Role of neurons and glia in the CNS actions of the renin-angiotensin system in cardiovascular control

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DISEASES OF THE CARDIOVASCULAR system are the predominant causes of mortality in the United States (128), and it is imperative that new and innovative strategies to combat these pathologies are developed. This can only be achieved with a thorough understanding of the systems that regulate cardiovascular control under normal and pathological conditions. The central nervous system (CNS) is crucial to maintaining cardiovascular homeostasis. Dysregulation of the neural systems controlling cardiovascular function can lead to chronic sympathetic nervous system activation and so-called “neurogenic hypertension”; a key contributor to the development of cardiovascular disease. It is well established that in many instances, the brain plays an important role in the onset and progression of hypertension via activation of the sympathetic nervous system. Further, the activity of the renin-angiotensin system (RAS) and of glial cell-mediated proinflammatory processes have independently been linked to this neural control and are, as a consequence, both attractive targets for the development of antihypertensive therapeutics. Although it is clear that the predominant effector peptide of the RAS, ANG II, activates its type-1 receptor on neurons to mediate some of its hypertensive actions, additional nuances of this brain RAS control of blood pressure are constantly being uncovered. One of these complexities is that the RAS is now thought to impact cardiovascular control, in part, via facilitating a glial cell-dependent proinflammatory milieu within cardiovascular control centers. Another complexity is that the newly characterized antihypertensive limbs of the RAS are now recognized to, in many cases, antagonize the prohypertensive ANG II type 1 receptor (AT1R)-mediated effects. That being said, the mechanism by which the RAS, glia, and neurons interact to regulate blood pressure is an active area of ongoing research. Here, we review the current understanding of these interactions and present a hypothetical model of how these exchanges may ultimately regulate cardiovascular function.

neurogenic hypertension; astrocyte; microglia; renin; angiotensin II

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Here, we review the role of neural and glial cells in the brain regulation of cardiovascular function by the RAS. To accomplish this, we first provide a brief overview of what is known regarding the localization of the RAS components to specific cell types within the brain. We then review the well-characterized impact of ANG II activation of neuronal AT1R in the brain regulation of cardiovascular function. Next, the role of communication between neurons and glia in the control of cardiovascular function is discussed, as is the impact that ANG II has on these interactions. The complementary influence of PRR signaling and the counterregulatory impact of other components of the RAS [e.g., angiotensin-converting enzyme 2, and angiotensin type 2 receptors (AT2R)] on these processes are also considered. Lastly, we present a hypothetical model of how the RAS may impact neuronal-glial interactions during cardiovascular pathology and how elucidating these interactions may lead to the development of novel antihypertensive therapeutics.

**Cellular Localization of the Renin-Angiotensin System Components Within the Brain**

Countless efforts have been made to provide a thorough characterization of the cellular localization of components of the RAS within the brain. These efforts have been somewhat complicated by the unreliability of angiotensin receptor antibodies (9, 73, 79) and the poor cellular resolution of angiotensin receptor autoradiography techniques. That being said, decades of research have led to a tremendous literature base that collectively localizes all of the integral components of the RAS to the various cell populations within the brain (36, 37, 42, 62, 83, 86, 105, 123, 151, 157, 180).

**Localization of components for ANG II synthesis.** The inability of circulating ANG II to effectively cross the blood-brain barrier (BBB) under normal, healthy conditions coupled with the dense localization of ANG II receptors to brain structures that are protected by the BBB has led to the prediction that ANG II can be synthesized within the brain to act in an autocrine or paracrine fashion at its CNS receptors. There are studies that have suggested that the production of ANG II occurs within the neurons themselves and that ANG II is generated intracellularly and on demand to act as a peptide neurotransmitter within neural circuits that regulate cardiovascular function (7, 68, 112). However, additional intricacies involved in CNS ANG II synthesis may be inferred by the localization of the components required for its production to multiple cell types within the brain, rather than exclusively to one cellular phenotype.

The ANG II precursor, angiotensinogen, is densely expressed within the CNS, and, in particular, within astrocytes (85, 151, 162, 180). Renin, which converts angiotensinogen to ANG I, is also localized to neurons and astrocytes; however, the low expression of this enzyme within the CNS has led to reservations in regard to its functional significance (83, 86, 87, 114, 142). On the other hand, the PRR, which binds (pro)renin, thereby sequestering and rendering it active to convert angiotensinogen to ANG I, is highly expressed in the brain, perhaps providing a mechanism by which ANG II may be synthesized within the CNS (37, 131, 132). Along these lines, this receptor is thought to be involved in the autocrine/paracrine actions of the RAS within several specific tissues, including the brain (37, 114, 164) and are also localized to microglia (169) and astrocytes (152). An additional mechanism by which ANG II may be generated within the brain, despite the low levels of renin observed within this tissue, involves another peptide derived from angiotensinogen, proangiotsensin-12, or ANG 1–12 (129). This peptide is also present in the brain and may allow for ANG II generation independent of renin (129).

Angiotensin-converting enzyme (ACE) is the next step in the generation of ANG II, and this enzyme is also present in the brain (36, 62, 157), with a particular abundance in the choroid plexus and in the capillary endothelial cells (5, 23) and also to a lesser extent in neurons within a few regions that are involved in the neural regulation of blood pressure, such as the subfornical organ (SFO) and paraventricular nuclei of the hypothalamus (PVN) (156, 182). Collectively, the localization of the RAS components required for the synthesis of ANG II within diverse cell types of the brain implies that a network of neuronal-glial interactions may be required for the synthesis of ANG II within the brain. Once ANG II is synthesized within the brain, it likely activates its AT1R and AT2R in BBB-protected nuclei to influence cardiovascular function and hydromineral balance.

