Neurohormonal Regulation of the Sympathetic Nervous System: New Insights into Central Mechanisms of Action

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To regulate blood pressure, the brain controls circulating hormones, which influence the brain by binding to brain neurons that lie outside the blood-brain barrier. Recent work has demonstrated that "cardiovascular" hormones are synthesized and released in the brain as neurotransmitters/neuromodulators and can, in some cases, signal through the blood-brain barrier. The renin-angiotensin system is a prototype for these newly appreciated mechanisms. The brain's intrinsic renin-angiotensin system plays an important role in blood pressure control. Angiotensin II in brain neurons affects other neurons both through activation of angiotensin receptors and via generation of nitric oxide and reactive oxygen molecules. Similarly, angiotensin in blood vessels activates endothelial nitric oxide, which can diffuse across the blood-brain barrier and thereby alter neuronal activity in cardiovascular control nuclei. The relative importance of these mechanisms to blood pressure control remains to be fully elucidated.

Introduction

For more than a century, researchers have known that circulating hormones regulate arterial pressure. Recent research has demonstrated that some hormones act via influences on the central nervous system (CNS). The prototype for most such interactions has been angiotensin II (AII), a circulating peptide that regulates cardiovascular homeostasis, including vascular function. AII has long been known to act via the CNS, but these interactions were typically mediated primarily via the circumventricular organs, areas of the brain that lack the blood-brain barrier and therefore can monitor peptides in the circulation. However, emerging evidence strongly indicates that AII and its active metabolites are capable of modifying neuronal activity in cardiovascular nuclei by other pathways. This paper reviews recent findings that show AII can bypass the blood-brain barrier through a vascular-brain signaling mechanism that involves AII-induced nitric oxide generation. Further data document an intrinsic renin-angiotensin system (RAS) in the brain that modulates neuronal activity. Both pathways appear to act in part through the generation of reactive oxygen species (Fig. 1).

Angiotensin and Hypertension

Hormonal imbalances have been long been recognized as contributors to hypertension, and probably the most thoroughly studied of these involve the RAS. Studies in the past 60 years have demonstrated that peripheral AII is intimately involved in volume homeostasis and blood pressure regulation, and that AII exerts a potent dipsogenic response, stimulates vasopressin release by the brain, and increases renal salt and water reabsorption. Several of the primary rodent models of hypertension display a strong linkage to AII (eg, spontaneously hypertensive rat [SHR], TGR[mRen2] rat, Dahl salt-sensitive rat, deoxycorticosterone acetate (DOCA)-salt hypertensive rat, and renal hypertensive rat) [1]. In these models, AII appears to raise arterial pressure, at least in part, through inappropriate volume retention or elevated peripheral resistance. These experimental models also have elevated sympathetic nervous system activity, leading many investigators to hypothesize a link between the RAS and sympathetic nervous system activity in hypertension. Thus, an overactive RAS may elevate arterial pressure directly through peripheral actions, through influences on CNS control of sympathetic nervous system activity and vasopressin release, and/or by blunting baroreceptor feedback to the brainstem.

Many investigators have dismissed a contribution of baroreceptors to hypertension, because baroreceptor denervation does not appreciably alter arterial pressure; it only increases lability of arterial pressure and heart rate. However, recent evidence implicates baroreceptors in the development

