HYPOTHALAMIC MEDIATION OF ACUTE INCREASES IN ARTERIAL BLOOD PRESSURE AND RENAL SYMPATHETIC NERVE ACTIVITY DURING ELECTRICAL STIMULATION OF THE LAMINA TERMINALIS

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ABSTRACT OF THESIS

HYPOTHALAMIC MEDIATION OF ACUTE INCREASES IN ARTERIAL BLOOD PRESSURE AND RENAL SYMPATHETIC NERVE ACTIVITY DURING ELECTRICAL STIMULATION OF THE LAMINA TERMINALIS

Discrete electrical stimulation of the organum vasculosum of the lamina terminalis (OVLT) produces sympathetically-mediated increases in peripheral resistance and arterial blood pressure (ABP). Since efferent fibers from the lamina terminalis innervate the kidney through polysynaptic connections, the present study determined whether electrical stimulation of the OVLT increased sympathetic outflow to the kidney. In anesthetized male, Sprague-Dawley rats (n=5) electrical stimulation of OVLT neurons produced frequency and current intensity dependent increases in renal sympathetic nerve activity (RSNA) and ABP that were abolished by ganglionic blockade with the nicotinic antagonist chlorisondamine (5mg/kg,i.v.). Since neurons from the OVLT terminate within the hypothalamic paraventricular nucleus (PVH), the present study also determined whether these connections mediate a portion of sympathetic and pressor responses to electrical stimulation of the OVLT. Bilateral inhibition of the PVH with the GABA\textsubscript{A} agonist muscimol (5mM/100nl) significantly attenuated the increase in ABP at all frequencies and current intensities. Spike-triggered averaging of RSNA revealed that PVH inhibition significantly blunted the RSNA responses to OVLT stimulation at 100, 200, but not 400µA. The present findings indicate that electrical stimulation of the OVLT increases RSNA and ABP and that these responses are partially mediated by the tonic activity of PVH neurons.

KEYWORDS: Sympathetic nervous system, blood pressure, paraventricular hypothalamic nucleus, organum vasculosum lamina terminalis

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February 15, 2008
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Thesis

Samuel Paterson Carmichael II

The Graduate School
University of Kentucky
2008
HYPOTHALAMIC MEDIATION OF ACUTE INCREASES IN ARTERIAL BLOOD PRESSURE AND RENAL SYMPATHETIC NERVE ACTIVITY DURING ELECTRICAL STIMULATION OF THE LAMINA TEMRINALIS

Thesis

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the College of Arts and Sciences at the University of Kentucky

By

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2008

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To Ian, Jean and Rebecca
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CHAPTER ONE

OVERVIEW

The sympathetic branch of the autonomic nervous system is essential in the control of blood pressure through vascular innervation in vertebrates. Acute activation of the sympathetic nervous system evokes increases in blood pressure, mediated by increased vascular smooth muscle tone \(^1,2\). Long-term elevation of sympathetic outflow to various vascular beds may contribute to the etiology and maintenance of chronic high blood pressure in the context of both human and experimental forms of hypertension \(^3,4\).

A major controller of sympathetic outflow to the vasculature is a region of the forebrain known as the hypothalamus. Hypothalamic efferent neurons project through mono- or polysynaptic pathways to preganglionic cell bodies in the spinal column. These cell bodies give rise to axons, which terminate within the sympathetic chain ganglia. Post-ganglionic fibers innervate major vascular beds, including renal, hind-limb and mesenteric. These neural connections provide a pathway by which stimulation of hypothalamic nuclei increases sympathetic nerve activity, total peripheral resistance and blood pressure \(^5-7\).

A significant portion of afferent signaling to the hypothalamus originates within the pre-optic area, immediately rostral to the hypothalamus. The anterior portion of the third cerebral ventricle, known as the lamina terminalis, contains nuclei which lack a complete blood-brain barrier and are sensitive to changes in circulating angiotensin II and plasma osmolality \(^8-11\). Acute stimulation of these nuclei with either angiotensin II or hypertonic saline increases sympathetic nerve activity and blood pressure as well as the release of vasopressin from the neurohypophysis \(^12-14\). Furthermore, chronic stimulation of the lamina terminalis has been implicated in both renal and salt-sensitive hypertension \(^15,16\). The current data suggest that the connection between the lamina terminalis and the
hypothalamus is critical to acute and chronic sympathetically-mediated changes in blood pressure.

The primary goals of this study are: 1) to determine whether electrical stimulation of the lamina terminalis increases renal sympathetic nerve activity (RSNA) and arterial blood pressure (ABP) 2) to investigate hypothalamic mediation of sympathetic outflow to stimulation of the lamina terminalis and 3) to determine whether circulating vasopressin potentiates the pressor response to stimulation of the lamina terminalis. The results of these experiments provide further insight into the mechanisms of neurally-mediated changes in blood pressure and contribute to a greater understanding of cardiovascular maladies, such as neurogenic hypertension.
CHAPTER TWO

INTRODUCTION

The lamina terminalis of the anteroventral wall of the third ventricle (AV3V) influences the release of vasopressin from the neurohypophysis and sympathetically-mediated changes in blood pressure\textsuperscript{12-14, 17, 18}. The circumventricular organs (CVO) of this region are exposed to plasma osmolality and hormone concentrations due to fenestrated capillaries, which dramatically increase the permeability of the blood-brain barrier (BBB). Two of these CVO, the subfornical organ (SFO) and the organum vasculosum of the lamina terminalis (OVLT), sense changes in plasma sodium chloride and hormone concentrations through the presence of several possible types of receptors\textsuperscript{19, 20}. Through anatomical connections to both humoral and autonomic regions of hypothalamic nuclei, these structures influence the release of vasopressin and sympathetically-mediated changes in blood pressure, respectively\textsuperscript{20-24}. The importance of these structures in the long-term regulation of blood pressure has been shown by their role in renal and salt-sensitive hypertension\textsuperscript{13, 15, 16}. Thus, a greater understanding of the mono- and polysynaptic connections arising from the lamina terminalis and their role in the control of sympathetic outflow is essential to understanding blood pressure regulation and the development of neurogenic hypertension.

A. Neuroanatomy of the Lamina Terminalis

Neuroanatomical research using various anterograde and retrograde tracing techniques has shown that the structures of the lamina terminalis, the SFO, OVLT and median preoptic nucleus (MnPO), all share substantial neuronal interconnections\textsuperscript{19, 21, 25-27}. Functionally, both excitatory and inhibitory neurons have been reported\textsuperscript{19}. Possible neurotransmitters mediating these connections include: glutamate, serotonin,
acetylcholine, γ-amino butyric acid (GABA), angiotensin II (ANGII), neotensin and neuropeptide Y. The SFO and OVLT both possess receptors to circulating hormones found within the blood plasma. In particular, densities of ANGII type-1 (AT1) receptors have been localized to both structures. \textit{In vivo} studies observing the expression of the proto-oncogene \textit{c-fos} showed increased protein expression within the SFO and OVLT to intravenous ANGII challenge. By comparison, more SFO neurons demonstrated Fos expression than OVLT neurons to ANGII. This finding suggests that sympathetic and pressor responses to ANGII or hypertonic saline may be primarily mediated by either nucleus. Furthermore, extracellular recording studies show an increase in the firing rate of SFO neurons to intracarotid ANGII infusion. Though ANGII sensitivity by these structures has been the focus of much investigation, examples of additional circulating hormones with receptors in the lamina terminalis include atrial natriuretic peptide, relaxin and somatostatin.

