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Neuroimmune Communication in Hypertension and Obesity: A new therapeutic angle?

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Abstract

Hypertension is an epidemic health concern and a major risk factor for the development of cardiovascular disease. Although there are available treatment strategies for hypertension, numerous hypertensive patients do not have their clinical symptoms under control and it is imperative that new avenues to treat or prevent high blood pressure in these patients are developed. It is well established that increases in sympathetic nervous system (SNS) outflow and enhanced renin-angiotensin system (RAS) activity are common features of hypertension and various pathological conditions that predispose individuals to hypertension. More recently, hypertension has also become recognized as an immune condition and accumulating evidence suggests that interactions between the RAS, SNS and immune systems play a role in blood pressure regulation. This review summarizes what is known about the interconnections between the RAS, SNS and immune systems in the neural regulation of blood pressure. Based on the reviewed studies, a model for RAS/neuroimmune interactions during hypertension is proposed and the therapeutic potential of targeting RAS/neuroimmune interactions in hypertensive patients is discussed. Special emphasis is placed on the applicability of the proposed model to obesity-related hypertension.

Keywords

angiotensin; renin; inflammation; sympathetic nervous system; microglia; blood pressure

1. Introduction

Hypertension is an epidemic health concern and a major risk factor for the development of cardiovascular disease, the leading cause of death in the USA. It has long-been established that high blood pressure is often accompanied by enhanced activities of the renin-

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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angiotensin system (RAS) and the sympathetic nervous system (SNS) and, over several decades, research efforts have led to the development and refinement of countless pharmacological antihypertensive therapeutics targeting these systems. Despite these efforts, which successfully reduce blood pressure in many hypertensive subjects, numerous patients remain unresponsive to available pharmacological interventions and are left with severely high blood pressure (Egan *et al.*, 2010). A large proportion of these patients have increased sympathetic outflow, parasympathetic withdrawal, and norepinephrine spillover, and surgical denervation of the kidney has shown promise as an effective intervention for these patients (Krum *et al.*, 2009; Schlaich *et al.*, 2009a; Schlaich *et al.*, 2009b; Esler *et al.*, 2010; Schlaich *et al.*, 2012). The implication is that their hypertension arises from neurogenic origins (Fisher and Fadel, 2010; Grassi, 2010; Grassi *et al.*, 2010).

The frequent ineffectiveness of available antihypertensive pharmacotherapies coupled with the neural origins of uncontrolled hypertension underscore the urgent need to develop new avenues for the pharmacological treatment or prevention of neurogenic hypertension. One promising prospect in this regard, stems from the relatively recent realization that hypertension is also an immune condition (Harrison *et al.*, 2008; Shi *et al.*, 2010; Zubcevic *et al.*, 2011; Marvar *et al.*, 2012). The nervous and immune systems are intricately connected and their reciprocal communication may contribute to the etiology of hypertension. Here we review the intimate relationship between these systems in the regulation of blood pressure and the potential roles of the SNS and RAS in these interactions.

Before discussing this RAS/ neuroimmune connection, we first briefly review the current understanding of the roles of the SNS and RAS in blood pressure control. Then, subsequent sections will consider the reciprocity between the nervous and immune systems, and how the RAS and SNS mediate these interactions. The role of the RAS, SNS and immune system in facilitating the causal relationship between obesity and hypertension is also considered. Finally, based on the reviewed studies a model for RAS/immune interactions in the neural control of blood pressure will be proposed and the clinical relevance of these regulatory systems for the development of new antihypertensive therapeutics will be discussed.

2. The brain renin-angiotensin system, sympathetic outflow and hypertension

Before discussing how the RAS, SNS and immune systems may interact to cause hypertension, it is appropriate to first review the roles of the SNS and RAS in blood pressure regulation. The SNS plays a key role in the pathophysiology of cardiovascular disease (Goldstein, 1983; Guyenet, 2006; Fisher and Fadel, 2010; Grassi, 2010; Grassi *et al.*, 2010). Hypertensive animals and patients exhibit elevated SNS outflow (Goldstein, 1983; Grassi, 1998; Guyenet, 2006; Joyner *et al.*, 2008) as well as, heightened vascular reactivity, characterized by greater vasoconstrictor responses to norepinephrine (Ziegler *et al.*, 1991).

Various environmental factors, such as exposure to stressful stimuli and high-fat diet-induced obesity, and humoral factors, including those synthesized by the RAS activate the SNS and lead to an increased susceptibility to hypertension (Oparil *et al.*, 2003; Dorresteijn *et al.*, 2012; Marvar and Harrison, 2012) and pharmacotherapies that target the SNS and RAS reduce blood pressure in many patients. Furthermore, a therapy that has recently proven effective for some individuals resistant to available antihypertensive pharmaceuticals is surgical denervation of the kidney (Krum *et al.*, 2009; Schlaich *et al.*, 2009a; Schlaich *et al.*, 2009b; Esler *et al.*, 2010). This surgical intervention is minimally invasive, as it uses a percutaneous catheter-based approach to ablate both afferent and efferent renal nerves, and it is highly effective, as it reduces sympathetic outflow to the kidney, reduces plasma renin activity and increases urine output, thereby reducing blood pressure without causing long-

term adverse events (Krum *et al.*, 2009; Schlaich *et al.*, 2009a; Esler *et al.*, 2010; Krum *et al.*, 2011).

The effectiveness of this surgical intervention solidifies the importance of the nervous system in uncontrolled hypertension and understanding the mechanism(s) by which the nervous system regulates blood pressure under normal conditions may therefore guide the refinement of existing and/or the development of new pharmacological therapeutics for this pathology. In this section, we review the neural circuitry controlling sympathetic outflow and blood pressure regulation and the impact of the RAS on these pathways.

2.1. The renin-angiotensin system

First, it is essential to outline the current understanding of the RAS. Classically, the RAS has been viewed as an endocrine system regulating cardiovascular function and hydromineral balance. Angiotensin-II (Ang-II) is considered the principle effector peptide of the system, which is formed from liver-derived angiotensinogen (AGT) through proteolytic cleavage first by kidney-derived renin (the rate-limiting enzyme for Ang-II synthesis) and then by lung-derived angiotensin-converting enzyme (ACE). Ang-II then acts predominantly at its type-1 receptor (AT1R), in variety of tissues to impact blood pressure regulation. Although this is the best characterized and perhaps the dominating pathway of the RAS, several additional nuances of the RAS have been revealed, that allow it to impact blood pressure in diverse ways.

This includes several endogenous mechanisms that counteract the hypertensive actions of AT1R. For example, AT1R activation can be opposed by stimulation of the angiotensin type-2 receptor (AT2R; Huang *et al.*, 1996; Bosnyak *et al.*, 2010; Steckelings *et al.*, 2012) and by the ACE2-Ang-(1-7)-Mas axis (Donoghue *et al.*, 2000; Katovich *et al.*, 2005; Der Sarkissian *et al.*, 2006; Diez-Freire *et al.*, 2006; Santos *et al.*, 2008). We have also determined that macrophage migration inhibitory factor (MIF) can oppose many of Ang-II's pathophysiological actions mediated by AT1R, working intracellularly via its thiol-protein oxidoreductase (TPOR) activity (Busche *et al.*, 2001; Sun *et al.*, 2007; Li *et al.*, 2008; Freiria-Oliveira *et al.*, 2012).