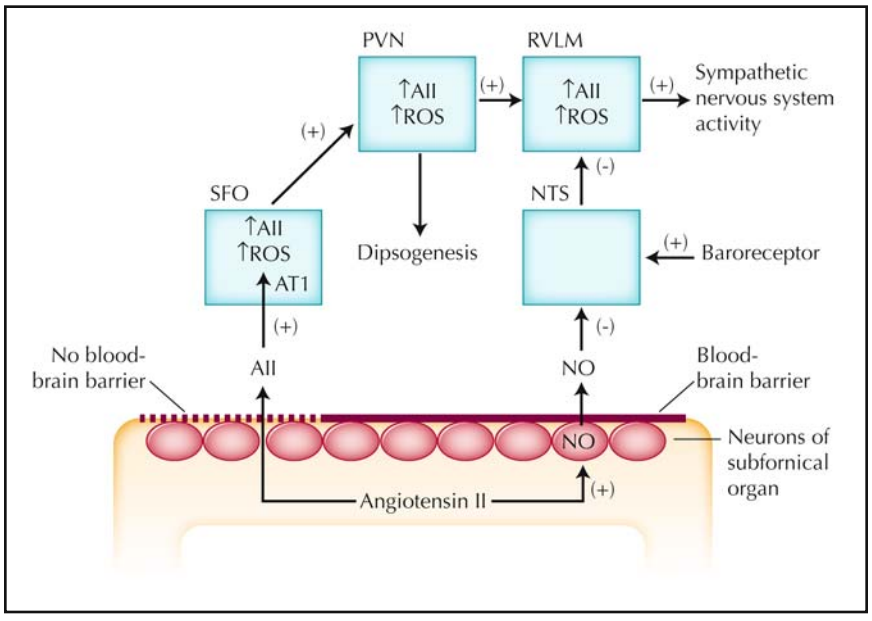

Figure 1. Angiotensin II (AII) can directly access AII type 1 (AT1) receptors on neurons in the subfornical organ (SFO), which lacks the blood-brain barrier (*dashed line*), thereby increasing the activity of neurons that project to the paraventricular nucleus of the hypothalamus (PVN), resulting in the release of AII and other transmitters. This stimulates drinking behavior and increases activity of neurons projecting to the rostral ventrolateral medulla (RVLM), resulting in an increase in sympathetic nervous system activity and elevation in arterial pressure. Neuronal activity in the SFO, PVN, and RVLM may be directly or indirectly altered by intrinsic brain AII binding to AII receptors and through AII-induced increases of reactive oxygen species (ROS) via stimulation of NADPH oxidase activity. AII can also stimulate formation of nitric oxide (NO) from vascular endothelial cells, which can diffuse across the endothelial blood-brain barrier (*solid dark line*) and stimulate intracellular mechanisms, resulting in a depression of baroreceptor sensitivity. *Plus sign* indicates stimulatory action; *minus sign* indicates inhibitory action. NTS—nucleus tractus solitarius.

and maintenance of hypertension. For instance, baroreceptors chronically reset to a higher "setpoint" when arterial pressure is chronically elevated. Once reset, the baroreceptor system defends the higher pressure until the setpoint is again adjusted [2]. Second, baroreceptor sensitivity is altered in many experimental models of hypertension, and baroreceptor impairment appears to precede the onset of hypertension [1]. A substantial amount of data indicates that AII inhibits baroreceptor function. For example, in response to an increase in arterial pressure due to phenylephrine infusion, activation of baroreceptors leads to a decrease in heart rate and inhibition of sympathetic nervous system activity. In contrast, following AII infusion, heart rate and sympathetic responses to the rise in arterial pressure are significantly blunted [3]. When rats are treated with an angiotensin II type 1 receptor (AT1) blocker, baroreflex sensitivity is restored [4]. Such an effect has been documented in several models of hypertension (eg, in SHR and TGR[mREN2]27 rats) [4,5]. Similarly, in the high-renin, two-kidney, one-clip hypertensive model and Lyon hypertensive rat, baroreflex control of heart rate and lumbar sympathetic nerve activity are suppressed [6–8]. In this model, treatment with an angiotensin-converting enzyme inhibitor restores sensitivity to that of normotensive controls. In contrast, angiotensinogen transgenic rats—TGR(ASrAOGEN), which are characterized by low levels of AII—have an enhanced baroreflex response compared with nontransgenic controls. As expected in this model, infusion of AII decreases sensitivity [9].

The observation that circulating AII inhibits baroreflex activity [4] suggests that AII binds to receptors in a circumventricular organ to exert this effect. Circumventricular organs lack a blood-brain barrier; therefore, neurons in these regions can detect and respond to circulating endocrine factors. Several circumventricular nuclei, including the organum vasculosum of the lamina terminalis, area postrema, subfornical organ, and median preoptic nucleus, display AII binding sites [1]. The area postrema is the closest of these nuclei to the "baroreceptor nucleus" (the nucleus tractus solitarius [NTS]), which is the site of afferent baroreceptor termination in the medulla. Thus, the area postrema is well situated to modulate baroreceptor input in response to circulating AII. Ablation of the area postrema abolishes AII-induced baroreflex desensitization in rabbits and eliminates AII-induced hypertension in rats [3,10]. Further, in SHR, removal of the area postrema prevents the beneficial effects of AII receptor blockade on baroreceptor function [11].