The OVLT and SFO are also sensitive to changes in plasma sodium chloride, shown both by \textit{in vitro} and \textit{in vivo} experiments. A recent study by Ciura and Bourque suggests that the transient receptor potential vanilloid 1 (TRPV1) is responsible for the osmosensitivity of OVLT neurons. Cell recordings of isolated OVLT neurons \textit{in vitro} showed that neuronal discharges to addition of hypertonic saline were absent from TRPV1 knockout mice compared to wild type controls. Furthermore, excitation to hypertonic saline in wild type neurons could be blocked by addition of a TRPV antagonist. These findings suggest that sympathetic and pressor responses to hyperosmotic stimulation of the lamina terminalis with sodium chloride may be mediated by this receptor. In all, the lamina terminalis is comprised of interconnected structures, which sense changes in plasma hormones and sodium chloride concentration through the
presence of receptors to these circulating substrates. Furthermore, these receptors may mediate changes in SNA and ABP to hypertonic saline and ANGII stimulation.

B. Hypothalamic and Medullary Efferents

In addition to interconnections within the lamina terminalis, the CVO send efferent projections to both magnocellular and parvocellular regions of the hypothalamus, including the supraoptic nucleus (SON) and the paraventricular hypothalamus (PVH) \(^{17, 22, 23}\). Magnocellular neurons of the SON and PVH terminate within the neurohypophysis to release vasopressin upon activation with ANGII and hypertonic saline \(^{34}\). Parvocellular neurons project to brainstem and spinal nuclei, including the rostral ventrolateral portion of the medulla oblongata (RVLM) and intermediolateral cell column (IML), respectively \(^{12, 18, 35-37}\). Neurons leaving the IML synapse within the sympathetic chain ganglia. From this point, postganglionic fibers innervate major vascular beds, including the renal, hindlimb and mesenteric circulations.

Recently, Sly et al. reported pseudorabies-positive cells within the lamina terminalis 4 days after injection of the virus into the renal cortices of anesthetized rats \(^{5}\). Interestingly, viral progeny were localized to the PVH 1 day prior to infection of the lamina terminalis \(^{5}\). In similar experiments, microinjection of pseudorabies into the posterior pituitary revealed virus-positive cell bodies in the SON and PVH following 16 hours incubation \(^{38}\). Furthermore, infected neurons were located in the dorsal cap of the OVLT and on the peripheral edges of the SFO 20-28 hours following microinjection \(^{38}\). Support for these findings is provided by in vivo extracellular recording studies in which stimulation of the SFO and OVLT excited cell bodies within the PVH \(^{39, 40}\). The available data indicate polysynaptic neuronal connections from the lamina terminalis to peripheral sympathetic fibers. Activation of these pathways with either ANGII or hypertonic saline
increases sympathetic outflow and blood pressure through increased peripheral resistance \cite{30, 31}. These pathways are diagrammatically summarized in Figure 1.
Figure 1. Schematic of neural circuitry under investigation. SFO, subfornical organ MnPO, median preoptic nucleus AC, anterior commissure OVLT organum vasculosum of the lamina terminalis ox, optic chiasm 3V, third ventricle PVH, paraventricular hypothalamic nucleus PP, posterior pituitary AP, anterior pituitary RVLM, rostral ventrolateral medulla IML, intermediolateral cell column.
C. Vasopressin Release

In pioneering experiments conducted by Verney and Jewell in the 1940’s and 50’s, infusion of hypertonic saline or sucrose, but not urea, increased vasopressin secretion from the neurohypophysis \(^{41, 42}\). Because urea readily equilibrates between the intra- and extracellular environment, it was an ineffective osmotic stimulus of vasopressin secretion. This observation led Verney to conclude that the release of vasopressin was mediated by dehydration of neurons within the forebrain.

In later studies involving electrolytic lesion of the AV3V, the lamina terminalis was suggested as the forebrain site responsible for vasopressin release after ANGII or hyperosmotic stimulation \(^{14, 43}\). Due to the magnitude of these lesions, information regarding the contribution of each nucleus to this response was limited. Studies utilizing discrete lesion of the OVLT and SFO revealed that destruction of either organ attenuated the release of vasopressin in response to hyperosmotic stimulation or ANGII \(^{34, 44, 45}\). However, ablation of both structures was necessary for elimination of vasopressin release to hypertonic saline \(^{34, 46}\). This finding suggests that both the SFO and OVLT are sufficiently osmosensitive to increase plasma vasopressin to an osmotic challenge. Indeed, this notion is supported by studies in which osmosensitivity was observed in neurons of both nuclei \(^{8, 9, 32}\). Altogether, these data present the lamina terminalis as the central forebrain site influencing vasopressin release from the neurohypophysis.

Though circulating vasopressin is widely recognized for its role in renal tubular reabsorption of water, studies have also suggested its influence in acute pressor responses to lamina terminalis stimulation. Mangiapane and Brody reported that increases in ABP after electrical stimulation of the SFO were augmented by circulating vasopressin \(^{2}\). Additionally, vasopressin (V1) receptors have been located in both the SFO and OVLT \(^{47}\) and cell recording studies have observed excitation of SFO neurons to the hormone \(^{48-50}\). Taken together, these data suggest that circulating vasopressin
contributes to pressor responses evoked by stimulation of the lamina terminalis, possibly through action upon the SFO and OVLT. A goal within the present study was to determine whether circulating vasopressin influences acute elevations in ABP to discrete electrical stimulation of the OVLT.

D. Dipsogenic responses

Drinking behavior has been observed in response to a variety of stimuli, including hyperosmotic plasma, ANGII, hypovolemia and dehydration\textsuperscript{19, 43, 51}. In the 1950’s, Andersson was the first to observe dipsogenesis to infusion of hypertonic saline in different regions of the goat hypothalamus\textsuperscript{52}. Later studies conducted by Epstein et al. noted an increase in water intake to intracranial injection of ANGII\textsuperscript{53}. Subsequent investigation showed that electrolytic lesion of the AV3V eliminated drinking responses to ANGII, hyperosmotic challenges, and dehydration\textsuperscript{43}. Though these experiments support the lamina terminalis in mediation of water intake, limited information was provided on the contribution of each nucleus.