Moreover, it is now acknowledged that the RAS is also an autocrine/ paracrine system within various tissues including adipose tissue (Yiannikouris *et al.*, 2012a; Yiannikouris *et al.*, 2012b) and the brain (Lenkei *et al.*, 1997; McKinley *et al.*, 2003; Cuadra *et al.*, 2010). Components of the RAS are present within brain regions that control cardiovascular function, but are protected from the circulating RAS by the blood brain barrier (BBB) suggesting that brain-derived Ang-II may contribute to SNS activity and may act as a neurotransmitter in these regions (Bains *et al.*, 1992; Li and Ferguson, 1993). Pharmacological or genetic manipulations of the RAS in these protected brain regions have profound effects on physiology and behavior, further supporting the prevalence of a functional brain-specific RAS (Morimoto *et al.*, 2002; McKinley *et al.*, 2003; Grobe *et al.*, 2008; Grobe *et al.*, 2010; de Kloet *et al.*, 2011; Yamazato *et al.*, 2011). However, the level of renin within the CNS is low at best (Bader and Ganten, 2002; Lavoie *et al.*, 2004), calling into question the mechanism/source of Ang-II's presence in the brain. In this regard, the (pro)renin receptor (PRR) which is highly expressed in the brain may mediate extracellular generation of Ang-II in the brain by binding and sequestering (pro)renin, thereby increasing its catalytic activity and propagating localized cleavage of AGT (Cuadra *et al.*, 2010).

2.2. Neural circuitry controlling the sympathetic nervous system and blood pressure

Sympathetic stimulation of the heart, vasculature and kidneys increases blood pressure by elevating cardiac output, vascular resistance and fluid retention, respectively, while

inhibiting the SNS produces the opposite effects (Goldstein, 1983; Grassi, 1998; Grassi *et al.*, 1998; Burke *et al.*, 2012). Several forebrain and hindbrain nuclei control sympathetic outflow to these tissues, and are regulated by the RAS. Of particular importance are the paraventricular nucleus of the hypothalamus (PVN), subfornical organ (SFO), rostral ventral lateral medulla (RVLM) and nucleus of the solitary tract (NTS), all of which contain receptors for the RAS; however, there are many other brain regions that influence blood pressure via projections to and from these key cardiovascular regulatory nuclei. For more comprehensive reviews of the neural circuits regulating sympathetic outflow please refer to (Dampney, 2004; Guyenet, 2006).

The circulating RAS can influence the SNS and blood pressure by acting peripherally at presynaptic AT1R to augment norepinephrine release onto specific tissues (Abboud, 1974; Cassis and Dworkin, 1991; Cassis, 1993), and by acting centrally at receptors in circumventricular organs (CVOs), such as the SFO. The SFO contains receptors for numerous circulating factors and is densely populated with AT1R, enabling the brain to monitor and respond to changes in systemic RAS activity. The levels of AT1R within the SFO are regulated by hypertensive stimuli and increased AT1R within CVOs reduces baroreflex sensitivity, increases sympathetic tone and elevates blood pressure (Zucker *et al.*, 2009; Braga, 2011; Nunes and Braga, 2011; Hilzendege *et al.*, 2012). Moreover, lesion of the SFO or interference with pathways activated by Ang-II/AT1R specifically within the SFO (e.g., ER stress or oxidative stress) attenuate the elevated SNS activity and blood pressure that accompany experimental hypertension (Zimmerman *et al.*, 2004a; Zimmerman *et al.*, 2004b; Hendel and Collister, 2005; Cao *et al.*, 2012; Osborn *et al.*, 2012; Young *et al.*, 2012). The SFO then sends direct projections to the PVN, as well as other cardiovascular regulatory sites to impact blood pressure (Lind *et al.*, 1984; Li and Ferguson, 1993; Krause *et al.*, 2008; Marvar *et al.*, 2010; Krause *et al.*, 2011).

The PVN lies at the heart of the regulation of many homeostatic systems, including those controlling blood pressure, energy balance and stress responsiveness. Neurons of the PVN are divided into distinct magnocellular and parvocellular sub-nuclei (Swanson and Kuypers, 1980). Magnocellular neurons express vasopressin or oxytocin and, although activation of these neurons primarily leads to the release of these neuropeptides from the posterior pituitary into the systemic circulation, some of these neurons are spinally-projecting and influence blood pressure by regulating SNS activity. Parvocellular PVN neurons are classified as preautonomic or neurosecretory and regulate sympathetic tone and neuroendocrine secretion, respectively (Swanson and Kuypers, 1980). PVN neurons receive and integrate signals from CVOs, brainstem nuclei, and from cortico-limbic brain regions that are sensitive to various stimuli such as changes in the systemic milieu or psychological stressors, and respond to them by regulating SNS activity and neuroendocrine secretion.

In addition to influencing the PVN via activating CVOs with excitatory afferents in the PVN, Ang-II can also act locally within the PVN to enhance SNS activity through many mechanisms. AT1R are located post-synaptically on preautonomic glutamate neurons of the PVN and Ang-II can directly activate these neurons to perpetuate increases in SNS activity (Jiang *et al.*, 2009; Kleiber *et al.*, 2010). Additionally, AT1R are located presynaptically within the PVN, stimulation of which reduces GABA release, thereby activating RVLM and spinally-projecting preautonomic neurons through presynaptic disinhibition (Li *et al.*, 2003; Li and Pan, 2005). Ang-II also acts at AT1R, possibly on immune cells of the PVN (Shi *et al.*, 2010), to induce oxidative stress and inflammation which feed-forward to further enhance sympathetic outflow and hypertension. Evidence that Ang-II acts at the immune cells of the PVN to perpetuate increases in SNS activity is discussed in detail in subsequent sections. AT1R activation in the PVN is opposed by the endogenous counter-regulatory ACE2-Ang(1-7)-Mas axis via the overexpression of ACE2 specifically within the PVN

(Sriramula *et al.*, 2011) and by the overexpression of MIF specifically within the PVN (Sun *et al.*, 2004; Li *et al.*, 2006), both of which reduce blood pressure in spontaneously hypertensive rats (SHR; a widely utilized model of hypertension).

Excitation of preautonomic PVN neurons increases blood pressure, via synapses onto RVLM neurons and preganglionic neurons in the intermediolateral cell column (IML) of the spinal cord (Swanson and Kuypers, 1980; Cechetto and Saper, 1988; Shafton *et al.*, 1998; Pyner and Coote, 2000) that receive inputs from many brain structures and regulate sympathetic outflow to cardiovascular tissues (Dean *et al.*, 1992; Ding *et al.*, 1993; Madden and Sved, 2003). Upon excitation, RVLM neurons release glutamate, catecholamines and other transmitters to regulate sympathetic outflow via their actions on preganglionic IML neurons (Guyenet, 2006; Bourassa *et al.*, 2009). Moreover, barosensitive sympathetic efferents are regulated in large part by the RVLM (Dampney *et al.*, 2002) and hyperactivity of RVLM barosensitive neurons controlling renal sympathetic nerve activity leads to hypertension, by permitting blood pressure to increase without decreasing heart rate. Hypertensive stimuli, such as Ang-II and high-salt intake, modulate the excitability of these neurons (Stocker *et al.*, 2006; Adams *et al.*, 2008; Bourassa *et al.*, 2009). RVLM AT1R activation potentiates increases in blood pressure, while ACE2 acts to reduce hypertension in SHRs. Importantly, salt intake tonically activates RVLM AT1R and enhances the sympathoexcitatory responses to RVLM administered Ang-II, highlighting an important interaction between the humoral and environmental regulators of sympathetic outflow (Adams *et al.*, 2008).