**Cellular localization of receptors mediating ANG II action.** ANG II binds to either the AT1R or the AT2R to exert its physiological actions, and these receptors are found within the CNS (42, 67, 77, 92–94, 105, 124, 195). Whereas humans contain only AT1R and AT2R, the AT1 subtype within rodents can be further subdivided into the angiotensin type 1a receptor (AT1aR) and type 1b receptor (AT1bR), whose genes are present on separate chromosomes but are highly homologous (119, 160). Within the brain of rodents, AT1R is primarily present in its AT1R form; however, in some cases, AT1bR has been detected in the brain, and it is also abundant in the anterior pituitary and adrenal cortex (27, 95, 105).

In rodents, AT1R (primarily AT1aR) are abundant in regions that are traditionally known to regulate blood pressure and hydromineral balance, including the PVN and SFO (105), while AT2R are densely expressed in a number limbic and thalamic regions that indirectly regulate cardiovascular function, as well as some regions that play a more direct role in cardiovascular homeostasis, such as the nucleus of the solitary tract (NTS) (42, 77, 92, 93). Despite the seeming consensus regarding the localization of these receptors to specific brain nuclei, the cellular localization of the angiotensin receptors within the brain has been a matter of debate. In contrast to immunohistochemistry (IHC) and in vitro studies that have localized AT1R and AT2R to non-neuronal cells in the brain (108, 127, 207), genetic mouse models and advances in in situ hybridization provide evidence that, at least under normal conditions, these receptor subtypes are predominantly neuronal and are absent from glia (33, 42, 67). In support of a predominantly neuronal localization of AT1aR within a cardiovascular control center, Fig. 1 depicts dual in situ hybridization and immunohistochemistry studies localizing this receptor to HuC/D-positive neurons in normotensive rat PVN, but not to Iba1-positive microglia or GFAP-positive astrocytes. As can be observed in this figure, although microglia and astrocytes...
Fig. 1. AT1R localizes to neurons but not astrocytes and microglia in the paraventricular nucleus (PVN). Representative images through the PVN of normotensive Sprague-Dawley rats depicting AT1aR mRNA as punctate green dots (in situ hybridization via RNAscope) combined with dual-label immunohistochemistry for the neuronal marker HuC/D, shown in red, and either the microglial marker Iba-1 (A–C) or the astrocytic marker GFAP (D–F), both shown in magenta. Combined in situ hybridization and immunohistochemistry were conducted as described in detail in de Kloet et al. (42). Scale = 10 μm.

themselves lack AT1aR expression, they are situated in close proximity to AT1aR-containing neurons. These cells are, therefore, positioned to interact intimately with one another.

Importantly these highly specific and sensitive in situ hybridization and genetic techniques for the localization of ANG II receptors have not yet been extended to models of cardiovascular pathophysiology, in which it is possible that AT1R and/or AT2R may become expressed on the non-neuronal cell types of the brain. However, it is probable that these technological advancements may be utilized in future studies to definitively ascertain the impact of cardiovascular pathology on the cellular localization of ANG II receptors in the brain. Along these lines, there are a number of previous in vitro and some in vivo studies that have suggested a role for angiotensin receptors on non-neuronal brain cells in the development and progression of hypertension, and these studies will be discussed in subsequent sections.

**Cellular localization of the ANG 1–7 receptor, Mas.** Other nontraditional components of the RAS, such as the ANG 1–7 receptor, Mas, are thought to counter-regulate ANG II’s actions at its type-1 receptor and are detected within the brain (75, 91, 212). Like the AT1R and AT2R, the Mas is a G protein-coupled receptor that is found in various areas of the brain, including the hippocampus, amygdala, forebrain, piriform cortex, olfactory bulb, thalamus, and portions of the hypothalamus (25, 60, 125, 212). Although these previous studies revealed that the Mas was primarily localized to neurons within these regions (60), others have observed Mas localization to glial cells as well (150), implying that this axis may directly and/or indirectly impact numerous cell types in the brain to ultimately influence physiology.

**Renin-Angiotensin System Actions Mediated via Neurons to Regulate Cardiovascular Function**

Because of the overwhelming acceptance that receptors for RAS-mediated actions are localized to neurons, much research has focused on the effects of their activation on the neuronal regulation of cardiovascular function, and these neuronal actions have been extensively reviewed elsewhere (54, 58, 135, 141) and will, therefore, only briefly be discussed here. Importantly, and as described above, the prohypertensive AT1R and PRR and the antihypertensive AT2R and Mas are localized to neurons within brain regions both directly and indirectly involved in cardiovascular function. These regions include those that are protected by the BBB (e.g., the NTS), as well as those that are termed circumventricular organs (CVOs), which serve as an interface between the systemic circulation and the brain.

The endocrine RAS is traditionally known to impact the neural control of cardiovascular function via the activation of AT1R in CVOs, such as the SFO (54–56, 101, 136). These CVO neurons then, in turn, send projections to and influence the activity of numerous cardiovascular control centers that are generally protected by the BBB, including the PVN (55, 101). Upon activation of these CVO projections, the release of traditional neurotransmitters (e.g., glutamate) from their nerve terminals and onto neurons situated in downstream cardiovascular control nuclei is modulated, and sympathetic outflow and blood pressure become elevated (161, 190). That being said, many of these downstream brain nuclei also densely express AT1R. As discussed in detail in Localization of components for ANG II synthesis, it is recognized that the RAS acts as an autocrine or paracrine system within a number of tissues, including the brain (37, 105, 123), where it has been speculated that brain-derived ANG II serves as a neurotransmitter within circuits that regulate cardiovascular function (7, 112). Although, as described in detail above, the mechanism by which ANG II would be synthesized within the brain and then released from nerve terminals is a topic of ongoing research, the putative ANG II neurotransmitter action is an intriguing method by which BBB-protected AT1R may impact physiology, and it likely involves coordination among numerous cell types in the brain.