In a series of experiments conducted by Thrasher et al., the OVLT was ablated prior to systemic administration of hypertonic saline or ANGII in dogs. Infusion of either substance in lesioned animals failed to evoke normal dipsogenic responses\textsuperscript{34, 45}. Similarly, in studies performed by Simpson et al., selective ablation of the SFO blocked increases in water intake to systemic infusion of ANGII in the rat\textsuperscript{54}. In contrast, McKinley and colleagues reported that lesion of either the OVLT or SFO did not decrease water intake to hypertonic saline infusion or water deprivation. In these experiments, only lesions encompassing the majority of the lamina terminalis abolished acute water ingestion responses to intravenous hypertonic saline\textsuperscript{55}. Similar to vasopressin secretion, appropriate dipsogenic responses to ANGII or hypertonic saline likely require the
participation of all lamina terminalis nuclei, though incomplete ablation may result in a residual increase in water intake.

E. Lamina Terminalis and Sympathetically Mediated Cardiovascular Changes

In addition to influencing vasopressin release and dipsogenic behaviors, the structures of the lamina terminalis mediate changes in ABP through excitation of the sympathetic nervous system. Mangiapane and Brody were the first to demonstrate that electrical stimulation of either the SFO or OVLT increases ABP through increased vascular resistance. Moreover, blood pressure elevation and hemodynamic effects were the result of sympathetic activation, as intravenous infusion of a ganglionic blocking agent abolished these responses \(^1,2\).

In the context of hyperosmotic stimulation, human and animal studies have reported elevations in sympathetic nerve activity to acute increases in plasma osmolality \(^12,56\). Chen and Toney showed that acute intracarotid infusion of hypertonic saline evoked significant increases in both RSNA and ABP \(^31\). Furthermore, these responses were attenuated following blockade of AT1 receptors within the PVH \(^31\). These data are supported by experiments in which chemical inhibition with a GABA\(_A\) agonist or blockade of glutamate receptors within the PVH blunted increases in lumbar SNA (LSNA) to hypertonic saline \(^57\). In this same preparation, knife cuts separating the CVO from the hypothalamus abolished lumbar sympathoexcitation to hypertonic saline \(^57\). Taken together, the data suggest that the forebrain CVO are integral to changes in SNA and ABP to hypertonic saline. Moreover, a portion of these responses are mediated by synaptic transmission in the PVH.

In contrast to hypertonic saline, ANGII has shown inconsistent affects upon SNA. In one study, plasma norepinephrine concentration either decreased or remained unchanged to chronic intravenous ANGII infusion in conscious dogs \(^58\). In a conflicting
study, chemical sympathectomy prevented increased systolic blood pressure to chronic subcutaneous infusion of ANGII$^{59}$. Furthermore, structural changes in arterial walls to ANGII infusion observed in control animals were not apparent in sympathectomized rats$^{59}$. One possible explanation for these findings, as suggested by Osborn et al.$^{13}$, could be the involvement of the baroreflex. Because the baroreflex may exert an inhibitory influence upon the sympathetic nervous system to systemic infusion of ANGII$^{60}$, interruption of the baroreflex circuit would likely lead to consistent ANGII-mediated sympathoexcitation. Indeed, intravenous infusion of ANGII following chronic sino-aortic denervation increases both ABP and LSNA in unanesthetized rats$^{30}$.

In summary, the CVO of the lamina terminalis mediate increases in sympathetic outflow and ABP to changes in plasma sodium concentration and ANGII. However, two questions remain: 1) whether discrete stimulation of individual lamina terminalis nuclei evokes quantifiable increases in SNA and 2) whether these excitatory signals are relayed through the PVH. In the present set of experiments, these questions will be tested through application of electrical current to the OVLT and recording of RSNA and ABP. The results will provide further insight into the efferent connections of the lamina terminalis and its role in acute changes in SNA and ABP.

F. Lamina Terminalis and Hypertension

Long-term elevations in sympathetic nerve activity have been observed in both human and experimental forms of hypertension$^{3,4}$. Because of its influence on sympathetic outflow and ABP, the lamina terminalis has been investigated as a potential mediator of neurogenic hypertension$^{13,15,18}$. Though acute changes in SNA and ABP are attributed to activation of forebrain CVO, the question remains whether the lamina terminalis is involved in the development and maintenance of elevated blood pressure.
Previous studies have connected the lamina terminalis to both Grollman renal wrap and Goldblatt renal arterial coarctation hypertension\textsuperscript{15, 61, 62}. In both forms, activation of the renin-angiotensin system plays a key role in the genesis of chronically elevated blood pressure. In one study, electrolytic lesion of the AV3V prior to induction of two-kidney, one-clip Goldblatt hypertension diminished acute pressor responses and prevented the maintenance of elevated blood pressure\textsuperscript{15}. Furthermore, lesion of the AV3V prior to or 6 weeks following one-kidney renal wrapping failed to produce or normalize the high blood pressure, respectively\textsuperscript{61, 62}. Though the peripheral vasopressor effects of ANGII may also play a role, these data suggest that a significant portion of the chronically elevated blood pressure in Grollman or Goldblatt hypertension is supported by the action of ANGII upon the lamina terminalis.

More recently, studies have investigated a role for the lamina terminalis in salt-sensitive hypertension\textsuperscript{16, 63, 64}. The observation of decreased ABP to intracarotid infusion of hypotonic saline was first reported by Scherrer in 1963\textsuperscript{65}. Similar studies performed by Schad and Seller showed that dilution of intracranial osmolality reduced RSNA in baroreceptor denervated cats\textsuperscript{66}. In support of these findings, O'Donaughy and Brooks suggested that elevated plasma sodium acts upon forebrain circumventricular nuclei to increase sympathetic outflow in salt-sensitive hypertension\textsuperscript{16, 64}. In these experiments, a deoxycorticosterone-salt (DOCA) pellet was implanted following unilateral nephrectomy. Drinking water was replaced with saline (1\% NaCl and 0.2\% KCl) \textit{ad libitum} and blood pressure increased by ± 50mmHg in DOCA-salt but not control animals\textsuperscript{16}. On the day of experiments, hypotonic saline was infused through the carotid artery and evoked depressor responses in hypertensive DOCA-salt animals, but not sham-operated, rats.

Though the development of hypertension supported by chronically elevated SNA remains a source of ongoing research, the available data suggest that repeated stimulation of the OVLT and SFO by elevated ANGII and plasma sodium chloride may
play a critical role in the development of the hypertension\textsuperscript{15, 16, 61, 62}. In this model, long-term excitation of lamina terminalis nuclei by elevated plasma ANGII or sodium chloride influences sustained elevation in sympathetic outflow to major vascular beds. Prolonged increases in total peripheral resistance lead to the onset of hypertension. Despite the possible involvement of additional factors, such as plasma volume expansion and the direct vasoactive effects of ANGII\textsuperscript{67}, the available data indicate significant influences by the sympathetic nervous system in these forms of hypertension.

