC, Franssen CF, de Jong PE, Bakker SJ, Navis G, Gansevoort RT (2014) Kidney function and plasma copeptin levels in healthy kidney donors and autosomal dominant polycystic kidney disease patients. Clinical journal of the American Society of Nephrology : CJASN 9:1553-1562.

Zoumakis E, Chrousos GP (2010) Corticotropin-releasing hormone receptor antagonists: an update. Endocrine development 17:36-43.

Appendices

Appendix 1.

Supraoptic nucleus vasopressin neurons are activated in PKD but chronic V1A receptor blockade is not anti-hypertensive

Underwood C.F., Phillips J.K. & Hildreth C.M

Abstract

Polycystic kidney disease (PKD) is characterised by the progressive enlargement of multiple renal cysts and hypertension. In PKD, higher circulating vasopressin levels drive cyst growth and is suspected to contribute to hypertension. However, it is currently unknown what the neural origins are and also whether inhibiting the vascular action of this neurohormone is anti-hypertensive. We first tested the hypothesis that the supraoptic nucleus (SON) contains more activated vasopressin neurons in a PKD rat model, the Lewis polycystic kidney (LPK) rat. Using immunohistochemistry, we found that 85% more vasopressin neurons expressed Fos/Fra immediate-early genes, a reporter of neuronal activity, in LPK relative to control Lewis animals (P=0.01, n=10). Then, the contribution of peripheral V_{1A} receptors to the development of hypertension in LPK was assessed by chronically treating animals with OPC-21268, an orally-active V_{1A} receptor antagonist. The arterial pressure of OPC-21268-treated LPK rats was comparable with that of untreated LPK rats throughout the treatment period. Our data indicate that the SON contains a greater proportion of activated vasopressin neurons in LPK rats; however, it is unlikely that V_{1A} receptor antagonists would be beneficial for the management of hypertension in PKD.

Introduction

Polycystic kidney disease (PKD) is a prevalent monogenetic disorder characterised by the progressive accumulation of multiple renal cysts and the development of hypertension (Chapman et al., 2010; Zittema et al., 2012). In PKD, the neurohypophysial hormone vasopressin is present in higher plasma concentrations (Danielsen et al., 1986b; Danielsen et al., 1986a; Michalski and Grzeszczak, 1996; Zittema et al., 2014) and serves to accelerate cyst expansion (Wang et al., 2008; Torres et al., 2012). In addition, elevated vasopressin levels have been suggested elevate arterial pressure in PKD via the activation of vascular V_{1A} receptors (Torres, 2008a; Wüthrich et al., 2017), but whether chronic V_{1A} receptor antagonism protects against hypertension in this condition has never been tested.

The magnocellular neurons that synthesise and secrete vasopressin into the circulation are distributed between two major sites in the mammalian brain: the supraoptic (SON) and paraventricular (PVN) hypothalamic nuclei (Brown et al., 2013). These neurons are sensitive to changes in extracellular fluid osmolality and, to a lesser extent, blood volume and pressure through synaptic connections with the forebrain osmoreceptors and visceral receptors conveying blood volume and pressure (baroreceptor) information (Bankir, 2001; Brown et al., 2013). In rodents, salt-loading (Choe et al., 2015a) and renin-angiotensin system over-activation (Korpal et al., 2017) elevates the discharge of SON vasopressin neurons, reflecting enhanced osmotic drive and/or an elimination of baroreceptor inhibition. In these conditions V_{1A} receptor antagonism chronically lowers arterial pressure suggesting that a greater basal section of vasopressin contributes to the hypertension observed (Choe et al., 2015a; Korpal et al., 2017).