Another mode by which ANG II has been proposed to activate AT1R on neurons within cardiovascular control centers that are normally protected by the BBB is via passive transport of circulating ANG II across a compromised (leaky) BBB. To this point, there are various pathological conditions that are accompanied by increased BBB permeability (16, 78, 140).
122, 126), and it is possible that regions that are highly vascularized, such as the PVN (174, 186), are particularly susceptible to such a mechanism by which circulating ANG II can access CNS receptors. In support of this notion, hypertension causes an ANG II-dependent increase in BBB permeability that then leads to the facilitated access of endocrine ANG II to brain regions typically considered to be protected from circulating factors (16). The interpretation is that there exists an ANG II-contingent feed-forward mechanism potentiating hypertensive actions by facilitating the access of ANG II to cardiovascular control centers of the brain.

In any event, regardless of the origin of ANG II within the brain, once levels of this peptide become elevated within cardiovascular control centers and neuronal AT1R are activated, the outcome is in many instances an increase in sympathetic outflow and blood pressure. As a consequence, neuronal ANG II/AT1R actions are by and large thought to facilitate neurogenic hypertension; granted, there is also evidence for ANG II/AT1R-dependent antihypertensive mechanisms within distinct brain nuclei such as the NTS (165) and the caudal ventrolateral medulla (3). Further, many currently prescribed ANG II receptor blockers cross the blood-brain barrier and are thought to exert some of their antihypertensive actions via the blockade of AT1R within the brain (18).

In regard to the neural circuitry of RAS-mediated cardiovascular actions, the PVN is one brain region containing AT1Rs that are thought to facilitate neurogenic hypertension. Neurons of the PVN can be divided into a number of functionally distinct subgroups based on their anatomical location and neurochemical phenotype. Parvocellular PVN neurons, many of which express AT1R (105), are classified as neurosecretory or preautonomic. The neurosecretory neurons project to the median eminence and, upon stimulation, release factors such as corticotropin-releasing hormone and thyrotropin-releasing hormone, which are integral components of the hypothalamic-pituitary-adrenal axis and the hypothalamic-pituitary-thyroid axis, respectively. Preautonomic neurons influence cardiovascular function by controlling sympathetic nervous system activity via projections to the hindbrain [i.e., rostral ventrolateral medulla (RVLM)] and spinal cord (31, 35, 196). Magnocellular PVN neurons express vasopressin (AVP) or oxytocin (187), and their activation results in the release of AVP and oxytocin from the posterior pituitary and into the circulation. Although the specific predominating roles for the subgroups of neurons within the PVN are established, there is some overlap and crosstalk between the subtypes of neurons of the PVN. For example, preautonomic neurons can also express markers that are specific to the other subregions of the PVN, such as AVP. As a consequence, AVP neurons in the PVN may impact blood pressure via effects on sympathetic outflow, as well as their effects on AVP secretion. Moreover, there is a growing body of evidence that indicates that various cell types of the PVN communicate with one another via the dendritic release of neuropeptides (51, 117, 172, 178). This phenomenon allows for the effective integration and coordination of responses that are mediated by the PVN, and this area is reviewed in detail elsewhere (117, 178).

In regard to ANG II’s actions at preautonomic neurons of the PVN, two potential mechanisms have been proposed for the neuronal AT1R-mediated excitation of these neurons and consequent increases blood pressure: 1) ANG II stimulation of AT1R on RVLM-projecting PVN neurons to cause their depolarization (31) and 2) presynaptic disinhibition of preautonomic neurons via the activation of AT1R on GABAergic nerve terminals that contact them (106, 107). Although, there is some evidence to support both of these mechanisms, the above-mentioned impediments to definitively localizing AT1R to specific neuronal phenotypes have cast uncertainty upon these propositions, as it is not entirely clear from anatomical studies that the AT1R are localized to the correct neuronal phenotypes to substantiate the involvement of these mechanisms. Further, it is also unclear as to whether these ANG II actions are direct neuronal effects or are mediated via actions of ANG II at other cell elements. That said, whether one or both of the above-described mechanisms is, indeed, at play can perhaps be definitively ascertained by the utilization of advanced genetic techniques, such as optogenetics and AT1R reporter mice. Regardless, the end result of either of these proposed mechanisms of ANG II actions within the PVN would be an excitation of the preautonomic PVN neurons and subsequent increase in blood pressure via projections onto neurons of the RVLM and the intermediolateral cell column of the spinal cord, and the available literature is supportive of an excitatory role for AT1R activation on preautonomic neurons (32, 35, 148, 163, 185).

Another important point is that the central RAS has been implicated in the regulation of the magnocellular AVP system, which likely also contributes to ANG II’s hypertensive effects (115, 199, 213). Elevations in brain ANG II (or ANG III) lead to activation of AVP neurons causing a rise in systemic AVP levels that can be attenuated by administration of AT1R blockers either via the intracerebroventricular route or specifically within magnocellular neuron-containing areas of the brain (199, 213, 214). Further, several recent studies have indicated that AVP may play an important role in RAS-dependent hypertension. For example, the hypertension observed in mice with transgenic activation of the brain RAS is AVP-dependent (115). On the basis of data from BAC transgenic reporter mice, it is unlikely that magnocellular neurons of the PVN (or supraoptic nucleus) contain AT1R or AT2R (42, 67), and the impact of ANG II on AVP secretion may be mediated by ANG II receptor-positive neural connections arising from upstream brain nuclei, such as the organum vasculosum of the lamina terminalis or SFO (57, 193). Alternatively, it may also involve non-neuronal cell types in the brain, and this notion will be discussed in the subsequent sections. While these stimulatory effects of ANG II on AVP cell secretion are likely mediated by the AT1R (34, 199, 213), several lines of evidence support the notion that activation of AT2R acts in opposition to these hypertensive and AVP secretion stimulatory effects. For example, whole body genetic deletion or pharmacological blockade of AT2R (using PD123,219) in mice leads to increased pressor responses to centrally administered ANG II. These experimental manipulations also augment ANG II-induced AVP release, reflected by an increase of plasma AVP levels (80, 113) and decreased pituitary AVP content (118). That being said, the therapeutic utility of AT2R agonists has only recently begun to be evaluated in this regard.