G. Summary and Rationale for Proposed Work

The past 50 years have produced a significant body of research implicating the lamina terminalis in fluid and electrolyte balance and blood pressure regulation. Its receptors and position external to the blood brain barrier enables the brain to monitor plasma osmolality and circulating humoral factors, such as ANGII. Transduction of these chemical and hormonal signals by the CVO activates downstream hypothalamic pathways, leading to the release of vasopressin and changes in sympathetic outflow and ABP\textsuperscript{13, 14}.

Despite these findings, information regarding the contribution of individual forebrain CVO to acute changes in SNA and ABP remains limited. Mangiapane and Brody were the first to document pressor and hemodynamic responses to electrical activation of the lamina terminalis structures\textsuperscript{1, 2}. Other Investigators demonstrated the involvement of the sympathetic nervous system in these responses through intravenous ganglionic blockade. However, the behavior of post-ganglionic sympathetic fibers to activation of individual lamina terminalis nuclei remains unclear. Therefore, one goal of the present study is to quantify activation of RSNA in support of acute pressor responses to direct electrical stimulation of the OVLT.
Circulating vasopressin may augment the pressor responses observed to electrical stimulation of the CVO. Since systemic application of an antagonist to vasopressin receptors decreased pressor responses to electrical stimulation of the SFO, a portion of the acutely elevated blood pressure could be attributable to vasopressin release that in turn increases peripheral resistance. Thus, a second goal of the present study is to assess the role of circulating vasopressin in acute sympathetic and pressor responses to electrical stimulation of the OVLT through systemic administration of an antagonist to vasopressin receptors.

Anatomical and functional studies have presented the PVH as a major downstream target of the lamina terminalis. Blockade of AT1 and glutamate receptors within the PVH decreased RSNA and LSNA activation to intracarotid hypertonic saline. Because osmosensitivity has been observed in the SFO and OVLT, excitation of RSNA may represent the contribution of both nuclei. Thus, one question that remains is whether the PVH mediates excitation of renal sympathetic outflow after discrete OVLT stimulation. A third goal of the present study is to test this hypothesis through electrical stimulation of the OVLT while recording RSNA and ABP prior to and following chemical inhibition of the PVH.

In conclusion, the lamina terminalis mediates acute SNA and ABP responses to changes in plasma osmolality and ANGII. Furthermore, a role for the lamina terminalis in renal and salt-sensitive hypertension has been shown by several studies. These findings suggest that this region of the forebrain may be influential to the development and maintenance of chronic hypertension. Future studies investigating etiological factors, such as behavioral and structural changes in these neuronal populations, are needed to fully evaluate this possible role. Taken together, the findings of the present study represent foundational research into the regulation of the sympathetic nervous system and ABP by the lamina terminalis.
CHAPTER THREE

METHODS

Animals: Male Sprague-Dawley rats (300-400 g, Charles River Laboratories) were housed in a temperature controlled facility (22-23 °C) with a 14:10h light-dark cycle (lights on at 7 am). Animals were provided with tap water and standard laboratory rat chow ad libitum. Experiments were performed in accordance with guidelines set by the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of Kentucky Institutional Animal Care and Use Committee.

General Methods: On the day of experiments, rats were anesthetized with isoflurane (2-3% in 100% O₂) and instrumented with femoral arterial and venous catheters to measure arterial blood pressure and infusion of a paralytic agent (gallamine triethiodide: 25 mg/kg per hour at 25 µL/h in 5% dextrose, Sigma Aldrich), respectively. An additional catheter was placed in the jugular vein for administration of intravenous anesthesia (750 mg/kg urethane and 75 mg/kg α-chloralose). A retroperitoneal incision was made to record RSNA using stainless steel electrodes, as described previously. After tracheal cannulation, rats were paralyzed and artificially ventilated with oxygen-enriched room air. End-tidal P_{CO₂} was maintained between 4.0-5.0% by adjusting ventilation rate (70-100 breaths/min) or tidal volume (2-3 ml). Body temperature was maintained at 37±1°C using a heating pad.

OVLT Stimulation and PVH Inhibition: Animals were placed into a stereotaxic head frame with the incisor bar positioned 3 mm below the interaural line. After a small craniotomy, a stimulating electrode was angled 8° from the midsagittal plane and lowered into the OVLT at the following coordinates with reference to bregma and the dorsal surface of the brain: 1.0 mm lateral, 0-1.2 mm rostral, and 7.6-8.0 mm ventral. Initially,
the OVLT was located by a pressor response to electrical stimulation of 50 Hz at 200 µA. Once baseline variables stabilized for 30 min, electrical stimulation was applied to the OVLT at various frequencies (10, 50 and 100 Hz) and intensities (100, 200 and 400 µA) for 5 seconds. At the conclusion of each experiment, sites were marked by a small lesion produced by DC current at 50 µA for 10 sec.

In experiments investigating the role of the PVH in OVLT stimulation, a second glass micropipette filled with the GABA<sub>A</sub> receptor agonist muscimol (5 mM 100nl in aCSF) was lowered into the PVH at the following coordinates in reference to bregma: 1.8 mm caudal to bregma, 0.5-0.7 mm lateral to midline, and 7.6-7.8 mm ventral to the dorsal surface of the cortex. Drugs were bilaterally injected into the PVH using a pneumatic picopump.

In order to quantify the magnitude of the sympathetic responses to electrical stimulation, single pulses (1.0 ms duration, 1 Hz) were applied to the OVLT for 60 sec. Spike-triggered averages of RSNA were generated from 60 sweeps at 3 different stimulation intensities (100, 200, and 400 µA). OVLT stimulation at these parameters was conducted both before and after ganglionic blockade with the nicotinic antagonist chlorisondamine (5 mg/kg, i.v.).

**Experimental Design:** To determine whether activation of the sympathetic nervous system is responsible for pressor responses to electrical stimulation of the OVLT, synaptic transmission at the sympathetic chain ganglia was blocked with chlorisondamine (5 mg/kg, i.v.). In a second group of animals, the PVH was bilaterally inhibited with the GABA<sub>A</sub> receptor agonist muscimol. Similarly, electrical stimulation of brain regions peripheral to the OVLT were made in order to control for any cardiovascular changes due to fibers of passage through the OVLT. In animals receiving control microinjections of aCSF bilaterally into the PVH, experiments were conducted in vasopressin-blocked
animals (Manning compound [d(CH$_2$)$_5$Tyr$^2$(ME)Arg$_8$]-vasopressin, 10 µg/kg/ml, i.v.) $^{69}$. Microinjections of either muscimol or aCSF into the PVH contained rhodamine beads for later histological verification of injection sites. Baroreflex function was also assessed with sodium nitroprusside (10 µg/kg in 100 µl, i.v.) before and after intravenous administration of Manning compound (Table 1).

Histology: At the conclusion of experiments, rats were euthanized with a lethal injection of saturated KCl and were perfused transcardially with 50 ml of 4% paraformaldehyde in 0.1 M phosphate-buffered saline. Brains were removed, post-fixed overnight in 4% paraformaldehyde at 4 ºC, and transferred to 30% sucrose for 2-3 days. Forebrains were sectioned at 50 µm on a vibratome, sections were mounted on glass slides, and counter stained with cresyl violet. PVH injection sites were confirmed by the localization of rhodamine beads.