The Lewis Polycystic Kidney (LPK) rat is a PKD model that displays elevated circulating vasopressin levels (Underwood et al., 2017) and marked hypertension (Hildreth et al., 2013b). We have previously found that both acute intravenous administration of a selective V_{1A} receptor antagonist (Chapter 2) (Underwood et al., 2018) and chronic high water-intake (to suppress vasopressin release) (Chapter 4) reduces arterial pressure in LPK rats, consistent with the view that vasopressin maintains a component of the hypertension exhibited in these animals. While the central nervous system origins of high vasopressin release in LPK animals, and PKD more generally, are unknown, previously we demonstrated that the PVN is not responsible for the V_{1A} receptor-dependent hypertension observed in LPK rats (Chapter 2) (Underwood et al., 2018). This therefore raises the possibility that magnocellular neurons

located in the SON are overactive and contributing to the hypertension observed in PKD via the release of vasopressin. The present study was therefore undertaken to address the following questions: (1) do SON vasopressin neurons display a greater level of ongoing activity in PKD?; and (2) does long-term inhibition of peripheral V_{1A} receptors attenuate the development of hypertension in PKD?

Methods

Male LPK and Lewis control rats were sourced from the Animal Resource Centre, Murdoch, Western Australia, Australia, and housed in standard living conditions with a 12-hour light/dark cycle and access to chow and water ad libitum. Experiments were performed in accordance with The Australian Code of Practice for the Care and Use of Animals (8th Edition, 2013) and approved by the Macquarie University Animal Ethics Committee.

Study 1: Do SON vasopressin neurons display a greater level of ongoing activity in PKD?

Male LPK and Lewis rats (13-14 weeks; *n* = 5 per strain) were deeply anaesthetised with sodium pentobarbital (100 mg/kg i.p.) and transcardially perfused with 300 ml ice-cold saline followed by 10% neutral-buffered formalin. Thereafter the brain was removed, post-fixed at 4 ºC in 10% neutral-buffered formalin for a period of 12 hours and then stored in TPBSm (10 mM Tris-HCL, 0.1 M phosphate-buffered saline [PBS], and 0.5% merthiolate) until further processing. Forebrains were sectioned at 50 µm in the coronal plane using a vibrating microtome (VT1200S, Leica).

To assess chronic activation of SON vasopressin neurons, one in four sequential sections were processed to visualise vasopressin neurons using a guineapig polyclonal vasopressin antibody (1:2000; T-5048, Peninsula Laboratories International Inc., CA, USA) and Fos/Fra-positive nuclei using a rabbit polyclonal antibody (1:1000; K-25, Santa Cruz Biotechnology, Santa Cruz, CA). Antigens were visualised simultaneously using a combination of immmofluorescence (vasopressin) and 3,3'-diaminobenzidine (DAB; Fos/Fra). Free-floating sections were washed in PBS containing 0.3% hydrogen peroxide for 10 minutes followed by three successive 15 minute PBS washes. Sections were then incubated for 1 hour at 4 ºC in a blocking solution containing avidin block (20%; Vector Laboratories, CA, USA) and serum (5% normal goat, 5% normal donkey and 20% avidin block in TPBSm containing 0.3% Triton-X-100). Sections were then transferred to the primary antibody solution which contained a biotin blocking agent (20%, Vector Laboratories) and serum (5% normal goat, 5% normal donkey and 20% avidin block in TPBSm containing 0.3% Triton-X-100). Following primary antibody incubation for 72 hours at 4 ºC, sections were washed (PBS; 3x15 minutes) and subsequently incubated for 24 hours at 4 ºC in a solution containing Cy3-congugated donkey anti-guineapig IgG (1:500; Jackson ImmunoResearch) and biotin-conjugated goat anti-rabbit IgG (1:500; Jackson ImmunoResearch) in TPBS with 1% serum. For chromogenic detection of biotin, sections were subsequently washed (PBS; 3x15

minutes) incubated overnight at 4 ºC in extravidin-peroxidase (1:1000; E-2886, Sigma Aldrich, Australia), washed (PBS; 3x15 minutes) and then underwent the DAB reactions for 75 seconds (SK-4100; Vector Labs). Sections were mounted onto glass sides and cover-slipped with fluorescent mounting medium (Agilent, CA, USA). Images were then captured at 10x magnification using an Axio Imager Z1 (Zeiss, Germany).