Research by Tan et al. [12••] indicates that microinjection of AII into the area postrema blunts baroreceptor sensitivity, and microinjection of an angiotensin-converting enzyme inhibitor into the area postrema inhibits this AII effect. Furthermore, ablation of the area postrema greatly reduces AII-induced baroreflex desensitization. Microinjection of an angiotensin-converting enzyme inhibitor directly into the adjacent NTS enhances baroreflex sensitivity, and microinjection of AII into the NTS blunts baroreflex sensitivity. The latter effect occurs whether the area postrema is intact or lesioned, suggesting that neurons in the NTS can respond to AII both directly and indirectly via the circumventricular organ. Because the NTS has a blood-brain barrier that AII cannot penetrate, circulating AII does not gain access to the NTS neurons under normal conditions. However, AT1 receptors in the NTS appear to mediate some AII-induced alterations in baroreflex control [13–15]. The discovery of an endogenous AII system in the brain has shed new light on the pathways that affect these AII receptors in the NTS.

The Endogenous Brain Renin-Angiotensin System

In addition to effects on the circumventricular organs, the ability of AII to modify arterial pressure also involves an endogenous brain renin-angiotensin system. In the past two decades, research has demonstrated that the brain contains all the necessary components for RAS signaling, including angiotensinogen, renin, and angiotensin-converting enzyme [1,16]. The localization pattern of these components in the brain suggests that angiotensinogen is released from glial cells and modified by neuronal renin and angiotensin-converting enzyme to form AII (described in the study by Sakai et al. [17••]), which in turn can act as a paracrine neuromodulator or be released as a neurotransmitter. The existence of an endogenous brain RAS signaling system is further supported by the widespread distribution of neuronal AII receptors in almost all the nuclei involved in cardiovascular regulation [16]. These include the paraventricular nucleus (PVN), parabrachial nucleus, rostral ventrolateral medulla (RVLM), and the NTS, along with widespread distribution in all the circumventricular regions [1]. Although these studies provided detailed localization of an intrinsic RAS in the CNS, research is only beginning to elucidate its role in cardiovascular control.

The endogenous RAS has been studied extensively in relation to the subfornical organ. Circulating AII binds to receptors in the subfornical organ and elicits a potent dipsogenic response, which is transmitted to other neurons in the brain at least in part by subfornical organ neurons, using AII as a neurotransmitter. During the past 5 years, Davisson et al. [18] have developed novel lines of transgenic mice that express human renin (hREN) and/or human angiotensinogen (hAGT) genes, and in which the endogenous matching genes have been deleted in selected areas of the brain. Their findings in these models demonstrate that arterial pressure is increased in mice expressing either hREN or hAGT. In both models, interventricular administration of losartan (an AT1 antagonist) ameliorates this hypertension [18]. Because both circulating and tissue RAS components are elevated in these models, the resulting hypertension could be due to intrinsic brain AII and/or circulating AII. To differentiate these alternatives, they used a single transgenic mouse that expressed hAGT flanked with loxP sites [19••]. In this model, the angiotensinogen gene is fully functional unless an Ad/Cre adenovirus is present, in which case the gene is inactivated. Injecting renin into the cerebral ventricle of these mice increases arterial pressure and decreases heart rate without changing circulating angiotensin levels. Intracerebral losartan injection blocks this response. In contrast, Ad/Cre adenovirus microinjection into the subfornical organ renders the hAGT gene nonfunctional and abolishes the blood pressure and heart rate responses to intercerebral renin injection. In this model, intraventricular injection of AII increases arterial pressure, demonstrating that the transgene does not alter the normal response of the neurons to AII. These results support a role for the intrinsic RAS of the subfornical organ in mediating cardiovascular responses to central angiotensin, and also provide a useful tool to test the role of endogenous AII in the brain.