Data Analysis: In order to quantify changes in blood pressure, 30 seconds of baseline activity was recorded prior to electrical stimulation and compared to a 1 second peak in ABP. Similarly, changes in RSNA were evaluated by spike-triggered averaging and 100 ms of baseline activity was compared to a 10 ms peak response. Figures 2, 3 and 8 were analyzed by 2 way ANOVA with F ratios. Mean differences were determined using the least significant range of the student Newman-Keuls evaluation (P<0.05). Figures 4 and 5 were analyzed by paired t-test (P<0.05).
CHAPTER FOUR

RESULTS

OVLT Stimulation Before and After Ganglionic Blockade: Electrical stimulation of the OVLT at various frequencies (10, 50, 100 Hz) and current intensities (100, 200, 400 µA) evoked frequency-current intensity dependent pressor and RSNA responses (Figures 2 and 3). Following intravenous administration of the ganglionic blocker, chlorisondamine (5 mg/kg, i.v.), pressor and RSNA responses evoked by electrical stimulation of the OVLT were abolished (Figures 2 and 3; p<0.05). OVLT stimulation sites were defined functionally by pressor responses to a brief pulse train (50 Hz, 1 ms pulse duration, 3 s, 100-400 µA). Figure 2A depicts raw traces of ABP before and after electrical stimulation of the OVLT at various frequencies and current intensities. At each frequency, black upper traces indicate baseline ABP stimulation before and grey traces following ganglionic blockade. Mean ABP responses are summarized in part B of Figure 2 (n=5). Similarly, Figure 3A illustrates spike-triggered averaging of an example of RSNA responses to electrical stimulation of the OVLT. Black traces indicate baseline, whereas grey traces indicate stimulation following ganglionic blockade. Group RSNA data are summarized in part B (n=5). Following ganglionic blockade, electrical stimulation failed to evoke a change in either ABP or RSNA.
Figure 2. ABP responses to electrical stimulation of the OVLT (A) before (black) and after (grey) infusion of the ganglionic blocker, chlorisondamine (5 mg/kg, i.v.). Group data are summarized in (B) (n=5). Group data were analyzed by 2-way ANOVA with F ratios. Mean differences were determined using the least significant range of the student Newman-Keuls evaluation (P<0.05).
Figure 3. Spike-triggered averaging of RSNA (A) before (black) and after (grey) infusion of the ganglionic blocker, chlorisondamine (5 mg/kg, i.v.). Group data are summarized in (B) (n=5). Group data were analyzed by 2-way ANOVA with F ratios. Mean differences were determined using the least significant range of the student Newman-Keuls evaluation (P<0.05).
**PVH Inhibition Experiments:** Bilateral inhibition of the PVH with the GABA\textsubscript{A} agonist muscimol (5 mM, 100 nl) significantly attenuated the pressor (n=6) and RSNA (n=4) responses to electrical stimulation of the OVLT at nearly all frequencies and current intensities (p<0.05) (Figures 4 and 5). The exception to this finding is the RSNA data from the greatest magnitude of electrical stimulation (400 µA), which was not significantly decreased following muscimol microinjection. However, ABP responses from this current intensity were significantly reduced following muscimol treatment. Figure 4A shows raw traces of ABP to electrical stimulation of the OVLT. At each frequency, black upper traces indicate baseline ABP stimulation prior to muscimol microinjection and grey traces indicate ABP following drug treatment. Mean ABP responses are summarized in part B of Figure 4 (n=6). Similarly, Figure 5A illustrates spike-triggered averaging of an example of RSNA responses to electrical stimulation before and after muscimol microinjection. Black traces indicate baseline, whereas grey traces indicate OVLT stimulation following drug treatment. Group RSNA data are summarized in part B (n=4). With the exception of 400 µA, PVH muscimol significantly reduced RSNA responses to electrical stimulation of the OVLT (p<0.05).

**Control Experiments:** Electrical stimulation of the OVLT increased ABP and RSNA similarly in rats prior to and post microinjection of aCSF into the PVH (Figures 6 and 7). Prior to bilateral microinjection of aCSF (100 nl) into the PVH, animals were pretreated with the V1 receptor antagonist, Manning compound (10 µg/kg/ml, i.v.). Figure 6A shows example traces of pressor responses evoked by electrical stimulation of OVLT. At each frequency, black upper traces indicate baseline responses prior to bilateral aCSF microinjection and grey traces indicate changes in ABP to electrical stimulation following microinjection. Mean ABP responses are summarized in part B of Figure 6 (n=3). Similarly, Figure 7A illustrates spike-triggered averaging of an example of RSNA
responses to electrical stimulation before and after aCSF microinjection. Black traces indicate baseline, whereas grey traces indicate stimulation following control microinjection. Group RSNA data are summarized in part B (n=3). At each current intensity, ABP and RSNA increased similarly before and after microinjection of aCSF into the PVH of all three Manning Compound pretreated rats.
Figure 4. ABP responses to electrical stimulation of the OVLT (A) before (black) and after (grey) bilateral microinjection of GABA<sub>A</sub> agonist, muscimol (5 mM, 100 nl) into the PVH. Group data are summarized in (B) (n=6). Significant differences were evaluated by paired t-test (P<0.05).
Figure 5. Spike-triggered averaging of RSNA (A) before (black) and after (grey) bilateral microinjection of the GABA\textsubscript{A} agonist, muscimol (5 mM, 100 nl) into the PVH. Group data summarized in (B) (n=4). Significant differences were evaluated by paired t-test (P<0.05).
Figure 6. ABP responses to electrical stimulation of the OVLT (A) before (black) and after (grey) bilateral microinjection of artificial CSF (100 nl) into the PVH with Manning Compound pretreatment (10 µg/kg/ml, i.v.). Group data are summarized in (B) (n=3). At each current intensity, ABP increased similarly before and after aCSF in all 3 rats tested.
Figure 7. Spike-triggered averaging of RSNA (A) before (black) and after (grey) bilateral microinjection of aCSF (100 nl) into the PVH with Manning Compound pretreatment (10 µg/kg/ml, i.v.). Group data are summarized in (B) (n=3). At each current intensity, RSNA increased similarly before and after aCSF in all 3 rats tested.
**Manning Compound:** Manning compound, a vasopressin type-1 antagonist, (10 µg/kg/ml, i.v.) was administered to 5 animals and ABP and RSNA responses were compared between pre- and post-treatment. Pressor and sympathetic responses to electrical stimulation of the OVLT were not significantly different following infusion of Manning compound (Figure 8; p<0.05). Figure 8A shows summary graphs of pressor responses evoked by electrical stimulation, black and grey bars indicating pre- and post-administration of drug, respectively. Changes in RSNA to electrical stimulation before and after Manning compound treatment are summarized in Figure 8B (n=5). Prior treatment with Manning compound did not affect the pressor or sympathetic responses to electrical stimulation of the OVLT (p<0.05).