The total number of vasopressin neurons and Fos/Fra-positive nuclei was quantified using the analyse particles function in ImageJ (Fiji) (Schindelin et al., 2012). For each image, all detections were overlayed on the raw image and manually corrected to ensure accurate detection of nuclei. The number of vasopressin neurons with Fos/Fra nuclei was manually counted in overlayed images. For each animal, a total of 4-6 sections containing the SON were analysed bilaterally over the range of -0.48 to -1.44 mm Bregma and averaged.

Study 2: Does long-term inhibition of peripheral V_{1A} receptors attenuate the development of hypertension in PKD?

6-week-old LPK (n = 14) male animals were anaesthetised with isoflurane and a radiotelemetry probe (PAC10 or HDX10; Data Sciences International, St Paul, MN, USA) implanted into the aorta via the femoral artery and probe body positioned in the subcutaneous space of the inner hind-leg. Animals were administered carprofen (2.5 mg/kg s.c) and cephazolin (50 mg/kg i.m.) and allowed to recover for at least 5 days. Following surgery LPK animals were randomised to an untreated (n = 8) or OPC-21268 (30 mg/kg p.o. 2.d administered in 70% sweetened condensed milk 1 ml/kg; Otsuka Pharmaceuticals, Japan; n = 6) treatment group. The control LPK group are the same untreated cohort reported in Chapter 4, with telemetry data sampled from an alternative day. The dose of OPC-21268 was chosen from previous publications (Yamamura et al., 1991; Burrell et al., 1994) and from a pilot study which found that treating Lewis animals with this dose for one week produced a 70% reduction in the diastolic pressor response to an intravenous vasopressin bolus (100 ng/kg) compared to untreated Lewis rats (13 ± 2 vs. 43 ± 5 mmHg, n = 4 per group; $P < 0.01$). Animals were treated from 6 to 13 weeks of age.

Arterial blood pressure was recorded continuously for 5 minutes every 15 minutes over a 24-hour period weekly from 7-13 weeks of age. From the arterial pressure waveform, SBP and HR were derived online using Dataquest ART (Data Sciences International) and exported into Excel (Microsoft Office, Microsoft Corporaton, WA, USA). All data acquired during

each 24-hour recording period was averaged to obtain a single value for each animal per week.

At the end of the treatment period, LPK rats treated with OPC-21268 were anaesthetised with urethane (1.3g/kg i.p., Sigma) and an intravenous vasopressin bolus (100 ng/kg) was administered to confirm effective V_{1A} receptor blockade. The diastolic pressor response was 67% lower in OPC-21268 treated LPK rats (n = 5) compared with naïve LPK rats (n = 6) previously reported (Chapter 2) (Underwood et al., 2018); 12 ± 6 vs. 36 ± 3 mmHg*; P <* 0.01), therefore confirming effective V_{1A} receptor blockade.

Statistical analysis

All data are expressed as mean ± SEM. GraphPad Prism version 8 (Graphpad software, Inc., La Jolla, CA, USA) was also for all statistical analysis. An unpaired Student's t-test was used to detect strain differences in cell counts. Radiotelemetry data were analysed with a repeated measures mixed-effects model. Holme-Sidak's correction was used for all multiple comparisons. Statistical significance was set at $P \leq 0.05$.

Results

LPK rats have a greater percentage of Fos/Fra+ SON vasopressin neurons

LPK rats showed a ~70% increase in Fos/Fra nuclei in the SON compared to Lewis controls (Figure 1). Likewise, while a similar number of vasopressin neurons were observed in LPK and Lewis animals (79 ± 6 vs. 67 ± 3 neurons per SON, $t_8 = 1.93$, $P = 0.09$), there was a ~85% increase in the number of Fos/Fra+ vasopressin neurons in LPK rats (Figure 1). These data support the hypothesis that SON vasopressin neurons are chronically over-activated in LPK rats.