The NTS is also an important integrative site for many homeostatic systems, including the regulation of circulation. It allows for the monitoring of the peripheral milieu as it receives input from sympathetic afferents, arterial baroreceptors, chemoreceptors, volume receptors and more. This site then mediates the sympathetic chemo- and baroreflexes. In hypertension arterial baroreceptors are reset to a higher pressure. Accordingly, abnormalities in the receipt, processing or integration of this input within the NTS could then contribute to enhanced blood pressure. AT1R are densely localized to the NTS and AT1R activation within the NTS stimulates GABAB receptor expression and dampens the baroreflex, thereby contributing to hypertension (Paton and Kasparov, 1999; Polson *et al.*, 2007; Yao *et al.*, 2008). Conversely, utilizing virally-mediated gene transfer techniques we have recently determined that counteracting AT1R activation via the NTS-specific overexpression of ACE2 (Yamazato *et al.*, 2011) or MIF (Freiria-Oliveira *et al.*, 2012) improves baroreceptor heart-rate reflex in SHRs, further corroborating protective roles for these systems in blood pressure control.

Neurons of the PVN, NTS, RVLM and IML receive projections from many other brain regions to regulate SNS activity and cardiovascular function and dysregulation of these neural circuits by genetic, humoral and environmental factors then contributes to the onset of hypertension.

3. Brain RAS, SNA and mobilization of the peripheral immune system

There is considerable cross-talk between the immune and nervous systems, such that alterations in the immune system can influence signaling within the nervous system while the brain can regulate immune function. This reciprocal communication occurs via several mechanisms and both the CNS's ability to alter immune function and the immune system's ability to regulate neuronal function can impact cardiovascular and metabolic regulation. In this section, we focus on the impact of peripheral immune activation on cardiovascular and metabolic homeostasis, as well as the potential role of the brain RAS and the SNS in mediating the mobilization of the peripheral immune system during these pathologies.

3.1. Peripheral immune activation during hypertension and obesity

Hypertension and predisposing conditions, such as obesity, have independently become acknowledged as chronic inflammatory conditions, with immune factors accumulating in the brain and peripheral tissues. In the periphery, hypertensive and obesigenic stimuli initiate innate and adaptive immune responses in cardiovascular (e.g., vasculature and kidney) and metabolic (e.g., adipose tissue and liver) tissues that exacerbate the pathologies (Guzik *et al.*, 2007a; Guzik *et al.*, 2007b; Crowley *et al.*, 2010; De Miguel *et al.*, 2010; Madhur *et al.*, 2010; Dorresteijn *et al.*, 2012; Marvar *et al.*, 2012; Sell *et al.*, 2012). Hypertensive stimuli increase the levels of macrophages and activate T-cells that secrete pro-inflammatory cytokines, promote vasoconstriction and enhance sodium retention (Guzik *et al.*, 2007a; Crowley *et al.*, 2010; De Miguel *et al.*, 2010; Madhur *et al.*, 2010; De Miguel *et al.*, 2011a; Harrison *et al.*, 2011; Harrison *et al.*, 2012; Marvar *et al.*, 2012). Moreover, macrophage and lymphocyte infiltration of adipose tissue and ectopic fat storage in liver and other tissues are hallmarks of obesity that lead to elevated circulating levels of immune factors which impair blood pressure and glucose regulation (Hotamisligil, 2003; Weisberg *et al.*, 2003; Xu *et al.*, 2003; Hotamisligil, 2006; Caspar-Bauguil *et al.*, 2009; Bennett *et al.*, 2012).

A causal relationship between activation of the peripheral immune system and hypertension is reflected by evidence that interfering with immune responses leads to perturbations in metabolic and cardiovascular homeostasis. In this regard, immune factors, such as TNF α (Sriramula *et al.*, 2008; Tran *et al.*, 2009), IL-6 (Brands *et al.*, 2010) and T-lymphocytes (Guzik *et al.*, 2007a; Marvar *et al.*, 2010) are necessary for Ang-II-induced hypertension. Disruption of the innate immune system by eliminating macrophages and neutrophils via the Cre-lox-mediated specific expression of diphtheria toxin receptor in all LysM cells reduces oxidative stress, improves vascular function and reduces blood pressure (Wenzel *et al.*, 2011). Pharmacological interference with adaptive immunity by administration of mycophenolate mofetil, which suppresses T-cells, decreases blood pressure (De Miguel *et al.*, 2010). Further, mice with genetic deficiencies in the adaptive immune system (i.e., SCID mice or mice that lack lymphocytes [RAG1 $^{-/-}$ mice]) are resistant to many forms of experimental hypertension (Guzik *et al.*, 2007a; Crowley *et al.*, 2010; Marvar *et al.*, 2010). Adoptive transfer of T-cells, not B-cells in the RAG1 $^{-/-}$ mice restores hypertension (Guzik *et al.*, 2007a; Marvar *et al.*, 2010).

Collectively, these studies point to an integral role for both the adaptive and innate immune systems in hypertension and accumulating evidence suggests that the brain, via the SNS, mediates this immune activation during hypertension.

3.2. A role for the brain RAS and sympathetic nervous system in the control of the peripheral immune system during hypertension

The brain can control peripheral immune function via neural or neuroendocrine mechanisms. The SNS innervates both primary and secondary lymphoid organs and thereby regulates the immune system (Elenkov *et al.*, 2000). Additionally, the immune cells express receptors for and are influenced by many neuroendocrine systems, including the hypothalamic pituitary adrenal axis and the RAS. The RAS facilitates brain-immune system cross-talk via a neuroendocrine mechanism and by acting in specific brain nuclei to enhance the SNS mobilization of the peripheral immune system and these interactions impact blood pressure regulation. As discussed above, hypertension and pre-disposing obesity are often associated with increased activities of the SNS, the RAS and the peripheral immune system and this can have deleterious effects on cardiovascular and metabolic homeostasis. It is possible that a brain RAS-mediated SNS mobilization of the peripheral immune system represents a facilitating mechanism for hypertension and obesity.

Models of hypertension are associated with enhanced SNS driven immune responses. In this regard, Harwani *et al.* recently ascertained that the pre-hypertensive SHR exhibits proinflammatory, while the normotensive WKY rat exhibits anti-inflammatory, TLR7/8 or 9 responses to cholinergic stimulation (Harwani *et al.*, 2012). Further, Ang-II modulates immune function by activating receptors expressed on peripheral immune cells and by acting in the brain to promote SNS outflow. Elevated brain Ang-II enhances pro-inflammatory splenic cytokines, through an SNS dependent mechanism (Ganta *et al.*, 2005). During obesity and hypertension, the SNS is also positioned to contribute to the rise in immune factors specifically within metabolic and cardiovascular tissues, which then act locally to exacerbate dysfunction. Metabolic tissues, such as fat, express adrenergic receptors and activation of these receptors initiates local inflammation (Bartness and Song, 2007; Wang *et al.*, 2011). Of relevance, perivascular adipose tissue is particularly highly innervated by the SNS and it is possible that adrenergic induced immune activation in perivascular fat that occurs during obesity and contributes to the progression of co-morbid hypertension (Guzik *et al.*, 2007b). Similarly, SNS activation of cardiovascular tissue initiates local inflammatory responses that exacerbate pathology (Rodriguez-Iturbe *et al.*, 2002; Levick *et al.*, 2010; De Miguel *et al.*, 2011a; De Miguel *et al.*, 2011b).