**Sodium Nitroprusside:** Sodium nitroprusside, a vascular smooth muscle vasodilator (10 µg/kg, i.v.), was administered to animals prior to intravenous infusion of Manning compound (10 µg/kg/ml). Depressor responses evoked by nitroprusside administration were not significantly different following infusion of Manning compound (Table 1A; p<0.05). Similarly, increases in RSNA evoked by nitroprusside infusion were not significantly altered following infusion of Manning compound (Table 1B; p<0.05).

**Histological Analysis:** Electrical stimulation sites within the OVLT and microinjection sites within the PVH were verified histologically. Figure 9 indicates anatomical levels of the OVLT. Marked sites indicate the tip of the stimulating electrode verified by visualization of OVLT lesion with bright-field microscopy. Figure 10 indicates anatomical levels of the PVH. Microinjection sites were verified histologically via fluorescent microscopy.
Figure 8. Summary data of change in MAP and RSNA to OVLT electrical stimulation before (black) and after (grey) infusion of Manning Compound (AVPx, 10 µg/kg/ml, i.v., n=5). Groups were analyzed by 2-way ANOVA with F ratios. Mean differences were determined using the least significant range of the student Newman-Keuls evaluation (P<0.05).
Table 1. ABP (A) and RSNA (B) responses to sodium nitroprusside (10 µg/kg, i.v.) administration prior to and post infusion of Manning Compound (10 µg/kg/ml, i.v.). ABP is expressed as MAP in units of mmHg and RSNA is expressed in millivolts (mV). Changes in RSNA are also expressed as a percent of baseline. Values are ± SE. *Significant difference from baseline (P<0.05).

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Figure 9. Anatomical levels of the OVLT with stimulation sites indicated. *DBB* diagonal band of Brocha, *AC* anterior commisure, *LV* lateral ventricle, *3V* third ventricle, *OX* optic chiasm.

+ 1.2 Bregma

+ 0.7 Bregma

+ 0.5 Bregma

-0.7 Bregma
Figure 10. Anatomical levels of the PVH with microinjection sites indicated. 3V third ventricle, AH anterior hypothalamus, PVH (parvo) parvocellular region of the PVH, PVH (magno) magnocellular region of the PVH, f fornix.
CHAPTER FIVE

DISCUSSION

The present study investigated the control of RSNA and ABP by the lamina terminalis and the neural pathways connecting this region to the hypothalamus. To accomplish this, the OVLT was electrically stimulated while RSNA and blood pressure responses were recorded in anesthetized, Sprague-Dawley rats in the absence and presence of ganglionic blockade and pharmacologic inhibition of the PVH. The major findings in this study were: 1) electrical stimulation of the OVLT evoked increases in RSNA and ABP, which were abolished by ganglionic blockade 2) bilateral inhibition of the PVH blunted pressor responses to OVLT electrical stimulation and 3) systemic administration of a vasopressin antagonist prior to OVLT stimulation did not affect sympathomimetic and/or pressor responses. Taken together, the data suggest that electrical activation of the OVLT increases RSNA and ABP through connections with the PVH and independent of vasopressin acting upon systemic receptors.

Electrical stimulation of the OVLT increased RSNA and ABP in a frequency and current intensity dependent fashion. Pressor responses were a direct result of increased postganglionic sympathetic outflow, since ganglionic blockade with chlorisondamine abolished both the increases in RSNA and blood pressure at all levels of OVLT stimulation (Figure 2). To verify that increased RSNA and ABP resulted from OVLT activation, control experiments stimulated sites immediately juxtaposed to this nucleus. Passage of test currents (50 Hz, 200 µA) into the rostral-caudal, medial-lateral and dorsal-ventral planes exterior to the OVLT, evoked either a sub-maximal increase or no change in ABP. Pressor responses were observed 1.2 mm rostral of bregma in the plane of OVLT, and these responses reached a maximum between 0.5 and 0.7 mm rostral of bregma. These coordinates corresponded with the known location of the OVLT.

At the conclusion of all experiments, the OVLT was electrolytically lesioned for
histological verification of electrode placement at all stimulation sites. Analysis of brain sections for electrolytic lesions verified successful electrode placement within the boundaries of the OVLT (Figure 9). Altogether, the results support the hypothesis that activation of the OVLT is responsible for increases in RSNA and ABP.

Previous experiments attempted to determine a role for the lamina terminalis in blood pressure regulation through electrical stimulation of the anteroventral wall of the third ventricle (AV3V)\textsuperscript{71,72}. In anesthetized Sprague-Dawley rats, electrical stimulation within the AV3V resulted in depressor responses, increases in hindquarter blood flow, and decreases in both mesenteric and renal blood flow\textsuperscript{71,72}. Two possible explanations for these results include: 1) spread of current from the stimulation site and 2) position of the stimulating electrode. Due to the magnitude of electrical current (~1.5 mA), multiple nuclei may have been activated within the lamina terminalis. Subsequent reports would reveal that stimulation of the OVLT and SFO increases ABP and depressor responses were only evoked by stimulation of the MnPO \textsuperscript{1,2}. However, decreases in mesenteric and renal blood flow were noted to stimulation of either the OVLT or SFO \textsuperscript{1,2}. Thus, the depressor and hemodynamic responses to stimulation of the AV3V may represent the contribution of multiple, functionally distinct nuclei.

The second caveat of these experiments is the position of the stimulating electrode. Given the placement of the electrode tip within the third ventricle, the cerebral spinal fluid may conduct current to periventricular tissue adjacent to the AV3V. In doing so, current may activate other nuclei or fibers of passage also influencing blood pressure. Thus, attributing changes in blood pressure to activation of AV3V nuclei alone is difficult. Altogether, these findings represent early attempts to characterize blood pressure regulation by the structures of the AV3V.

Later studies utilizing discrete electrical stimulation of the lamina terminalis were the first to describe roles for the individual nuclei in hemodynamic and blood pressure
changes. In anesthetized Sprague-Dawley rats, electrical stimulation (constant current, 200 µA) of the SFO and OVLT increased ABP, whereas the median preoptic nucleus (MnPO) produced either a slight depressor response or no change in ABP \(^1,^2\).

Interestingly, the hemodynamic responses to stimulation of the SFO and OVLT were not identical. SFO stimulation increased resistance in the mesenteric, hindquarter and renal vascular beds, whereas OVLT stimulation only increased resistance to the mesenteric and renal circulations \(^1,^2\). This finding suggests that activation of either nucleus may influence sympathetic outflow to specific vascular beds. In all cases, changes in vascular resistance and ABP were prevented following ganglionic blockade with intravenous chlorisondamine. These findings were the first to show that electrical stimulation of the OVLT or SFO produces sympathetically-mediated changes in peripheral hemodynamics.

The data from the present study affirm and extend these reports, indicating for the first time that activation of the OVLT increases sympathetic outflow to the renal vasculature and these sympathomimetic responses are associated with increased ABP.