The projections from the preautonomic portion of the PVN to the hindbrain and spinal cord are generally glutamatergic, and the release of glutamate from their synapses onto RVLM neurons and preganglionic neurons in the intermediolateral cell
column of the spinal cord leads to increased sympathetic outflow to cardiovascular tissues and a rise in blood pressure (32, 43, 48, 120, 148, 163, 185). Furthermore, the RVLM contributes to the regulation of baroresponsive sympathetic efferents (38), and hyperactivity of RVLM-baroresponsive neurons controlling renal sympathetic nerve activity exerts similar pressor effects. AT1Rs are also localized to the RVLM (2), as well as other hindbrain cardiovascular control brain nuclei, such as the NTS (another important integrative site for many homeostatic systems). Activation of AT1R within these hindbrain regions has been thought to contribute to neurogenic hypertension, via mechanisms that involve abnormalities in the receipt, processing, or integration of information concerning sympathetic afferents, arterial baroreceptors, chemoreceptors, and volume receptors (1, 140, 145, 211).

There is evidence that the PRR is localized to neurons and that its activation influences cardiovascular homeostasis, ultimately contributing to neurogenic hypertension (110, 111, 164). Administration of (pro)renin into the brain of mice increases blood pressure, an effect that is reversed by the deletion of the PRR specifically from neurons (111). Further, neuronal PRR deletion prevents DOCA-salt hypertension in mice (111). Again, the implication of these previous studies is that neuronal PRR are critical for the development of hypertension. On the other hand, much like AT1R activation, PRR can also have diverse effects on blood pressure, depending on the brain nuclei in which PRR becomes activated. In other words, PRR stimulation can be pressor or depressor, depending on the specific population of neuronal PRR that are impacted. For example, PRR deletion in all neurons and in specific forebrain nuclei (e.g., supraoptic nucleus) is anti-hypertensive, suggesting that the PRR within the entire brain and within these specific areas is pressor (110, 111, 164). On the other hand, activation of this receptor specifically within the NTS has also been associated with an antihypertensive response that is nuclear factor-κB (NF-κB)-dependent, but AT1R-independent (215). Additionally, there is also evidence for (pro)renin/PRR actions within non-neuronal cells of the brain, which will be discussed in subsequent sections.

It has been suggested that AT1R activation can be opposed by stimulation of AT2R (20, 82, 177) and by the ACE2-(ANG 1–7)-Mas axis (44, 47, 50, 98, 158). At least under normal, nonhypertensive conditions, AT2Rs within or adjacent to cardiovascular control nuclei, are exclusively neuronal (42), and Mas have similarly been localized, in large part to neurons (60). As would then be expected, these putative cardioprotective limbs of the RAS directly impact the neuronal control of cardiovascular function. Consistent with this, AT2R activation via the administration of the selective agonist Compound 21 or Mas activation via the administration of ANG 1–7 within the brain reduces blood pressure (20, 22, 64, 69, 208). Conversely, blockade of either AT1R or Mas specifically within the brain via the delivery of the AT2R antagonist (PD123,319) or the Mas antagonist (A-779) elevates blood pressure, as well as sympathetic nerve activity to specific tissues (63, 64, 137). Furthermore, additional studies have extended these findings by determining specific brain nuclei that are involved in the antihypertensive actions of the Mas and AT2R. For example, overexpression of ACE2, an enzyme required for ANG 1–7 formation, within the NTS (209) or PVN (176) leads to similar antihypertensive responses. In regard to AT2R, its antihypertensive actions have been postulated to be mediated by the RVLM (65) and the NTS (17), among other brain areas containing AT2R (42). Despite these lines of evidence, suggesting that AT2R and Mas-mediated mechanisms are antihypertensive, there are also studies that have revealed that ANG 1–7 administered into specific brain regions (e.g., the RVLM or PVN) enhances cardiac sympathetic afferent reflex and increases sympathetic outflow and blood pressure via Mas activation (76, 109, 171).

Important questions are whether these countering effects of AT1R and Mas over AT1R-mediated cardiovascular effects are physiologically relevant, and under what physiological conditions might they occur. These questions are relevant because, thus far, actions of AT2R and Mas have been uncovered under pharmacological conditions. With regard to AT2R, it is known that AT1R and AT2R are present at different levels of expression within various cardiovascular control centers. It is generally accepted that AT1R are more abundant than AT2R within many of the cardiovascular control centers of the brain, and, therefore, AT1R actions, in general, are thought to predominate. Nonetheless, AT1R actions are potentially dampening these AT1R-mediated responses, such that if AT1R is blocked pharmacologically or deleted genetically, AT1R actions are often augmented (21, 84, 113). It is also important to note that although these receptors are present within the same brain nuclei, they are primarily localized to separate cells within these sites (42). Thus, antagonistic actions of these receptors within or near cardiovascular control centers are likely opposite or offsetting, rather than mediated through the same neuron. Although brain AT1R and AT2R may have opposite effects on cardiovascular control and there is anatomical evidence that shows they are found in close proximity to each other, the physiological conditions that might precipitate predominance of one receptor subtype over the other, or increased expression of one versus the other, are not established. Nonetheless, we feel that the AT1R does, indeed, play a physiological role in countering AT1R effects. Perhaps the ratio of AT1R to AT2R is important in determining the extent of ANG II’s hypertensive actions. Further, we hypothesize that AT2R can be exploited pharmacologically to also counter AT1R-mediated hypertension.