CVOs are in an ideal position to mediate reciprocal communication between the nervous and immune systems as they can monitor peripheral levels of inflammation, Ang-II and other hypertensive stimuli and then facilitate the activation of the immune system. These brain regions are potent regulators of cardiovascular function and hydromineral balance and they likely play a causal role in the SNS mobilization of the peripheral immune system (peripheral activation of T-cells and vascular inflammation) during hypertension (Lob *et al.*, 2010; Marvar *et al.*, 2010; Marvar *et al.*, 2012). Commensurate with this, electrolytic lesion of the anterioventral third ventricle region prevents experimental hypertension (Marvar *et al.*, 2010) and blocks the activation and vascular infiltration of T-cells in response to chronic Ang-II infusion (Marvar *et al.*, 2010). Marvar and colleagues have further delineated a role of oxidative stress specifically within the SFO, as the loss of superoxide dismutase 3 within this brain region leads to T-cell activation, enhanced inflammation and reactive oxygen species in peripheral vessels, and enhanced sympathetic outflow and hypertension (Lob *et al.*, 2010).

Furthermore, our group has recently published studies supporting a brain-SNS-bone marrow connection, originating in the PVN that plays a facilitating role in the progression of Ang-II-induced neurogenic hypertension (Jun *et al.*, 2012). Specifically, we have found that elevations in Ang-II decrease bone marrow-derived endothelial progenitor cells and increase bone marrow-derived inflammatory cells, thereby compromising vascular repair and facilitating the progression of hypertension. It is likely that this pathway not only activates the peripheral immune system, but also mobilizes brain immune cell progenitors during hypertension.

Based on these studies, it is clear that the SNS mobilization of the peripheral immune system is involved in various forms of experimental hypertension, including stress-related high blood pressure (Marvar and Harrison, 2012; Marvar *et al.*, 2012). Since obesity can also be considered a stressor, it is possible that a similar mechanism contributes to obesity-related hypertension.

4. Recruitment and activation of immune cells within the CNS during neurogenic hypertension

Another mechanism by which the immune and nervous systems interact is via the activation of resident immune cells and/or the recruitment of peripheral immune cells to the neural

parenchyma. The healthy CNS is thought to be “immune-privileged,” in that there is a lack of parenchymal dendritic cells, which are critical for antigen uptake, migration to draining lymph nodes and presentation to naïve T-cells. Despite this immune-privilege that protects the brain from inflammatory reactions that may damage non-regenerating neurons, the immune system impacts the brain during several pathologies. For instance, the nervous system clearly responds to acute systemic infections by initiating sickness behavior, which is characterized by decreased locomotor activity and food intake (Langhans, 2007) and is considered an adaptive response to facilitate survival during the challenge (Dantzer and Kelley, 2007). Additionally, when the brain becomes injured, for example, by a stroke or a neurodegenerative disease, resident and recruited exogenous immune cells act to remove damaged tissue and protect the remaining neurons. The magnitude of the insult likely governs whether the immune cells are of central origin or are recruited from the periphery and although the immune system is clearly in place to protect the host from antigens, immune cells also release factors that exacerbate pathologies, including hypertension and metabolic disease.

4.1. Hypertension and brain inflammation

Several lines of evidence suggest that immune factors are capable of acting in the brain to intensify hypertension. In this regard, models of hypertension are associated with increased brain immune factors while models of neuroinflammation are coupled with increased blood pressure (Wu *et al.*, 2012). Ang-II is a potent regulator of the immune system centrally and peripherally and induces brain inflammatory responses that are critical for the development and progression of hypertension. Ang-II increases PVN expression of proinflammatory cytokines (Shi *et al.*, 2010; Shi *et al.*), such as IL-1 β , that activate PVN neurons (Yang *et al.*, 1997) and increase sympathetic outflow (Kannan *et al.*, 1996). Ang-II also activates NF κ B within the PVN and this is required for Ang-II's hypertensive effects (Kang *et al.*, 2009; Cardinale *et al.*, 2012). On the other hand, expression of the anti-inflammatory cytokine, IL-10, is reduced during Ang-II infusion, and its overexpression attenuates Ang-II-induced hypertension (Shi *et al.*, 2010). Similarly, the cytokine MIF, working intracellularly via its intrinsic TPOR activity, opposes Ang-II actions and reduces blood pressure in hypertensive animals (Busche *et al.*, 2001; Sun *et al.*, 2004; Li *et al.*, 2006; Li *et al.*, 2008; Freiria-Oliveira *et al.*, 2012). Other models of hypertension, such as the SHR, as well as predisposing conditions such as obesity or stress-related disorders, are also associated with increased hypothalamic expression of inflammatory factors that may represent a causal link among these pathologies (Jankord *et al.*, 2010; Agarwal *et al.*, 2011). Moreover, during hypertension, different brain regions important for the regulation of blood pressure also exhibit a proinflammatory milieu. For example, within the brainstem, microarray comparison between SHR and WKY NTS (Waki *et al.*, 2007; Waki *et al.*, 2008b, a) revealed an upregulation of junctional adhesion molecule-1 [JAM-1], overexpression of which is sufficient to increase blood pressure in normotensive rats. In addition, the LPS-induced model of systemic inflammation is associated with neuroinflammation and oxidative stress specifically in the RVLM that leads to neurogenic hypertension (Wu *et al.*, 2012). The implication is that in addition to influencing blood pressure via direct actions on neurons, many hypertensive stimuli also initiate a rise in proinflammatory factors that then augment sympathetic outflow.

4.2. Blood brain barrier

The blood brain barrier (BBB), which on one hand protects the CNS from deleterious peripheral immune reactions, on the other hand regulates much of the neuroimmune exchange by serving as an intermediary for the peripheral immune system's ability to impact the brain (Banks, 2005; Banks and Erickson, 2010). Incidentally, one important mechanism by which the peripheral immune system can directly interact with the CNS is through a

compromised blood brain barrier (BBB). Certain threats, including homeostatic challenges that cause hypertension or obesity, compromise the integrity of the BBB which can then lead to the recruitment of peripheral immune cells to the brain parenchyma. For example, chronic administration of hypertensive doses of Ang-II or consumption of high-fat diet increase the permeability of the BBB (Kanoski *et al.*, 2010; Zhang *et al.*, 2010; Davidson *et al.*, 2012).

Also of relevance, peripheral immune system macrophages are associated with the perivascular space, the CVOs, the choroid plexus and the meninges of the brain. Certain brain regions that are key regulators of metabolism and blood pressure, such as the PVN and the arcuate nucleus of the hypothalamus (ARC), are highly vascularized and it is possible that these regions are particularly susceptible to factors that jeopardize the BBB. Further, it is possible that the abundance of immune factors within these regions in response to hypertensive and obesigenic stimuli occurs secondary to a threatened integrity of the BBB.

Importantly, several cytokines can also effectively be transported bi-directionally across the intact BBB. Moreover, peripheral cytokines can activate receptors situated on the BBB, thereby disrupting transport systems of the BBB and/or leading to the release of paracrine factors, such as prostaglandins, that can impact various cell-types of the CNS (Yu *et al.*, 2010).

4.3. Resident Immune Cells of the Brain

Other important sources for the elevations in central sympathoexcitatory pro-inflammatory factors during hypertension are the resident immune cells of the brain, which are capable of both sensing and responding to hypertensive stimuli. Microglia and astrocytes are the brain's resident innate immune cells (reviewed in Ransohoff and Brown (2012)) that respond to various threats to homeostasis, including hypertensive stimuli (Shi *et al.*, 2010; Thaler *et al.*, 2012). While it is clear that the interactions among the cell-types of the nervous system contribute to the maintenance of homeostasis, it is also possible that these exchanges contribute to metabolic and cardiovascular pathologies.