To determine whether circulating vasopressin affected the magnitude of pressor responses to electrical stimulation of the OVLT, the vasopressin receptor type 1 antagonist Manning compound (10 µg/kg/ml i.v.) was administered following OVLT stimulation. Subsequent electrical stimulation of the OVLT showed that blockade of systemic vasopressin receptors had no affect upon RSNA or ABP responses (Figure 8). The dosage of Manning compound was similar to previous studies and was shown to prevent pressor responses to intravenous administration of vasopressin (20 ng) \(^16,^64,^69\). These findings are consistent with the results of a recent report in which RSNA and ABP responses to intracarotid infusion of hypertonic saline were not different before and after intravenous administration of Manning compound \(^69\). These results suggest that circulating vasopressin does not augment sympathetic and pressor responses to acute electrical or chemical stimulation of the lamina terminalis.
However, conflicting results from another study suggest that pressor responses to electrical stimulation of the SFO were attenuated following intravenous administration of a vasopressin antagonist. One explanation for this discrepancy is the duration of stimulation. In the present study, electrical current was passed through the OVLT for 5 seconds at various frequencies and current intensities (see methods). In similar studies by Mangiapane and Brody, the SFO was stimulated for 60 seconds at a frequency of 32 Hz and 200 µA. Thus, the duration of applied current in these experiments may have increased circulating vasopressin to a greater concentration than the present study. To this end, circulating vasopressin augmented pressor responses observed in these reports.

Prior studies describing vasopressin receptors within the SFO and OVLT suggest the presence of a central mechanism whereby circulating vasopressin may directly affect sympathetically mediated changes in blood pressure. To test this hypothesis, Smith and Ferguson microinjected vasopressin (5 pmol in 0.5 µl) into the SFO of urethane anesthetized rats. Vasopressin decreased blood pressure and this depressor response was abolished by intravenous infusion of a vasopressin antagonist. Though this finding suggests that circulating vasopressin might act upon the lamina terminalis, it may not contribute to sympathetically-mediated blood pressure elevation.

The second major finding of the present study is that the PVH mediates pressor and RSNA responses to OVLT electrical stimulation. Bilateral microinjection of the GABA agonist muscimol (100 nl) into the PVH significantly blunted pressor responses to electrical stimulation of the OVLT at all frequencies and current intensities (Figure 4). Similarly, renal sympathetic responses were blunted following muscimol treatment at all stimuli with the exception of 400 µA. Dosages of muscimol (0.5 nmoles) were within the effective range for PVH inhibition (0.05-1.5 nmoles), as described previously. Microinjection sites within the PVH were verified by the presence of fluorescent...
rhodamine beads at the injection sites. In control groups, microinjections of similar volumes of aCSF into the PVH evoked no change in RSNA and ABP (Figure 6). Taken together, these results suggest that the PVH significantly influences sympathetic and pressor responses to electrical stimulation of the OVLT.

This connection between the lamina terminalis and the PVH in the context of sympathetic nerve activity and ABP is supported by both anatomical and functional studies. Anatomical data have shown that the PVH receives dense axonal projection from nuclei within the lamina terminalis. In a recent study, positive cell bodies were observed within the lamina terminalis following injection of the transneuronal retrograde label pseudorabies virus into the renal cortex of a rat. Infection of the PVH was noted prior to the lamina terminalis, suggesting a common neuronal pathway. Functionally, activation of the PVH with either chemical or electrical stimulation has been shown to evoke increases in sympathetic outflow and ABP. Studies utilizing knife cuts caudal to the lamina terminalis eliminate changes in lumbar SNA to hypertonic saline and attenuate ABP to intravenous ANGII, respectively. Furthermore, chemical blockade of either AT1 or glutamate receptors within the PVH decreases RSNA or LSNA responses to hypertonic saline, respectively. In concert with the present findings, the available data support a role for the PVH in the mediation of sympathetic signaling from the lamina terminalis.

Interestingly, the present study shows that the level of RSNA and ABP inhibition decreases with increasing frequency and current stimulation of the OVLT. Several alternative explanations may account for this outcome: 1) muscimol was not an effective inhibitor of neurotransmission in the PVH 2) some neurons from the lamina terminalis do not synapse within the PVH 3) electrical current overwhelmed chemical inhibition of the PVH, evoking a residual increase in RSNA and ABP and 4) alternative pathways exist.
that also support changes in sympathetic outflow and ABP to stimulation of the lamina terminalis.

The neurotransmitter γ-aminobutyric acid (GABA) is an inhibitory neuropeptide that hyperpolarizes cell bodies through induction of an inward chloride current. Muscimol mimics the inhibitory action of GABA through selective binding to the endogenous ionotropic GABA$_A$ receptor on the cell body$^{79,80}$. In an in vitro study of rat hippocampal neurons, Segal and Barker reported channel opening durations of 22.9 ms and 38.8 ms for GABA and muscimol, respectively$^{81}$. Functional studies show that bilateral microinjection of muscimol (0.05-1.5 nmol) into the PVH decreases sympathetic nerve activity and ABP in anesthetized rats for a period of at least 120 minutes$^{74-76}$. Altogether, these data support the notion that muscimol effectively inhibits neural transmission in the PVH.

Reduced inhibition of SNA and ABP responses to OVLT stimulation may also suggest the presence of fibers of passage. These axons may pass directly from the lamina terminalis to brainstem or spinal targets without synapsing in the PVH. Because muscimol inhibits neurons though GABA$_A$ receptors on cell bodies, these fibers of passage would be unaffected. Therefore, RSNA and ABP responses following chemical inhibition of the PVH at higher frequencies and current intensities could be attributable to such projections.

The third alternative suggests that chemical-induced hyperpolarization of PVH cell bodies is overwhelmed by increased neurotransmitter release at PVH synaptic junctions. Frequency and current intensity affect the rate of neuronal discharge and the population of activated neurons, respectively. Thus, with increasing frequency and current intensity, more neurons are recruited and discharge at a faster rate. At synaptic junctions within the PVH, this leads to a greater release of neurotransmitter and frequency of excitatory post-synaptic potentials arriving at the cell body. As a result, hyperpolarization due to
muscimol-induced chloride influx may be progressively overwhelmed, evoking an action potential.

A final possibility for the lack of complete inhibition is the presence of alternative pathways mediating a portion of the sympathetic and pressor responses to OVLT stimulation. Early studies conducted by Enoch and Kerr in anesthetized cats utilized a combination of electrical stimulation and brain sectioning to describe two distinct vasopressor pathways within the hypothalamus. The lateral pathway, arising from the suprachiasmatic nucleus and medial forebrain bundle, passes through the lateral and ventromedial (VMH/ME) hypothalami to terminate in the lateral mesencephalic tegmentum. The periventricular pathway begins in the posterior hypothalamic area and connects to the periaqueductal grey region. Follow-up studies performed in anesthetized rats have demonstrated a link between these hypothalamic pathways and activation of the lamina terminalis. Fink and colleagues observed that electrolytic lesion of the VMH/ME attenuates hemodynamic changes to electrical stimulation of the AV3V. Similarly, ablation of the periaqueductal grey blunted hemodynamic responses to AV3V electrical stimulation. Moreover, Johnson et al. showed that destruction of the VMH/ME prevents the onset of Grollman renal wrap hypertension. Altogether, these reports indicate alternative pathways, which may contribute to sympathetic and pressor responses to electrical stimulation of the OVLT and thus account for the reduction of PVH muscimol inhibition with increasing magnitude of OVLT electrical stimulation.