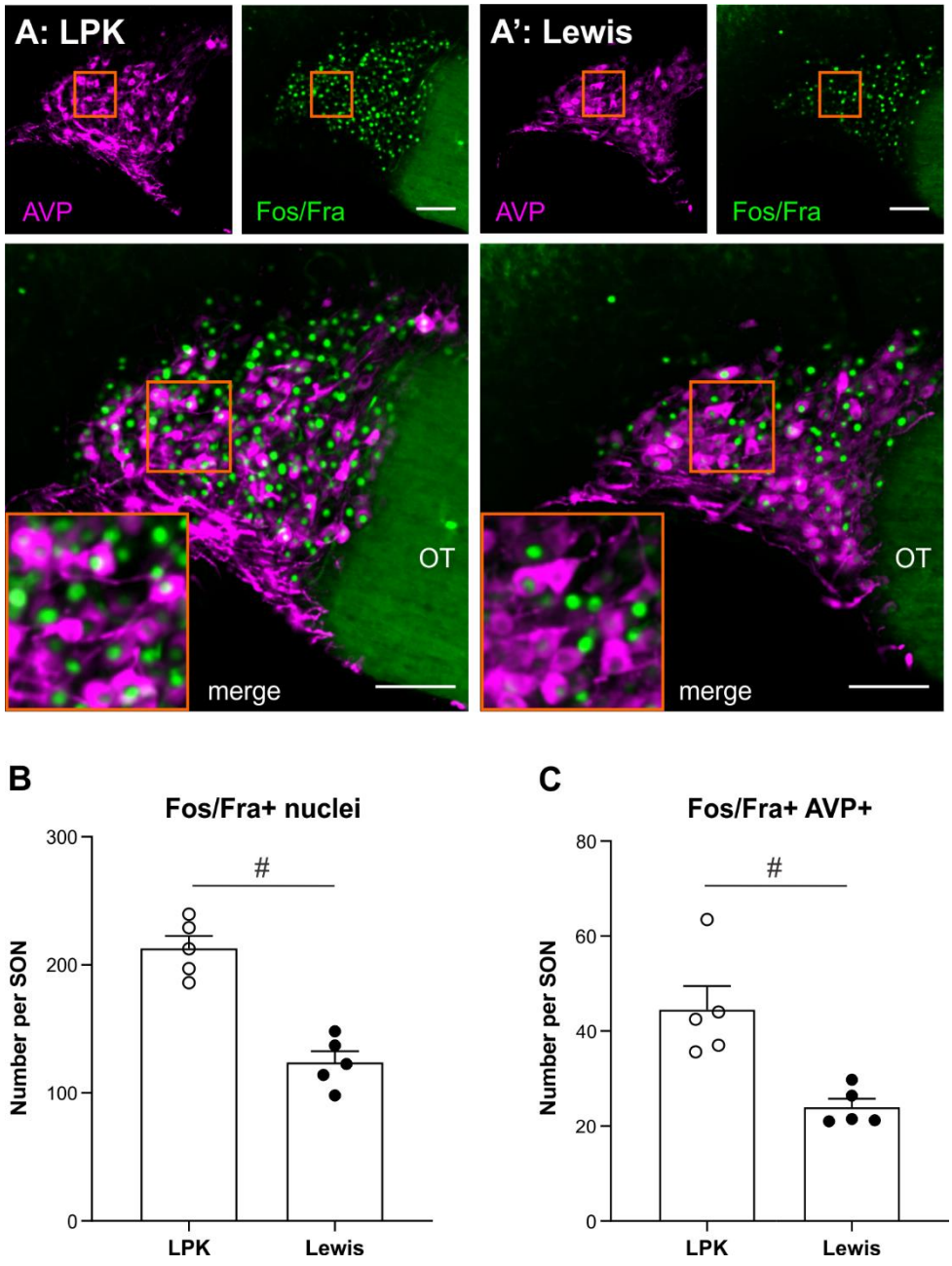

Figure 1. Representative images illustrating the distribution of Fos/Fra nuclei (green) and vasopressin (AVP) neurons (magenta) in the SON of a LPK rat (**A**) and a Lewis rat (**A'**). The top panels shows the pseudocoloured single images of vasopressin (left) and Fos/Fra (right). The bottom panel shows the merged image with the inset indicated in orange. Scale = 50 µm for all panels. Grouped data for Fos/Fra nuclei (**B**) and Fos/Fra positive vasopressin neurons (**C**) in the SON. OT, optic tract. #, $P < 0.05$ strain difference. $n = 5$ per strain.

Chronic V_{1A} receptor antagonism does not lower blood pressure in LPK rats

As we previously reported that acute intravenous administration of OPC-21268, a selective V_{1A} antagonist, lowers arterial pressure in LPK but not in Lewis rats (Chapter 2), we examined whether 7 weeks oral treatment with OPC-21268 reduced the development of hypertension in LPK animals. Figure 2 illustrates the weekly 24-hour average level of SBP and HR in both groups during the treatment period. There was an age-related increase in SBP in untreated and treated LPK animals ($P < 0.0001$). Contrary