Microglia—Microglia are of mesodermal/mesenchymal origin, originating most likely from the bone marrow, and are distributed throughout the healthy and injured CNS (Kettenmann *et al.*, 2011). Under normal conditions, 'resting' microglia have a small soma and long fine processes that act to survey their microenvironment for pathogens and other disturbances (Badoer, 2010; Kettenmann *et al.*, 2011). They sense threats to homeostasis, and respond to them by rapidly becoming activated and migrating to the damaged tissue (Nguyen *et al.*, 2002; Shi *et al.*, 2010; Thaler *et al.*, 2012). Upon activation microglia undergo a phenotypic change, transforming into cells with enlarged cell bodies and ramified processes (Kreutzberg, 1996; Nakajima and Kohsaka, 2001; Shi *et al.*, 2010). Activated microglial cells then facilitate neuroprotection by removing debris, clearing dead cells and secreting neurotrophic factors. However, chronic recruitment and activation of microglia can also be maladaptive and contribute to impaired neuronal function (Nakajima and Kohsaka, 2001; Badoer, 2010; Thaler *et al.*, 2012)

There are extensive communications between microglia and neurons, facilitating the ability of microglia to sense disturbances to the nervous system, and to regulate the development, structure and function of neural connections (Kettenmann *et al.*, 2011). Gap junctions allow the bidirectional exchange of ions and small molecules between neurons and microglia (Eugenin *et al.*, 2001; Dobrenis *et al.*, 2005). Microglia make contact with synaptic structures (Wake *et al.*, 2009) and thereby have the capability to sense synaptic activity. Further, upon injury, microglia play a role in reactive synaptogenesis (Bessis *et al.*, 2007).

Microglia express toll-like receptors TLRs, which are receptors of the innate immune system that recognize molecules that are conserved among many pathogens. Importantly, a number of TLRs are known to recognize lipid-containing motifs and TLR-4 is well-known to recognize lipopolysaccharides (LPS). Microglia also express receptors for numerous chemokines, cytokines, neurotransmitters (e.g., glutamatergic, GABAergic and adrenergic) and neurohormones (e.g., angiotensin-II, glucocorticoids and mineralocorticoids) (reviewed by Kettenmann *et al.* (2011)), aiding their ability to sense disturbances to homeostasis. Additionally, various factors are released by microglia that either support the survival of remaining neurons or induce neuronal death. Of relevance, microglia are known to respond to and produce factors that influence cardiovascular and metabolic homeostasis (Shi *et al.*, 2010; Thaler and Schwartz, 2010; Thaler *et al.*, 2012; Yi *et al.*, 2012a; Yi *et al.*, 2012b).

Under normal conditions, microglia lie in close proximity to preautonomic PVN neurons (Figure 1), placing them in an ideal position to regulate sympathetic outflow. During experimental hypertension, brain regions important for regulating sympathetic outflow, such as the PVN and NTS, are infiltrated with additional microglia and the levels of proinflammatory factors and reactive oxygen species within these regions rise (Waki *et al.*, 2008b, a; Shi *et al.*, 2010; Zubcevic *et al.*, 2011; Cardinale *et al.*, 2012), and have the potential to influence PVN neuron activity. Several lines of evidence suggest that the RAS facilitates these interactions. Hypertension produced by chronic infusion of Ang-II is associated with AT1R-mediated increases in microglial activation and cytokine levels in the PVN (Shi *et al.*, 2010). Further, the central administration of minocycline, an anti-inflammatory antibiotic that reduces microglial activation, decreases Ang-II-induced hypertension (Shi *et al.*, 2010). This has led us to hypothesize that microglial activation in response to Ang-II stimulation results in an inflammatory response (increases in cytokine, chemokine, ROS production) that can, via a paracrine action, modulate the activity of associated PVN preautonomic neurons (Shi *et al.*, 2010). Our preliminary data, generated from microglia isolated from rat hypothalamus, support this idea and suggest that there are two components to the Ang-II induction of microglia in the PVN during hypertension. A direct effect of Ang-II at AT1R located on microglia to elicit their activation, and an indirect effect via neuronal AT1R to cause secretion of monocyte chemoattractant protein-1 (MCP-1; or CC-chemokine ligand-2 [CCL2]) which then binds to its receptors (CCR2) on microglia and stimulates their migration towards PVN preautonomic neurons. In addition, it has been shown that AT1R are expressed on microglia and angiotensin receptor blockers reduce LPS-induced inflammation *in vitro* (Miyoshi *et al.*, 2008). Conversely, the recent generation of a transgenic AT1R reporter mouse indicates that, under normotensive *in vivo* conditions, brain AT1R expression is almost exclusively neuronal (Gonzalez *et al.*, 2012). However, it is possible that during hypertension, the expression pattern of AT1R changes and that microglia begin to express the receptor. Then again, it is also possible that microglia sense neuronal AT1R stimulation, and that this triggers their activation in response to Ang-II.

Astrocytes—Astrocytes also participate in innate immune reactions within the nervous system. They express TLRs and produce proinflammatory factors that can act on cells within the brain, as well as anti-inflammatory cytokines and neurotrophic factors, such as CNTF, which promote the repair of the injured CNS (Van Der Voorn *et al.*, 1999; Panenka *et al.*, 2001; McKimmie and Graham, 2010; Allaman *et al.*, 2011; Gorina *et al.*, 2011; Kang *et al.*, 2012; Werry *et al.*, 2012). Astrocytes regulate neuronal function via several mechanisms including the release of glutamate, ATP and other signaling molecules. Astrocytes buffer CNS potassium, remove excess cytotoxic glutamate and modulate blood flow (Gurden *et al.*, 2006; Belanger *et al.*, 2011; Ransohoff and Brown, 2012). Astrocytes also express the GABA transporter 3, which removes GABA and thereby contributes to GABA tone in various neural circuits, including those that regulate sympathetic outflow and blood pressure (Park *et al.*, 2009). Moreover, astrocytes and microglia interact to regulate

one another's function and the function of neurons (Kloss *et al.*, 1997; Bezzi *et al.*, 2001a; Bezzi *et al.*, 2001b; Belanger *et al.*, 2011; Liu *et al.*, 2011). They secrete factors that modulate the activity of microglia and they are impacted by factors secreted by microglia (DeWitt *et al.*, 1998; Yang *et al.*, 1998; Bezzi *et al.*, 2001a). Importantly, astrocytes, like microglia, lie in close proximity to preautonomic PVN neurons (Figure 1) and are capable of sensing and responding to hypertensive and obesigenic stimuli (Buckman *et al.*, 2012; Gerber and Bale, 2012; Thaler *et al.*, 2012). Consequently, it is conceivable that astrocyte-neuron and/or astrocyte-microglia interactions contribute to blood pressure regulation.

Another mechanism by which astrocytes participate in the neuroimmune regulation of blood pressure depends on the fact that they represent a key cellular compartment for RAS actions within the brain. Angiotensinogen is primarily expressed within astrocytes (Stornetta *et al.*, 1988; Intebi *et al.*, 1990 & Deschepper, 1990; McKinley *et al.*, 2003; Sherrod *et al.*, 2005) and astrocyte AGT is critical for the integrity of the BBB (Kakinuma *et al.*, 1998). Astrocytes also express AT1R and Ang-II can elicit immune responses via their activation (Kandalam and Clark, 2010; Finsen and Owens, 2011; Fuchtbauer *et al.*, 2011). Furthermore, there is some evidence linking the protective effects of the ACE2-Ang-(1-7)-Mas axis's on cardiovascular function to astrocytes, as these cells likely mediate Ang-(1-7)'s cardiovascular regulatory actions in the RVLM (Guo *et al.*, 2010). On the other hand, although it is clear that AT1R are present on astrocytes and that activation of astrocytic AT1R induces inflammation, at present, the Ang-II-AT1R pathway within astrocytes has not been directly linked to the regulation of cardiovascular function.