In summary, the lamina terminalis is a forebrain region influential in sympathetically-mediated changes in blood pressure. Activation of its individual nuclei evokes unique hemodynamic responses in end-organ vasculature. The present study contributes two major findings to the understanding of lamina terminalis function: 1) activation of the OVLT increases sympathetic outflow to the renal nerve and ABP and 2) the full expression of these responses is dependent upon synaptic signaling at the PVH.
These results are consistent with previous studies, which show acute increases in sympathetic outflow and blood pressure to hypertonic and ANGII stimulation of the lamina terminalis\textsuperscript{13,69}. However, the sensation of circulating factors, mono- and polysynaptic pathways, and neurotransmitters involved in signaling from the lamina terminalis require further investigation. Insight into these underlying mechanisms will contribute to a greater understanding of how sympathetic outflow to the vasculature is regulated. From this, a possible role for the lamina terminalis in the etiology of hypertension may be more thoroughly described.
CHAPTER SIX
PERSPECTIVES AND FUTURE IMPLICATIONS

The affect of dietary sodium upon blood pressure has been a source of clinical and basic investigation in recent years. The results of the DASH (Dietary Approaches to Stop Hypertension) clinical trial showed that a reduction in sodium chloride intake effectively reduces blood pressure in both hypertensive and non-hypertensive individuals. Furthermore, high dietary sodium chloride increases the activity of the sympathetic nervous system to produce chronic blood pressure elevation in human and experimental forms of salt-sensitive hypertension. However, several questions that remain are: where/how is plasma sodium chloride sensed by the CNS and what are the CNS cellular mechanisms underlying salt-sensitive hypertension?

Studies have shown that the lamina terminalis, a region of interconnected nuclei along the anterior wall of the third ventricle, possesses sensitivity to changes in plasma sodium chloride concentration. Acute stimulation of these structures with hypertonic saline increases LSNA, RSNA and ABP in vivo and in vitro. Chemical inhibition or ablation of the OVLT significantly decreases LSNA and RSNA, but not ABP, to intracarotid infusion of hypertonic saline. In the context of salt-sensitive hypertension, intracarotid, but not intravenous, infusion of hypotonic saline decreased ABP in DOCA salt-sensitive rats. Furthermore, this model of hypertension is supported by increased sympathetic outflow due to elevated plasma sodium chloride concentration. Altogether, these data implicate the lamina terminalis as a major center for osmosensitivity, with influence over sympathetic outflow and ABP.

One mechanism within the lamina terminalis implicated in its osmosensitivity is the TRPV1 receptor. Depolarization to osmotic stimulation was not observed in OVLT cells from TRPV1 knockout mice and these responses in wild type neurons could be blocked with a TRPV antagonist. Moreover, intracarotid infusion of aTRPV1 agonist evoked
increases in ABP in anesthetized rats. A remaining question is whether these receptors mediate chronically elevated sympathetic outflow and ABP in hypertension. One set of experiments that could address this question involves selective inhibition of the TRPV1 receptor in DOCA-salt rats. A reduction in sympathetic outflow and ABP following TRPV1 inhibition would suggest that these receptors mediate a portion of the salt-sensitive hypertension.

Downstream of the lamina terminalis, anatomical studies have identified the PVH as a prominent efferent target. Renal cortical microinjections of pseudorabies virus revealed positive cell bodies within the PVH prior to lamina terminalis infection. Functionally, blockade of PVH AT1 receptors attenuated RSNA and pressor responses to intracarotid infusion of hypertonic saline. Similarly, antagonism of PVH glutamate receptors blunted LSNA responses to introduction of hypertonic saline in an in situ rat preparation. One remaining question is whether these receptors support the elevated sympathetic outflow observed in salt-sensitive hypertension. To address this question, microinjections of either losartan (selective AT1 antagonist) or kynurenic acid (non-selective ionotropic excitatory amino acid antagonist) could be made into the PVH of DOCA-salt rats. Subsequent decreases in SNA and ABP would suggest a role for these receptors in salt-sensitive hypertension.

Recently, a compelling argument made by Osborn et al. suggested that increased plasma sodium chloride alone might not be sufficient to generate neurogenic hypertension. Instead, chronic elevations in SNA and ABP may be the result of a synergistic interaction between sodium chloride and ANGII upon circumventricular organs. As primary evidence for this hypothesis, elevated plasma norepinephrine concentrations were observed following infusion of ANGII in rats on a high, but not normal, sodium chloride diet. Moreover, a high salt diet increased TPR and ABP in conscious dogs receiving chronic subpressor infusions of ANGII, while animals on a
normal salt diet were unaffected. Though the cellular mechanisms subserving this hypothesized interaction remain unclear, stretch-inactivated cation (SIC) channels and TRPV1 receptors have been proposed to play a role.

Elevated dietary sodium chloride upregulates the sympathetic nervous system to support chronically elevated blood pressure. The circumventricular organs of the lamina terminalis are an osmosensitive site, where elevated plasma osmolality may influence long-term changes in sympathetic outflow and ABP. However, the cellular mechanisms mediating osmosensitivity within these nuclei remain unclear, though current findings suggest the involvement of TRPV1 and/or SIC channels. Furthermore, sodium chloride and ANGII may act in concert upon these cellular targets to affect long-term changes in blood pressure and sympathetic nerve activity. Future experiments are needed to investigate these mechanisms along with the downstream receptors and substrates in support of osmotically-induced synaptic signaling in salt-sensitive hypertension. From this work, a greater comprehension of the etiology of salt-sensitive hypertension and possible anti-hypertensive therapies will be gained.
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VITA

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III. Awards

   1. Alumni Association Senior Book Award, Emory University, 2004
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   4. 2007 American Physiological Society Professional Skills Short Course – Bethesda, MD

IV. Professional Affiliations

   2006- American Physiological Society (Student Member)
   2006- Association of Biology Laboratory Educators

V. University, State and National Committees

   1. Delta Epsilon Iota – Academic Honor Society – University of Kentucky
   2. Biology Graduate Student Association, 2005-current
      a. Secretary, 2006-2007
   3. University of Kentucky Graduate Student Congress
      a. Representative – Department of Biology, 2007-current
      b. Health Care Committee
VI. **Teaching Activities**

A. Classroom, Seminar or Teaching Laboratory

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VII. **Publications**


VIII. **Abstracts**