to our initial hypothesis, the level of SBP of LPK rats treated with OPC-21268 was not significantly different from untreated LPK ($P = 0.15$; Figure 2A). HR decreased with age ($P < 0.0001$) and was similar in both treatment groups (adjusted $P = 0.23$; Figure 2B).

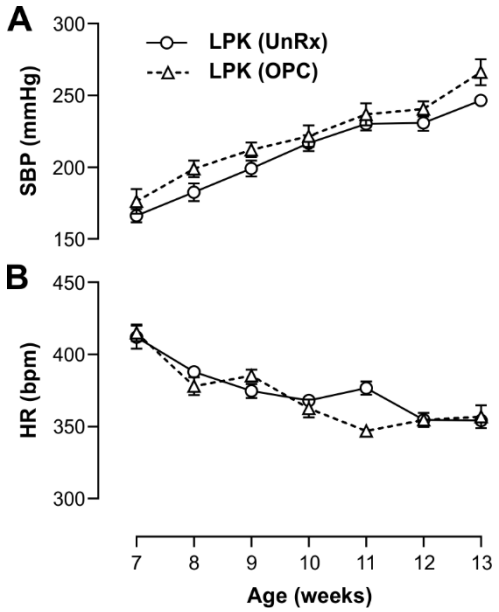

Figure 2. 24-hour weekly measurements of SBP (**A**) and HR (**B**) untreated (unRx) and OPC-21268-treated (OPC) LPK rats. Animals were treated from 6 to 13 weeks. $n \geq 7$ LPK untreated and $n \geq 5$ LPK OPC-21268-treated.

Discussion

This is the first study to show that the ongoing activity of SON vasopressin neurons is increased in a rodent model of PKD, the LPK rat. This finding may explain the higher circulating vasopressin levels observed in LPK animals (Underwood et al., 2017) and PKD patients (Zittema et al., 2014), and is of potential clinical relevance since vasopressin is a prominent driver of cyst growth (Torres, 2008a). However, we did not find that chronic V_{1A} receptor blockade produced lower levels of arterial pressure in LPK rats, suggesting that this intervention will not be directly beneficial for the management of hypertension in PKD.