4.4. Recruitment of Immune Cells to Specific Brain Nuclei

Many disturbances to homeostasis, including hypertension and obesity are associated with increased numbers of immune cells in distinct brain nuclei; however whether these cells are of central and/or peripheral origin is not always clear. Resident microglia may migrate within the brain and accumulate in damaged regions, while exogenous microglial progenitors, produced and mobilized from bone marrow, penetrate the brain and migrate to injured areas upon insult (Lawson *et al.*, 1992; Imai *et al.*, 1999; Malm *et al.*, 2005; Stalder *et al.*, 2005; Simard *et al.*, 2006; Imai *et al.*, 2007). Accumulating evidence suggests that the SNS regulates mobilization of microglial progenitors (Katayama *et al.*, 2006). To this end, interference with the SNS, via either the administration of a β -blocker or the disruption of catecholaminergic neurons, reduces the number of HSPCs mobilized by granulocyte colony-stimulating factor (Katayama *et al.*, 2006).

Once mobilized, resident microglia and microglial progenitors are directed to infiltrate specific injured brain areas, at least in part, by chemokine signaling. Chemokines and their receptors are vital for the recruitment of immune cells to specific sites of inflammation throughout the body and for development of inflammatory responses. For example, within the brain, MCP-1/CCL-2 stimulates its receptor, CCR2 to regulate the communication between neurons, astrocytes and microglia, thereby mediating the recruitment of both resident and peripheral immune cells to sites of injury within the CNS (Old and Malcangio, 2012). CCR2 is expressed on monocytes, and within the brain, predominantly on microglia. MCP-1/CCL2 is produced by glial cells (Glabinski *et al.*, 1996; Gourmala *et al.*, 1997; Van Der Voorn *et al.*, 1999) and is released from neuronal synaptic vesicles (Thacker *et al.*, 2009; Van Steenwinckel *et al.*, 2011) to mediate the recruitment of monocytes to injured brain tissue. Importantly, this system is critical for recruiting additional microglial progenitors to the brain parenchyma. Using green fluorescent protein transgenic bone marrow chimeric mice, Schilling and colleagues were able to differentiate between resident and infiltrating immune cells and determine that mice deficient in this pathway have reduced infiltrating immune cells, rather than resident microglia following transient cerebral ischemia (Schilling *et al.*, 2009a; Schilling *et al.*, 2009b; Schuette-Nuetgen *et al.*, 2012).

In addition to the recruitment of microglial progenitors to the CNS, peripheral immune cells are trafficked to the brain during certain pathologies, such as cerebral inflammatory diseases and peripheral organ inflammation (D'Mello *et al.*, 2009; Conductier *et al.*, 2010). For example, elevated peripheral TNF α signaling during hepatic inflammation enhances CCL2 production in microglia, leading to the recruitment of CCR2-expressing monocytes into the brain (D'Mello *et al.*, 2009).

Because of the critical role of MCP-1/CCL-2 in mobilization of immune cells in CNS disease states, it is possible that this system is important for immune activation and sympathoexcitation at the level of the PVN during hypertension. Under normal conditions, MCP-1/CCL2 is densely expressed in the PVN and other hypothalamic nuclei (Banisadr *et al.*, 2005). In addition, CCR2 is upregulated in the PVN during restraint stress, a challenge that causes elevations in blood pressure (Reyes *et al.*, 2003). These findings are consistent with a role for CCL2/CCR2 signaling in the recruitment of immune cells to the PVN during hypertension, and our preliminary data provide direct support for this idea. Specifically, we have demonstrated that central (intracerebroventricular) infusion of Ang II at a level that causes hypertension also increases MCP-1/CCL-2 expression in the PVN (Shi *et al.*, 2011). Furthermore, elevations in PVN MCP-1/CCL-2 expression are also observed in SHR (Shi *et al.*), animals that display enhanced Ang-II/AT1R signaling in the PVN. In summary, the data available thus far support the idea that a critical component of sympathoexcitation and neurogenic hypertension is activation of and recruitment of immune cells within and to the PVN, and MCP-1/CCL-2 may play a major role.

5. Roles of the sympathetic nervous, renin-angiotensin and immune systems in obesity-related hypertension

One important strategy to treat or prevent hypertension is to develop therapeutics that target predisposing conditions. In this regard, obesity is a principal risk factor for the development of hypertension and these ailments are very often co-morbid (Garrison *et al.*, 1987; Cassano *et al.*, 1990; Brown *et al.*, 2000; Timpson *et al.*, 2009; Weiss *et al.*, 2009; Armitage *et al.*, 2012). Importantly, both pathologies are independently associated with activation of the SNS and RAS (Grassi *et al.*, 1995; Alonso-Galicia *et al.*, 1996; Vaz *et al.*, 1997; Cooper *et al.*, 1998; Rumantir *et al.*, 1999; Grassi *et al.*, 2003; de Kloet *et al.*, 2010; Armitage *et al.*, 2012) and when obese individuals reduce sympathetic drive or RAS activity either pharmacologically or by losing weight, their blood pressure is decreased (Grassi *et al.*, 1998; Wofford *et al.*, 2001; Straznicky *et al.*, 2005). Obesity modulates the neural circuitry that controls sympathetic outflow and blood pressure and several mechanisms for the enhanced sympathetic drive during obesity have been proposed (Haynes, 2005; Stocker *et al.*, 2006; Purkayastha *et al.*, 2011; Hilzendeger *et al.*, 2012; Yiannikouris *et al.*, 2012a). Of importance, many of the factors that are thought to contribute to increased blood pressure during obesity are also associated with increased recruitment and activation of immune cells within the CNS.

It is widely-accepted that diet-induced and genetic models of obesity are accompanied by elevated inflammation in peripheral tissues and the brain (Xu *et al.*, 2003; Shoelson *et al.*, 2006; Cani *et al.*, 2007; Shoelson and Goldfine, 2009; Thaler and Schwartz, 2010; Mathis and Shoelson, 2011; Thaler *et al.*, 2012). In regards to the brain, high-fat diet consumption increases the expression of microglial and astrocytic markers within several brain regions (Pistell *et al.*, 2010). In the mediobasal hypothalamus (including the ARC and median eminence), high-fat feeding elevates inflammation, microglial activation and astrogliosis in as little as 1-3 days (Thaler *et al.*, 2012) and this inflammatory milieu persists after long-term high-fat diet feeding (Thaler *et al.*, 2012), implying that perhaps just one meal of high-fat diet or long-term high-fat diet feeding can have a deleterious impact on the CNS.

The enhanced inflammation within mediobasal hypothalamus is then thought to contribute to alterations in metabolism (Zhang *et al.*, 2008), glucose homeostasis (Posey *et al.*, 2009; Milanski *et al.*, 2012), and, more recently, to cardiovascular regulation (Purkayastha *et al.*, 2011). Consistent with this, Purkayastha *et al.* determined that hypothalamic I κ κ - β and NF κ B represents a key mechanism linking the consumption of high-fat diet to the dysregulation of energy, glucose and cardiovascular homeostasis. Of relevance, adenoviral activation of NF κ B within the mediobasal hypothalamus leads to elevated blood pressure, while its inactivation prevents obesity-associated increases in blood pressure. Further, inflammation specifically within proopiomelanocortin neurons, which are particularly important for the regulation of energy balance, may contribute to blood pressure regulation, as TNF α causes increases in blood pressure and I κ κ - β phosphorylation in proopiomelanocortin neurons, while I κ κ - β deletion in proopiomelanocortin neurons prevents TNF α and obesity-induced increases in blood pressure.