Because one of the primary roles of subfornical organ in angiotensin signaling is dipsogenesis, these investigators sought to determine whether the intrinsic RAS contributes to water homeostasis. To investigate this question, they modified their hREN and hAGT double-transgenic strain (SRA) so that hREN had a neural-specific promoter, ensuring expression only in neural tissue [17••]. Compared with nontransgenic mice, these mice displayed higher arterial pressure and had a threefold increase in water intake. Their urine and electrolyte excretion was increased, which resulted in a preference for saline (compared with water) consumption. Chronic intercerebral losartan administration decreased water intake and urine volume to a much greater degree in transgenic than nontransgenic mice. In contrast, subcutaneous losartan had no effect on drinking behavior or urine output. Immunocytochemistry demonstrated that AII immunoreactivity was increased only in the subfornical organ. These data suggest that the RAS in the subfornical organ plays a significant role in drinking behavior. Although these transgenic mice displayed elevated arterial pressure, suggesting a role for subfornical organ RAS in arterial pressure control, no blood pressure effects of losartan injections were reported, leaving the role of intrinsic AII in the subfornical organ on blood pressure control unclear.

Similar to the subfornical organ, the RVLM displays an intrinsic RAS, including significant expression of AII receptors. Although protected by the blood-brain barrier,

the RAS in this nucleus contributes to regulation of sympathetic nervous system activity and arterial pressure. The RVLM, which is a major site that controls sympathetic nervous system activity, receives baroreceptor-related input from the NTS and descending hypothalamic input, which together modulate the sympathetic tone generated by the RVLM. Microinjection of AII into the RVLM increases sympathetic activity and arterial pressure, suggesting a role for intrinsic AII in modulating RVLM output [20]. Microinjection of an adenovirus expressing a constitutively active AT1 receptor into the RVLM results in a significant increase in blood pressure, likely attributable to increased sympathetic outflow [21•]. Interestingly, the constitutively active AT1 receptors in this study were localized to the surrounding glial (vs neuronal) cells, suggesting that the glial cells can modify RVLM activity. Further support for a role of RVLM AT1 receptors in blood pressure and sympathetic nervous system control is provided by studies in SHR, Dahl-sensitive rats, and TGR(mRen2)27 rats, in which microinjection of AT1 receptor blocker into RVLM lowers arterial pressure [22–24]. However, other studies, particularly in normotensive and/or anesthetized animals, have failed to demonstrate that AT1 receptor blockade alters arterial pressure or elicits a depressor response [25••]. These discrepancies suggest that endogenous AII does not tonically alter RVLM control of sympathetic nervous system activity, except under conditions in which the RAS is disturbed [25••]. Some studies suggest that endogenous AII exerts both sympatho-excitatory and -inhibitory effects on the RVLM, thus modifying the set-point of sympathetic outflow and arterial pressure in an additive manner [25••]. In this model, any imbalance of endogenous AII could alter this push-pull relationship, and correspondingly change arterial pressure in either a hypertensive or hypotensive manner.

The PVN in the hypothalamus is a major source of input to the RVLM. Tagawa and Dampney [26] and Tagawa et al. [27] have demonstrated a role for AII in PVN modulation of RVLM activity. In anesthetized rats, activation of PVN neurons elicits an increase in arterial pressure and sympathetic activity. Microinjection of an AT1 receptor antagonist into the RVLM significantly reduces these responses, but blockade of glutamate and γ-aminobutyric acid (GABA) receptors in the RVLM does not alter the responses [26,27]. These results suggest that AT1 receptors in the RVLM mediate excitatory synaptic inputs from the PVN to the RVLM. Interestingly, PVN neurons that project to the RVLM express AT1 receptors and appear to be activated by AII. To elucidate the mechanism by which AII modulates PVN activity, Li and Pan [28•] used brain slices and whole-cell patch clamp techniques to measure cell responses to AII. After identifying PVN neurons that projected to the RVLM and used AII, they measured whole-cell current response to AII. AII increased activity of the PVN neurons, and losartan treatment eliminated the effect. In assessing the effect of AII on specific currents, the authors found that AII decreased the amplitude of evoked GABAergic inhibitory postsynaptic currents in a dose-dependent manner, and the addition of bicuculline blocked AII activation of PVN neurons. In contrast, AII did not alter excitatory currents. In a similar study, Chen and Pan [29] examined the effect of AII on the activity of RVLM-projecting neurons in the PVN. Using whole-brain slices, they demonstrated that application of AII decreased GABAergic postsynaptic inhibitory currents via a G-protein–dependent pathway. These results indicate that AII decreases GABAergic inhibition of PVN neurons, thereby increasing their firing rate and leading to excitation of RVLM neurons.