It is clear from the reviewed studies, as well as numerous other reports, that the RAS can exert powerful cardiovascular effects via direct actions on neurons. On the other hand, AT1R-neuronal-glial interactions are an emerging area of interest, as is the impact of PRR activation and the counter-regulatory AT1R and ACE2/ANG 1–7/Mas axis on neuronal-glial communications, and consequently, on cardiovascular function. These concepts are discussed in the subsequent sections.

Neuron and Glia Interactions in the Regulation of Cardiovascular Function by the RAS

In addition to direct effects of ANG II and other RAS components on neurons, the localization of the RAS elements to diverse cell types within the CNS brings to light the possibility that communication between neurons and glia mediates CNS RAS actions. It is a fact that neurons and glial cells interact with one another on many levels and thereby impact neurophysiology. Glial cells influence neurotransmitter release
and metabolism, while neurotransmitters and other factors released by neurons impact the activity of glial cells. Furthermore, since both microglia and astrocytes are considered the brain’s resident innate immune cells (149), there is potential for the RAS to initiate inflammatory responses within cardiovascular control centers of the brain via the activation of these non-neuronal cells, possibly modulating cardiovascular homeostasis.

Cross-talk between neurons, astrocytes, and microglia. Over recent years, the understanding of brain function has shifted from a predominant focus on neuron-neuron communication, to the reinforced appreciation that active cross-talk between the various cell types within the brain is an imperative component of signaling within the tissue. There are extensive communications between microglia, astrocytes, and neurons that facilitate the ability of microglia and astrocytes to sense disturbances to the nervous system and to regulate the development, structure, and function of neural connections (99). The impact of these glial-neuronal interactions on brain function have been extensively reviewed elsewhere (4, 179, 191).

Astrocytes have traditionally been considered the support cells or “glue” of the brain, and this accepted, but passive, role has often overshadowed the appreciation of their many essential active roles in brain function (153, 203). That being said, it is currently understood that astrocytes and neurons participate in a bidirectional communication that is essential for the coordination of synaptic transmission and the function of neuronal networks (4). Individual astrocytes contact and oversee hundreds to thousands of dendrites and synapses within various brain areas and are situated such that they can integrate a tremendous amount of synaptic activity (28, 74). This positioning allows them to regulate neurotransmitter diffusion into the extracellular space not only via the formation of a physical barrier to diffusion, but also via effects on neurotransmitter uptake (46, 144, 154, 191). For example, they are known to modulate GABA tone via its removal by the GABA transporter 3 within various neural circuits, including those that regulate sympathetic outflow and blood pressure (138). It is also established that astrocytes regulate the release of glutamate, ATP, and other signaling molecules from nerve terminals (139). Further, astrocytes buffer CNS potassium, remove excess cytosolic glutamate, and modulate blood flow (8, 71, 149).

Finally, astrocytes impact synaptic activity by themselves releasing “gliotransmitters” (121). Of particular relevance, there is accumulating evidence for a potential role of astrocyte-neuronal interactions in the regulation of cardiovascular function, in particular, and this is discussed in greater depth below.

Microglial cells similarly influence neuronal signaling. They make contact with synaptic structures and are, thereby, positioned to sense activity within neuronal circuits (200), and gap junctions between microglia and neurons allow the exchange of ions and small molecules between these cell types (49, 52). Further, microglia facilitate synaptogenesis subsequent to brain injury (13). Additionally, astrocytes and microglia interact with each other to regulate neuronal function (8, 14, 15, 100, 116). That is, astrocytes release factors that modulate microglial activity and vice versa (14, 45, 210).

It is well known that these two glial cell types respond to severe threats to the internal milieu of the brain, such as injury subsequent to stroke, by undergoing phenotypic transformations. On one hand, astrocytes are known to retract their processes in response to certain threats, thereby, in a sense, withdrawing their influence. Of particular relevance, this phenotypic shift can be observed within the magnocellular PVN during hyperosmotic challenges (197). On the other hand, in response to other threats (e.g., obesity and injury) astrocytes undergo a phenomenon that is often referred to as astrogliosis, which is characterized by hyperplasia and hypertrophy of these cells and can be observed via the upregulation of glial fibrillary acidic protein (GFAP) and an increased number of GFAP-positive cells within the site of injury (24, 26, 41, 143). Similarly, the microglial response to injury is characterized by a phenotypic shift from a “resting state” appearance, in which they contain long processes that are dynamically surveying the microenvironment for potential threats, to an “activated state” in which they respond to a potential threat by transforming into cells with enlarged cell bodies and ramified processes (102, 130, 167). Upon injury, they also migrate to and infiltrate the damaged tissue (133, 167, 192). Activated microglial cells then facilitate neuroprotection by removing debris, clearing dead cells, and secreting neurotrophic factors. However, chronic recruitment and activation of microglia can be maladaptive by contributing to impaired neuronal function or inducing neuronal death, as reviewed by several authors (6, 99, 130, 192). Of relevance, the “protective” microglia are often designated as M2 microglia, while the “maladaptive microglia” are often referred to as M1 microglia. Furthermore, once activated, both microglia and astrocytes perpetuate a proinflammatory milieu, and are, thereby, also thought to impact neuronal signaling.