The expression of the Fra immediate-early gene is constitutive in some SON neurons during basal conditions, but is robustly up-regulated during chronic exposure to hyperosmotic stimuli (Miyata et al., 2001). Here we observed that Lewis rats displayed a moderate level of Fos/Fra immunoreactivity in the SON, consistent with unchallenged Wistar rats (Miyata et al., 2001). Importantly, we found that significantly more SON vasopressin neurons showed Fos/Fra immunoreactivity in LPK rats, indicating that SON vasopressin neurons display a greater level of ongoing activity in this rat model of PKD. While we did not directly assess the release of vasopressin from SON neurons, it is pertinent to note that the secretion of vasopressin is dependent on, and directly related to, the activity of magnocellular vasopressin neurons (Bicknell, 1988). These observations are therefore consistent with the notion that a greater ongoing activity of SON vasopressin neurons may underlie the high level of vasopressin release typically observed in PKD (Danielsen et al., 1986b; Danielsen et al., 1986a; Michalski and Grzeszczak, 1996; Zittema et al., 2014). Although it was beyond the scope of the current study to determine the stimulus responsible for augmenting the activity of SON vasopressin neurons in LPK rats, we suggest that an elevation in extracellular fluid osmolality due to a urinary concentrating deficit (Phillips et al., 2007; Zittema et al., 2012; Jeewandara et al., 2015; Sagar et al., 2019) is a strong candidate, and is likely to be relayed to the SON via osmoreceptive neurons of the forebrain lamina terminalis, particularly the subfornical organ and median preoptic nucleus which we have previously found to contain more Fos/Fra positive nuclei in LPK rats (Chapter 4).

Our previous observations that acute systemic V_{1A} receptor antagonism lowers arterial pressure in anaesthetised LPK animals (Chapter 2) (Underwood et al., 2018), warranted investigation into whether chronic systemic V_{1A} receptor antagonism reduces hypertension in this PKD model. To that end, we found that LPK rats treated with OPC-21268 at a dose that

substantially blocked the pressor response to intravenous vasopressin showed comparable levels of arterial pressure as untreated LPK rats, indicating that V_{1A} receptor antagonism will not provide an anti-hypertensive benefit in PKD. This finding contrasts our previous acute study (Chapter 2) (Underwood et al., 2018) and other reports showing that chronic systemic administration of OPC-21268 or other V_{1A} receptor antagonists attenuate the development of hypertension in spontaneously-hypertensive rats (Yamada et al., 1994), deoxycorticosterone-salt rats (Burrell et al., 1994), salt-loaded rats (Choe et al., 2015a) and transgenic rats with renin-angiotensin system over-activation (Korpal et al., 2017), animal models that all display a two- to three-fold elevation in plasma vasopressin comparable to that seen in LPK rats (Mohring et al., 1977; Crofton et al., 1978; Ludwig et al., 1996; Underwood et al., 2017). It is possible that the lack of anti-hypertensive effect of chronic OPC-21268 in LPK animals may be due to potential off-target effects of OPC-21268 that result from chronically antagonising V_{1A} receptors outside of the vasculature. For example, OPC-21268 is lipophilic (Setiadi et al., 2018) and may therefore be able to penetrate the brain where V_{1A} receptors can have a net hypotensive (Smith and Ferguson, 1997) or hypertensive (Malpas and Coote, 1994) action depending on the region. We must also consider the possibility that the differential effect of OPC-21268 during acute versus chronic conditions in LPK rats reflects the use of urethane anaesthesia in our acute study which can elevate plasma osmolality and circulating vasopressin levels (Severs et al., 1981; Holbein and Toney, 2013). While we have not yet compared vasopressin levels in LPK rats during these different experimental conditions, we have noted that the plasma osmolality of LPK and Lewis rats anaesthetised with urethane (Chapter 4) is similar to those sacrificed following ketamine anaesthesia (Sagar et al., 2019), highlighting that the reported hyperosmotic effect of urethane is not as evident in our experimental conditions, possibly due to our fluid supplementation protocol.

In summary, we have provided evidence that vasopressin neurons located in the SON display a greater ongoing activity in PKD. Our results suggest however that chronic V_{1A} receptor antagonism is not an effective means to reduce blood pressure in PKD. Nevertheless, a greater understanding of the neurochemistry and dynamics of SON vasopressin neurons may lead to novel therapies to suppress the release of vasopressin and limit renal disease progression in PKD.

Appendix 2.

Animal Research Authority Protocols

Appendix 2 of this thesis has been removed as it may contain sensitive/confidential content