Angiotensin and Reactive Oxygen Species

AII receptors are widely distributed throughout cardiovascular centers in the brain, and these receptors respond to AII (described earlier). Therefore, researchers are examining the intracellular signaling pathway by which AII exerts its effects. Recent studies have focused on angiotensin-induced generation of reactive oxygen species. Reactive oxygen species—including oxygen ions, free radicals, and peroxides—are products of enzymes such as NADPH oxidase. As reactive oxygen species are generated, intracellular superoxide dismutase converts them into hydrogen peroxide. There are two forms of superoxide dismutase: mitochondrial superoxide dismutase, which contains manganese (MnSod), and cytoplasmic superoxide dismutase, which contains copper and zinc (CuZnSod). Because hydrogen peroxide is a potent free radical species, it must be quickly degraded by enzymes such as catalase, glutathione peroxidase, and peroxiredoxins.

Although their role in cell damage and apoptosis is well documented, increasing evidence suggests that reactive oxygen species also are involved in intracellular signaling pathways, including those used by AII. Campese et al. [30••] found that when AII was infused centrally, mean arterial pressure and renal sympathetic nerve activity were significantly elevated. When tempol (a superoxide dismutase mimetic) was coadministered with AII to reduce reactive oxygen species levels, the AII effects on arterial pressure and sympathetic nerve activity were abolished. Similarly, Zimmerman et al. [31,32••] observed that AII-induced pressor and drinking responses were accompanied by increased superoxide production in the subfornical organ, and that inducing superoxide dismutase overexpression via central administration of a superoxide dismutase transgene into the subfornical organ (causing overexpression localized to the subfornical organ) eliminated these responses. These results suggest that elevated AII can increase arterial pressure by increasing reactive oxygen species generation within subfornical organ neurons, thereby increasing activation of hypothalamic centers that control sympathetic nervous system activity. This is supported by

studies in cultured neuroblastoma cells in which AII induces a rapid increase in cytosolic calcium, whereas reducing reactive oxygen species generation via adenoviral overexpression of superoxide dismutase greatly reduces this calcium influx [33].

The importance of superoxide dismutase in AII signaling raises the question of how superoxide is generated in this pathway. Although a number of potential enzymes exist by which reactive oxygen species could be generated in response to AII, most data suggest NADPH oxidase. AII-induced NADPH oxidase activity occurs in areas similar to those that display endogenous AII. In NTS, AII infusion increases reactive oxygen species production, which is blocked by NADPH oxidase inhibitors [34•]. Nozoe et al. [35••] demonstrated that NADPH oxidase is elevated in the NTS of stroke-prone SHR, and selective inhibition of NADPH oxidase significantly reduces arterial pressure and heart rate in stroke-prone SHR but not in normotensive Wistar-Kyoto rats. In addition, CuZnSod was significantly lower in stroke-prone SHR, indicating a reduced ability to remove generated reactive oxygen species.

A similar role for NADPH oxidase generation of reactive oxygen species was demonstrated in the RVLM. Chronic intraventricular infusion of AII in rabbits increases renal sympathetic nerve activity and elevates AT1 receptor density, NADPH oxidase levels, and superoxide production in the RVLM [36]. Baroreflex control of heart rate is also impaired by the infusion. This suggests that NADPH-derived superoxide production in the RVLM contributes to elevated sympathetic activity in response to intracerebroventricular administration of AII. Similarly, Chan et al. [37•] observed that injection of AII into the RVLM increased glutaminergic excitatory postsynaptic potentials and activated the mitogen-activated protein kinase signaling pathway in RVLM neurons. These effects were attenuated by the addition of either an antisense oligonucleotide against NADPH oxidase or the superoxide dismutase mimetic tempol. These results suggest that AII enhances presynaptic glutamate release via NADPH oxidase–derived superoxide that activates the mitogen-activated protein kinase pathway. Other studies indicate that superoxide dismutase generation plays a role in AII signaling in the PVN. In PVN, superoxide dismutase treatment abolishes AII-induced decreases in GABA currents in cells projecting to the RVLM [29].