Proinflammatory and immune involvement in communication between neurons and glia. One mechanism by which the various neuronal and glial cell types of the brain communicate with one another is via the release and detection of proinflammatory or immune factors (39). Consistent with their roles in the innate immune system, they express the pattern recognition Toll-like receptors (TLRs; pattern recognition receptors) that identify molecules conserved among many pathogens [e.g., TLR-4 is well known to recognize lipopolysaccharides (LPS)], and they are capable of expressing and synthesizing numerous cytokines and chemokines, as well as their receptors (53, 90). As a consequence, microglia and astrocytes both sense and perpetuate a proinflammatory milieu within the CNS, and this glial-mediated rise in inflammation has a profound impact on neuronal activity.

Importantly, astrocytes and microglia are both localized to cardiovascular control centers of the brain, and both lie in close proximity to preautonomic (39) and to AT1R-containing PVN neurons. Within these areas, they are capable of sensing and responding to hypertensive stimuli to impact cardiovascular function (24, 66, 192). Consequently, it is possible that astrocyte-neuron and/or astrocyte-microglia interactions contribute to the inflammation within cardiovascular control centers of the brain that ultimately elevates blood pressure. During experimental hypertension, the PVN, as well as other brain regions important for regulating sympathetic outflow, such as the NTS, are infiltrated with additional microglia and astrocytes, the levels of proinflammatory factors and reactive oxygen species within these regions rise, and these events have the potential to influence neuronal activity within these regions (29, 167, 201, 202, 216). This neuroimmune communication between microglia, astrocytes, and neurons has then been suggested to facilitate neurogenic hypertension (30, 39).
In regard to cardiovascular function, increases in proinflammatory cytokines within brain nuclei that regulate cardiovascular homeostasis are generally considered to promote sympathetic nervous system activity and increase blood pressure. For instance, such is the case upon an elevation in proinflammatory cytokines specifically within the PVN. Blockade of proinflammatory cytokines within the PVN, for example, via the administration of a TNF-α receptor antagonist, etanercept, has the opposite impact on blood pressure (175). Furthermore, elevations in the levels of the anti-inflammatory cytokine, IL-10, within the PVN similarly reduce blood pressure (167). The implication is that the proinflammatory actions of ANG II within the brain may also contribute to the hypertensive actions of this peptide.

Evidence for astrocyte and microglial involvement in the neural regulation of cardiovascular function by ANG II and (pro)renin. Although it is recognized that ANG II can have a direct impact on neurons to regulate cardiovascular function, several lines of evidence indicate that the RAS also impacts cardiovascular function by facilitating the communication between neurons and glia (39). Along these lines, since astrocytes represent the key cell type within the nervous system that synthesize angiotensinogen (88, 123, 134, 166, 180), it is intriguing to hypothesize that astrocyte-generated angiotensinogen itself acts as a signaling moiety to facilitate this communication. Similarly, as it is evident that astrocytic angiotensinogen is critical for the integrity of the BBB (96), it is possible that the brain RAS plays a “gatekeeping” role, whereby it maintains and/or potentiates the access of endocrine ANG II and other circulating factors to the brain. In this regard, ANG II derived from astrocytic angiotensinogen has been found to activate AT₁R on endothelial cells to maintain BBB function (206).

Also of particular relevance, astrocytes and the RAS have independently been acknowledged to impact the magnocellular AVP system to influence blood pressure, and it is possible that an angiotensinergic-neuronal-glial communication impacts AVP signaling to ultimately regulate cardiovascular function (34, 46, 115, 191). As discussed above, astrocytes regulate neuronal function, in part, by influencing neurotransmitter diffusion into the extracellular space through a variety of mechanisms involving the formation of a physical barrier to diffusion and via neurotransmitter uptake (46, 144, 154, 191). Within the magnocellular region, in particular, astrocytes are involved in regulating excitatory/inhibitory neurotransmitter balance through such mechanisms, and the disruption of this balance is associated with disease conditions that are known to impact the RAS, such as heart failure (146, 147). For example, they influence crosstalk between NMDA and GABA A receptors in magnocellular neurons (147). Moreover, dehydration, which is known to impact the RAS and blood pressure, is associated with a structural remodeling of the neuronal-glial interactions within the magnocellular portion of the PVN, as well as the SON (197), and it is possible that a modulation of the neuronal-glial interactions within the magnocellular hypothalamus is integral to the RAS regulation of blood pressure. Furthermore, it is also possible that hypertensive stimuli induce such a remodeling of the astrocyte-neuron interactions within the preautonomic portion of the PVN and, thereby, also allows for altered angiotensinergic-neuronal-glial interactions [e.g., obesity (41)]. Along these lines, there is evidence that astrocytes do impact preautonomic neurons within the PVN in that they regulate the inhibitory GABA tone onto these neurons, thereby influencing renal sympathetic nerve activity, an important component of blood pressure regulation (138).

Another mechanism by which the RAS may impact neuronal-glial interactions is via an escalation of the proinflammatory milieu within cardiovascular control centers (10, 41, 167). Hypertension produced by chronic infusion of ANG II is associated with AT₁R-mediated increases in microglial activation and cytokine levels in the PVN (167). Furthermore, accumulating evidence has indicated that the beneficial properties of RAS blockade are attributable, in part, to anti-inflammatory actions (11, 155). That being said, unlike the general acceptance that ANG II receptors are localized to neurons, whether or not non-neuronal cells of the brain also express ANG II receptors under normal conditions in vivo is less clear. Consequently, whether ANG II impacts astrocytes and microglia directly via receptors expressed on these cell-types or indirectly via its effects on neuronal receptor activation is a subject of debate.