An unanswered question, however, is whether or not high-fat diet consumption leads to inflammation in other cardiovascular control regions of the brain, thereby contributing to obesity-related hypertension. There is some recent evidence that genetic and diet-induced obesity in mice leads to astrogliosis within the PVN (Buckman *et al.*, 2012). Moreover, our preliminary studies indicate that like chronic Ang-II administration, high-fat diet causes inflammation within the PVN.

Obesity is associated with adipose tissue dysfunction, characterized by hypertrophied adipocytes, increased adipose inflammation, infiltration of macrophages and by altered expression and secretion of various adiposity factors, collectively termed 'adipokines,' that may then act in the brain to control SNS activity. Of particular interest for this review, Ang-II can be considered an adipokine as it is produced by adipose tissue and its levels are positively-correlated with body mass in both humans and rodents (de Kloet *et al.*, 2010). Considering that Ang-II is a potent activator of the SNS, one potential mechanism for the augmented sympathetic drive during obesity is this increased level of Ang-II. AT1R blockade reduces SNS activity in obese hypertensive humans (Grassi *et al.*, 2003) and genetically obese rats have a greater magnitude in the decrease in blood pressure that follows AT1R blockade (Alonso-Galicia *et al.*, 1996). Overexpression of AGT specifically within adipose tissue leads to elevated blood pressure (Massiéra *et al.*, 2001; Kalupahana *et al.*, 2011), while adipose tissue-specific deletion of AGT prevents obesity-related hypertension (Yiannikouris *et al.*, 2012a).

Based on these findings and the observation that Ang-II induces hypothalamic inflammation in hypertensive models, it is intriguing to hypothesize that this peptide contributes to recruitment and activation of immune cells within the CNS during obesity which then facilitates sympathetic outflow and leads to obesity-related hypertension. Although several indirect lines of evidence suggest this may be the case, this hypothesis has not directly been tested. Moreover, this phenomenon is likely complex and several mechanisms contributing to obesity-related neuroimmune communication in hypertension have been proposed. For example, it is well known that other adipokines such as leptin and insulin can impact the SNS and increase immune factors in specific brain regions. Leptin acts at its receptors in cardiovascular control centers of the brain to increase sympathetic outflow to several tissues (Haynes *et al.*, 1999; Haynes, 2005; Mark *et al.*, 2009), to impair baroreflex control of heart rate (Arnold *et al.*, 2009) and to increase blood pressure Correia *et al.* (2001). Importantly, although a key mechanism for obesity is resistance to the appetite suppressing effects of leptin, the sympathoexcitatory effects of leptin are preserved during obesity (Mark *et al.*, 1999) and it is possible that leptin is a critical mediator of obesity-related hypertension. It is also apparent that, similar to Ang-II, leptin activates its receptor not only on neurons, but also on microglia and astrocytes, and it is possible that leptin's actions at these cell-types

may contribute to its sympathoexcitatory effects (Pinteaux *et al.*, 2007; Tang *et al.*, 2007; Lafrance *et al.*, 2010). Lastly, there are substantial interactions between adiposity factors, such as insulin and leptin, and Ang-II, suggestive of additional avenues by which the RAS can influence blood pressure during obesity (Cassis *et al.*, 2001; Hilzendege *et al.*, 2012).

6. A model for immune/central nervous system interactions during hypertension

Based upon the compelling evidence from the reviewed literature, it is tempting to speculate that an immune/central nervous system reciprocal interaction is critical for the regulation of cardiovascular and metabolic function. Hypertensive stimuli, such as obesity and chronic stress, cause increases in circulating factors, a key factor being Ang-II. These factors are then sensed by CVOs such as the SFO which then transmit these signals to the preautonomic region of PVN. This pathway then directly increases sympathetic outflow and blood pressure by stimulating spinally and RVLM-projecting neurons. During neurogenic hypertension, it is well known that there is also an enhancement of the direct neuronal actions of Ang-II via AT1R in the PVN, resulting in an over-stimulation of preautonomic neurons. Although the precise mechanism by which Ang-II becomes present in the brain is a subject of debate, there is substantial evidence that this peptide is synthesized by the brain-specific RAS (Lenkei *et al.*, 1997; Morimoto *et al.*, 2002; McKinley *et al.*, 2003; Grobe *et al.*, 2008; Cuadra *et al.*, 2010; Grobe *et al.*, 2010; de Kloet *et al.*, 2011; Yamazato *et al.*, 2011). Here we are proposing that the enhanced actions of Ang-II in neurogenic hypertension involve direct effects at microglia and possibly astroglia and sustained induction of both the central and peripheral immune systems. In terms of microglia, we suggest that Ang-II directly activates these cells, and indirectly (via MCP-1/CCL-2) released from neurons) causes their migration towards preautonomic neurons (Figure 2). It is possible that a similar scenario occurs with astroglia. This proinflammatory microenvironment within the PVN then stimulates the brain to signal via the SNS to mobilize the peripheral immune system and to the bone marrow to mobilize microglial progenitors that are recruited to the PVN, likely via a CCL2/CCR2-dependent mechanism. This increase in microglial progenitors within the PVN then contributes to the population of innate immune cells within the PVN, then feeding-forward to enhance and sustain elevations in blood pressure by further influencing the activity of preautonomic PVN neurons. The mechanism(s) by which microglia interact with preautonomic neurons to influence their activity has not yet been elucidated and is therefore an important avenue for future studies.

We also propose that hypertensive and obesigenic stimuli threaten the integrity of the BBB in the key cardiovascular and metabolic control regions, the PVN and ARC and that this is sensed by the innate immune cells of the brain, further exacerbating their responses, and contributing to the microglial activation and astrogliosis within these brain regions. It is possible that this astrogliosis and microglial activation initially occurs in attempt to protect the neurons from peripheral factors, but ends up being detrimental.

It is clear that during many conditions that pre-dispose to hypertension there are elevated levels of the peripheral RAS activity that is sensed by the brain. Although the focus here is on Ang-II, other factors, such as leptin, proinflammatory cytokines and fatty acids may also activate this pathway during obesity to exacerbate cardiovascular dysfunction. We believe that these collective neuroimmune interactions facilitate the progression of neurogenic hypertension.

7. Consideration of RAS/Neuroimmune System interactions as novel therapeutic targets in hypertension

Importantly, the reviewed studies reveal a promising new avenue for the development of therapeutics for patients with neurogenic hypertension. As discussed throughout this review, chronic immune activation, increases in SNS activity and enhanced RAS activity are common features of hypertension and reciprocal communication between these systems likely contributes to increased blood pressure. Therapeutics that target the RAS or SNS are already widely-utilized to reduce blood pressure in hypertensive patients. The concept that has only recently been appreciated is that the connection between the RAS/SNS and the immune system may serve as another opportunity for the development of anti-hypertensive therapeutics. In this regard, it has been hypothesized that AT1R blockade may have utility for the treatment of inflammatory brain disorders (Benicky *et al.*, 2009; Benicky *et al.*, 2011). Furthermore, numerous clinically-approved pharmacological therapeutics that target inflammatory cascades and are currently prescribed for other pathologies, may perhaps also be exploited to treat hypertension. For example, Etanercept, which inhibits TNF α actions, is widely utilized to treat autoimmune disorders and reduces blood pressure in rodent studies (Tran *et al.*, 2009; Venegas-Pont *et al.*, 2010)

Additional support for the probable therapeutic utility of targeting this pathway is reflected by the fact that this model may not only underlie hypertension, but it may also causally link hypertension to its pre-disposing conditions. While there are several pre-disposing conditions that may activate the proposed pathway, in this review, particular emphasis was placed on the applicability of the proposed model to obesity-related hypertension. Therefore, when evaluating the therapeutic potential of targeting RAS/neuroimmune system interactions during obesity-related hypertension, it is also important to consider, that the SNS, RAS and immune system are also involved in other related pathologies, such as obesity itself and diabetes (Hotamisligil, 2003, 2006; de Kloet *et al.*, 2010; Thaler and Schwartz, 2010). Developing a clear understanding of the overlap and distinction between the roles of the systems in the regulation of energy balance and glucose homeostasis versus their roles in the regulation of cardiovascular function may, therefore, also open avenues for the development of novel therapeutics to treat or prevent several aspects of the metabolic syndrome.