The above research supports reactive oxygen species generation as a contributor to AII-induced sympathoexcitation; however, reactive oxygen species generation is also increased in response to other mediators and in other hypertensive models. In vitro research suggests that endothelin stimulates superoxide production in sympathetic ganglia via endothelin (ET) B receptors [38]. A subsequent in vivo study by Lau et al. [39] found that the ETB-specific agonist safrotoxin increases superoxide formation in the inferior mesenteric ganglion, due in part to the pressor effects of safrotoxin. Similar results were demonstrated in DOCA rats, which display elevated NADPH oxidase activity and increased superoxide levels in sympathetic ganglia compared with normotensive rats [40]. In Dahl salt-sensitive rats, salt-loading increases mean arterial pressure, NADPH-dependent superoxide production, and NADPH mRNA levels in the brain [41]. In Dahl salt-sensitive rats, compared with salt-resistant rats, tempol administration into the brain induces significantly greater reductions in arterial pressure, renal sympathetic nerve activity, and heart rate. Thus, reactive oxygen species levels may contribute to elevated sympathetic nervous system activity induced by dietary sodium in this model.

Nitric Oxide

Nitric oxide is another agent that appears to modulate sympathetic nervous system activity and blood pressure control. Nitric oxide production from arginine is catalyzed by nitric oxide synthase (NOS), of which there are three isoforms: endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS). The eNOS and nNOS are distributed in the central nervous system, including regions responsible for cardiovascular regulation. Research suggests that nitric oxide modulates neuronal activity by altering neuronal responses to excitatory amino acids. One region of interest in relation to the actions of nitric oxide is the NTS, where neurons demonstrate nNOS immunoreactivity and express glutaminergic AMPA receptors [42]. Because the NTS is the site of initial baroreceptor input to the medulla and the NTS relays this information to the RVLM, nitric oxide generation in these neurons could contribute to baroreflex control of autonomic output. Dias et al. [42] have demonstrated that activity of AMPA-containing NTS neurons receiving vagal input is facilitated by nitric oxide. To test whether these neurons participate in baroreceptor and cardiopulmonary reflex control of autonomic tone, they studied the effect of nitric oxide blockade on the renal sympathetic nerve activity response to baroreceptor and cardiopulmonary reflex input [43••]. Microinjection of the glutamate agonists AMPA or NMDA decreased mean arterial pressure, heart rate, and renal sympathetic nerve activity, whereas pretreatment with the NOS antagonist L-NAME greatly reduced the responses to the glutamate agonists. To determine the functional significance of nitric oxide in baroreflex control of sympathetic activity, they further examined the effect of NOS inhibition on baroreflex and cardiopulmonary activation. Both baroreflex and cardiopulmonary reflex responses were attenuated when nitric oxide production was blocked. These results support the hypothesis that nitric oxide production facilitates glutaminergic signaling in baroreceptor and cardiopulmonary reflexes.

Although the work from Mifflin's laboratory demonstrates a role for nitric oxide in facilitating baro- and cardiopulmonary reflexes, several studies suggest the opposite. Waki et al. [44••] reported that eNOS

is overexpressed in SHR compared with normotensive Wistar-Kyoto rats. Blockade of eNOS activity by adenoviral-mediated gene transfer increased cardiac baroreceptor reflex sensitivity in both SHR and Wistar-Kyoto rats (defined by decreased cardiac sympathetic tone and increased vagal output). This suggests that basal levels of nitric oxide contribute constitutively to reflex control, which was not observed in studies by Dias et al. [43••]. Additionally, eNOS blockade decreased arterial pressure in SHR but not in Wistar-Kyoto rats. These studies suggest that baroreflexes and cardiopulmonary reflexes may be desensitized in SHR as a result of overexpression of eNOS, resulting in elevated nitric oxide levels in the NTS neurons.

Numerous other studies report conflicting results regarding nitric oxide's role in the control of arterial pressure by the brain. To further investigate these discrepancies, Wang et al. [45•] measured current responses to nitric oxide in rat brainstem slices and found that nitric oxide and a nitric oxide donor could induce excitatory and inhibitory responses, with a lower dose required to elicit an excitatory response. Soluble guanylate cyclase and a non–nitric oxide-dependent guanylate cyclase activator mimicked these effects. These studies indicate that the effects of nitric oxide are presynaptic, suggesting that nitric oxide modulates neurotransmitter release in NTS neurons. These observations also indicate that the response of the NTS neurons to nitric oxide is dose dependent: low concentrations of nitric oxide facilitate glutaminergic transmission in NTS neurons, whereas higher concentrations inhibit transmission. Careful consideration of the nitric oxide concentration to which neurons are exposed will be critical for the interpretation of future studies of its role in the control of arterial pressure by the brain.