In support of direct ANG II actions on non-neuronal cells, there are studies that have localized AT₁R to cultured microglia that were exposed to LPS (127) and to astrocytes both in culture and in vivo (61, 183, 184). However, as some of these previous localization studies (61, 183) were conducted using ANG II receptor antibodies, the presence of the ANG II receptors on these non-neuronal cell types should be verified using alternate methodologies (e.g., reporter mice or in situ hybridization). Further, there is evidence that ANG II can elicit immune responses via activation of these AT₁R (59, 61, 97). For example, in vitro, ANG II administration impacts cultured microglia and astrocytes by increasing transforming growth factor-β (TGF-β) expression, while angiotensin receptor blockers reduce inflammatory responses to ANG II and other stimuli (e.g., LPS) (103, 127). Moreover, in vivo, AT₁R blockade using candesartan, an angiotensin receptor blocker that crosses the BBB, leads to reduced inflammatory responses during immune challenges or hypertensive stimuli within specific brain regions that is often accompanied by decreased microglial activation (10, 103). However, these in vivo actions may or may not be due to direct actions on glia.

As discussed in detail above, there are studies that have determined that ANG II receptors are likely not localized to glia and are instead exclusively localized to neurons in the brain (42, 67, 134). Characterization of recently generated transgenic AT₁R and AT₂R reporter mice indicate that, under normotensive in vivo conditions, brain AT₁R and AT₂R expression is almost solely neuronal (42, 67). Although a lack of ANG II receptor expression on non-neuronal cells of the brain may rule out direct effects of ANG II on glial cells, it would not per se rule out a key role of ANG II in glial cell influence over cardiovascular homeostasis. It is possible that microglia (and astrocytes) sense neuronal AT₁R stimulation and that this then triggers their activation [i.e., an indirect mechanism by which ANG II influences glial cells via secretion of chemotactic factors from neurons (173, 204, 205)]. The prediction is that this feeds forward to further enhance hypertension, by promoting the above-described inflammatory response in glia that then further enhances sympathetic outflow. In this regard, there is some evidence for such a role for chemotactic proteins.
such as CC-chemokine ligand-2 (CCL2), as well as stromal cell-derived factor 1 and high-mobility group box 1 in these processes (168, 173, 204, 205).

There are several studies that would be congruent with either a direct or indirect influence of AT\textsubscript{1}R activation on microglia or astrocytes (40–42, 167, 192). For example, a study conducted by our research group revealed that the central administration of minocycline, an anti-inflammatory antibiotic that reduces microglial activation also decreases ANG II-induced hypertension (167). Furthermore, deletion of AT\textsubscript{1}R from neurons within the PVN of mice, leads to reduced microglial and inflammatory responses that occur subsequent to long-term high-fat diet consumption (40, 41, 192).

It is also possible that during hypertension, the expression pattern of AT\textsubscript{1}R changes and that microglia and/or astrocytes begin to express the receptor when challenged. Although, as described above, ANG II receptors are not detected in microglia or astrocytes of normotensive rodents under baseline conditions in situ (42, 67), some immunohistochemical studies have revealed localization of AT\textsubscript{1}R, AT\textsubscript{2}R, and ANG II labeling in microglia or astrocytes of the ischemic brain or in brains collected from rodents during other pathological conditions (89, 207). As mentioned throughout this review, however, the unreliability of many ANG II receptor antibodies (9, 79) has led to a hesitancy to rely solely on immunohistochemical data to conclude that ANG II receptors are localized to non-neuronal cells of the brain. Therefore, it will be necessary to verify these results using alternative localization techniques. In this regard, using another approach to determine the role of a potential glial AT\textsubscript{1}R receptor population in cardiovascular function, Isegawa et al. (89) recently determined that mice that have undergone Cre/Lox-mediated deletion of AT\textsubscript{1}R from GFAP-containing cells have an improved prognosis when subjected to myocardial infarction-induced heart failure. In particular, they found that this “knockout” mouse did not exhibit the increase in AT\textsubscript{1}R mRNA or protein (IHC and Western blot analysis) within the brain stem that wild-type mice exhibit in response to MI-induced heart failure. Importantly, under baseline, non-MI-induced heart failure, conditions, there was no difference in AT\textsubscript{1}R. The implication is that astrocytic AT\textsubscript{1}R is increased in response to heart failure and that this increase contributes to heart failure-induced mortality. Future studies need to be conducted to definitively determine the localization of ANG II receptors during such pathological conditions. Nonetheless, the plasticity of ANG II receptor expression in non-neuronal cell-types of the brain during disease states is an interesting potential mechanism by which ANG II/glial interactions may impact cardiovascular function. Such plasticity of ANG II receptor expression in non-neuronal cells may also be induced during the production of cell cultures, potentially explaining the observations that have demonstrated the presence of AT\textsubscript{1}R on microglia and astrocytes within in vitro models (127, 189).

As with ANG II, there is also evidence suggesting that (pro)renin impacts the non-neuronal cells in the brain, either directly or indirectly, to ultimately influence neuronal function. In this regard, (pro)renin elicits a direct proinflammatory action on microglial cells, mediated through NF-κB and potentiated by pretreatment with ANG II (169). Furthermore, we have found that (pro)renin exerts proinflammatory actions in cultured astrocytes of rats, an effect that is potentiated in astrocyte cultures generated from hypertensive rats (152).