Collectively, the reviewed studies provide support for a key RAS/neuroimmune interactions in the regulation of blood pressure are reveal a promising new avenue for the development of therapeutics for patients with neurogenic hypertension.

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Abbreviations

ACE	angiotensin-converting enzyme
ACE2	angiotensin-converting enzyme 2
AGT	angiotensinogen
Ang-II	angiotensin-II
Ang-(1-7)	angiotensin (1-7)
AT1R	angiotensin type-1 receptor
AT2R	angiotensin type-2 receptor
ARC	arcuate nucleus of the hypothalamus
BBB	blood-brain barrier
CCL2	C-C chemokine ligand type-2
CCR2	C-C chemokine receptor type-2
CVO	circumventricular organ
GFAP	glial fibrillary acidic protein
IL-10	interleukin-10
IL-1β	interleukin-1 β
IL-6	interleukin-6
IML	intermediolateral cell column
Iba-1	ionized calcium binding adaptor molecule 1
MIF	macrophage migration inhibitory factor
MCP-1	monocyte chemoattractant protein-1
NTS	nucleus of the solitary tract
PVN	paraventricular nucleus of the hypothalamus
PRR	pro-renin receptor
RAS	renin-angiotensin system
RVLM	rostral ventral lateral medulla
SFO	subfornical organ
SHR	spontaneously hypertensive rat
SNS	sympathetic nervous system
TPOR	thiol-protein oxidoreductase
TNFα	tumor necrosis factor- α

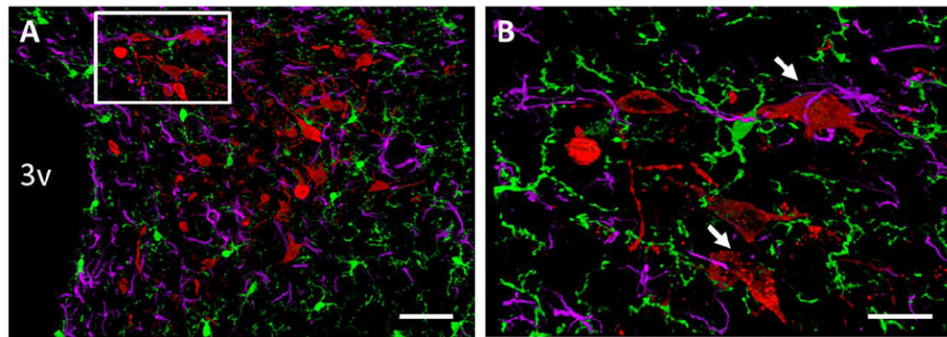


Figure 1. Interactions between microglia, astrocytes and preautonomic RVLM-projecting PVN neurons

(A) 20× and (B) 63× images of microglia (green), astrocytes (purple) and preautonomic RVLM-projecting neurons within an adult mouse PVN. Adult mice were injected bilaterally into the RVLM with the retrograde tract tracer, fluorogold. Seven days later mice were perfused and brains were processed for triple-label immunohistochemistry for Iba-1 (a microglial marker), GFAP (an astrocytic marker), and fluorogold (preautonomic neurons) using previously published protocols (Ulrich-Lai *et al.*, 2011; Thaler *et al.*, 2012). 3v = third cerebral ventricle. Arrows highlight interactions between microglia, astrocytes and RVLM-projecting neurons. Scale bars = 50 μm (A) and 20 μm (B).

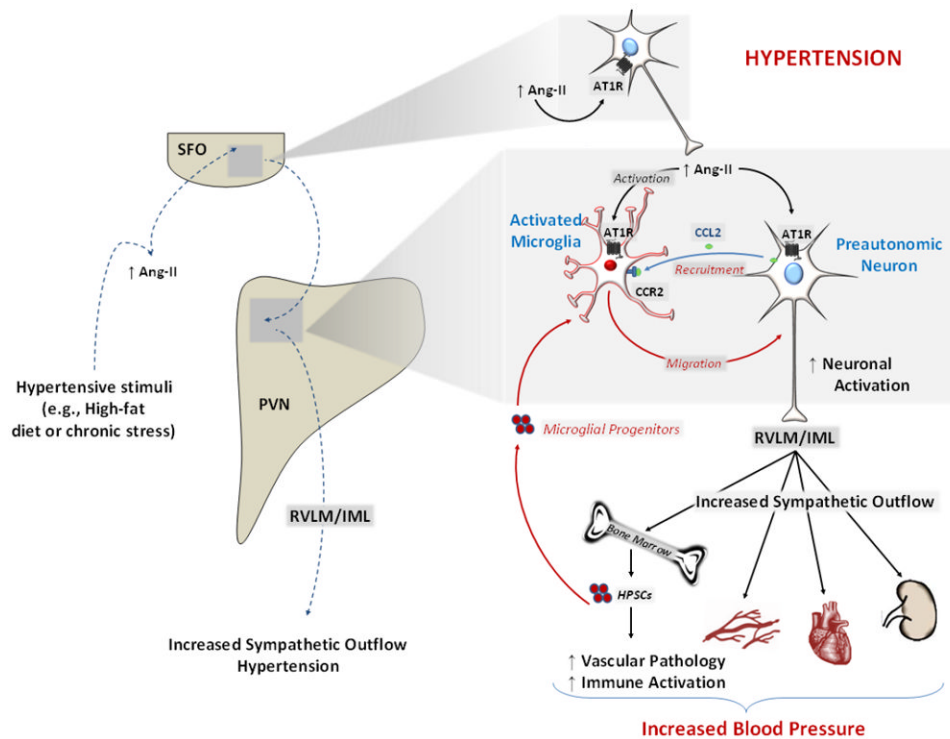


Figure 2. Proposed model for immune/central nervous system interactions during hypertension
 Hypertensive stimuli (e.g., obesity and chronic stress), cause increases in circulating factors (e.g., Ang-II) that are sensed by CVOs, such as the SFO. The SFO then transmits these signals to the preautonomic region of PVN, leading to stimulation of intermediolateral cell column (IML) and rostralventrolateral medulla (RVLM)-projecting neurons and resulting in increased sympathetic outflow and blood pressure. Furthermore, during neurogenic hypertension, the enhanced direct neuronal actions of Ang-II at AT1R in the PVN leads to an over-stimulation of preautonomic neurons. Direct effects of Ang-II at microglia leads to sustained induction of both the central and peripheral immune systems. We propose that initially Ang-II directly activates microglia, and indirectly (via MCP-1/CCL-2 released from neurons) causes their migration towards preautonomic neurons. This proinflammatory microenvironment within the PVN then stimulates the brain to signal via the SNS to mobilize the peripheral immune system and to the bone marrow to mobilize microglial progenitors that are recruited to the PVN, likely via a CCL2/CCR2-dependent mechanism. This increase in microglial progenitors within the PVN then contributes to the population of innate immune cells within this nucleus, feeding-forward to activate preautonomic PVN neurons thereby augmenting and sustaining the elevations in blood pressure.