On the other hand, there are several lines of evidence that have linked the neuroprotective effects of AT\textsubscript{2}R activation and of the ACE2-(ANG 1–7)-Mas axis to their ability to alter the interactions between neurons and non-neuronal cells in the brain (12, 198). For example, AT\textsubscript{2}R activation using the selective AT\textsubscript{2}R agonist Compound 21 attenuates microglial responses during autoimmune encephalomyelitis (81), and there is some evidence that astrocytic AT\textsubscript{2}R correlates with BBB breakdown during CNS inflammation (181), implying that AT\textsubscript{2}R may also play a role in the access of the peripheral RAS to the CNS. In regard to the ACE2-(ANG 1–7)-Mas axis, in addition to Mas’s documented expression in neurons, there is also evidence that this receptor is localized to microglia (150) and that its activation impacts microglial responses to stroke, implying that activation of this protective RAS either directly or indirectly influences these non-neuronal cells. It is possible that the putative protective effects of these systems in the CNS control of cardiovascular function similarly involve astrocytes and microglia (70).

Conclusions and Hypothesis

Although it has been suggested that ANG II receptors located on glial cells mediate certain cardiovascular actions of ANG II, we propose rather that cooperation between neurons and glia is integral to the RAS influence over the brain control of cardiovascular function under normal and hypertensive conditions. As discussed throughout this review, this premise stems, in part, from the realization that the components required for ANG II synthesis and action are not confined to a single cellular compartment within the CNS and are instead localized to diverse cell types throughout the tissue. Further, despite numerous research efforts to localize ANG II receptors to non-neuronal cells in the brain, their presence on cells other than neurons of the CNS in vivo is not definitively supported by the literature, particularly under normotensive conditions. In spite of this, there are numerous lines of evidence indicating that AT\textsubscript{1}R activation does, indeed, impact microglial activation and astrogliosis and that this is associated with cardiovascular dysfunction.

This profound impact of the ANG II on microglia and astrocytes coupled with the apparent lack of AT\textsubscript{1}R expression within these cell types under normal baseline conditions, further reinforces the appreciation of a probable intricate relationship between microglia, astrocytes, and ANG II receptor-containing neurons. Along these lines, we have previously determined, that both astrocytes and microglia lie in close proximity to preautonomic PVN neurons (39) and are, therefore, positioned to regulate sympathetic outflow. Here, we further determine that in normotensive Sprague-Dawley rats, microglia and astrocytes, although they themselves lack AT\textsubscript{1}R mRNA under normotensive conditions, are localized in close proximity to AT\textsubscript{1}R-containing neurons within the PVN. Consequently, these AT\textsubscript{1}R-containing neurons within the PVN are similarly positioned to interact closely with glial cells and perhaps respond to glial cell-generated or circulating RAS peptides, and it is possible that similar interactions are present in other cardiovascular control nuclei, as well.
Another important component of the RAS-neuron-glia interactions in cardiovascular control is that during hypertension, the activity of the RAS actions within the CNS rises. This elevation in central RAS actions is due to an increase in ANG II synthesis within the CNS and/or an increase in peripheral RAS access to the CNS. In regard to the CNS synthesis of ANG II, the PRR plays a key role in this and then in the subsequent activation of CNS AT1R. That being said, PRR’s influence over the neural control of blood pressure extends beyond its ability to increase ANG II levels locally, within cardiovascular control nuclei. That is, PRR activation is also linked to independent intracellular signaling cascades that facilitate neurogenic hypertension. Furthermore, PRR is likely expressed on neurons, microglia, and astrocytes during both normotensive and hypertensive conditions and, therefore, also has the potential to both directly and indirectly influence the neuronal-glial influence over cardiovascular function.

Regardless of the source of the ANG II within the brain, we propose the following model to help explain the respective roles of neurons and glia in the cardiovascular actions of RAS components under normal and hypertensive conditions. First, under normotensive conditions ANG II activates neuronal AT1R to increase sympathetic nerve activity and blood pressure (Fig. 2A). We further propose that during the normotensive state, (pro)renin can influence neuronal PRR to exert ANG II-dependent and -independent effects and ultimately increase sympathetic nerve activity and blood pressure. This action of (pro)renin may involve glial effects on RAS components to assist in the generation of ANG II. During hypertension (Fig. 2B), we expect that the elevation in the CNS ANG II leads to increased activation of neuronal AT1R in BBB-protected brain nuclei, with subsequent activation of preautonomic neurons to augment sympathetic outflow and blood pressure. Furthermore, we postulate that during hypertension, activation of neuronal AT1R leads to the secretion of certain factors (e.g., the chemotactic protein CCL2), which then bind to their receptors (e.g., CCR2) on microglia and stimulate their migration toward these neurons. This hypothesis has been described in detail in a previous review (39). Our model also incorporates the possibility that a certain plasticity exists in regard to the cellular localization of the AT1R in the brain, whereby under cardiovascular pathologies, AT1Rs come to be expressed on astrocytes and microglia (Fig. 2B). The hypothesis is then that activation of these non-neuronal AT1R directly facilitates astrogliosis and microglial activation, leading eventually to a perpetuation of neurally mediated cardiovascular dysfunction. Finally, we predict that in addition to enhanced neuronal effects during hypertension, (pro)renin acts via PRR on astrocytes and microglia to elicit astrogliosis and microglial activation, and subsequent neuronal activation via paracrine interactions.

**Perspectives and Significance**

Collectively, we believe that these RAS-mediated increases in neuronal, microglial, and astrocyte activity contribute to the perpetuation of neurogenic hypertension. Despite tremendous research efforts, hypertension and the often consequential morbidity or mortality associated with cardiovascular pathologies remain an immense problem for our society. Taken together, the reviewed studies highlight a promising avenue for the development of antihypertensive therapeutics. These studies suggest that one potential contributing mechanism to these pathologies is the RAS-mediated interactions between neurons and glia within cardiovascular control centers of the brain. These interactions are now an area of active research that may lead to new and innovative ways to combat treatment-resistant hypertension.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

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