Angiotensin II, Nitric Oxide, and Reactive Oxygen Species: How the Vasculature Signals the Brain

Paton and associates [46,47••] have introduced an intriguing mechanism by which circulating hormones can alter neuronal activity that includes both an endogenous AII signaling system in the brain and a mechanism by which circulating AII binds to and activates brain neuronal receptors outside the blood-brain barrier (in circumventricular organs). Both circulating and endogenous brain AII contribute to regulation of sympathetic activity through both mechanisms. The work of Paton et al. [46,47••] suggests that AII can bind to receptors on the vascular endothelium, and thereby induce the release of signaling molecules that can cross the blood-brain barrier and stimulate neurons in the brain. According to their hypothesis, AII stimulates AT1 receptors on blood vessels, and activation of these receptors causes the release of nitric oxide from endothelial cells and diffusion of nitric oxide across the blood-brain barrier, resulting in activation of neurons in the brain in a paracrine manner [46,47••].

Several lines of evidence indicate that an interaction between AII and nitric oxide in NTS alters baroreceptor responses [47••]. Using electron paramagnetic resonance spectroscopy (which traps nitric oxide as a stable form that is localized to the area of production), Paton et al. [47••] demonstrated that AII microinjection into the NTS stimulates local nitric oxide release, blunting baroreflex gain, and that microinjection of a nitric oxide donor similarly reduces baroreflex sensitivity. In this model, direct injection of AII does not alter baroreflex gain when an inhibitor of soluble guanylate cyclase is injected before AII, suggesting that AII acts via a nitric oxide-dependent pathway in the NTS. Using immunocytochemistry, this group also demonstrated that AT1 receptors are localized on the vascular endothelium in the NTS and on a few NTS neurons, supporting the ability of AII to modulate baroreceptor function via endothelial cell release of nitric oxide in the area of the NTS [46]. Differential central versus vascular signaling in the NTS may help explain the conflicting observations on the role of AII in the NTS discussed earlier [13,14].

Conclusions

Circulating AII acting via activation of brain neurons outside the blood-brain barrier is a well-known modulator of drinking behavior and cardiovascular function, but current research has uncovered novel AII signaling mechanisms that also appear to regulate these systems (Fig. 1). First, neurons in cardiovascular areas inside the blood-brain barrier have AII receptors on the surface, and these neurons use AII (produced via the endogenous brain RAS) to signal other cardiovascular neurons. This endogenous system may play an important role in some forms of hypertension. Although the precise mechanisms responsible remain to be fully elucidated, activation of NADPH oxidase and formation of reactive oxygen species appear to be critical in the AII pathway. Second, circulating AII may regulate cardiovascular neurons in the brain by activating eNO, which readily diffuses into the brain and alters activity of neurons in cardiovascular regulatory nuclei. Thus, circulating AII can circumvent the blood-brain barrier and activate signaling mechanisms in central nuclei. Whether this mechanism is important in cardiovascular nuclei other than those in the ventral medulla remains to be determined. Reactive oxygen species may also be signaling molecules in the vascular-brain signaling pathway, but this issue requires further exploration.

Disclosures

No potential conflicts of interest relevant to this article were reported.

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Central administration of tempol abolished AII-induced increases in arterial pressure and renal sympathetic nerve activity, indicating that central AII stimulates formation of reactive oxygen species in its signaling pathway.

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AII-induced pressor and drinking responses are accompanied by increased superoxide production in the subfornical organ, and superoxide dismutase overexpression in the subfornical organ eliminates these responses.

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AII infusion increases reactive oxygen species production in the NTS, and this is blocked by NADPH oxidase inhibitors, indicating that reactive oxygen species is produced via NADPH oxidase.

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Injection of AII into the RVLM increased glutaminergic excitatory postsynaptic potentials in RVLM neurons, and the addition of either an oligonucleotide against NADPH oxidase antisense or tempol abolished this effect.

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43.•• Dias AC, Vitela M, Colombari E, Mifflin SW: **Nitric oxide modulation of glutamatergic, baroreflex, and cardiopulmonary transmission in the nucleus of the solitary tract.** *Am J Physiol Heart Circ Physiol* 2005, **288:**H256–H262.

Microinjection of glutamate agonists into the NTS decreases baroreflex sensitivity, and pretreatment with NOS antagonist greatly reduces this effect. These results suggest that nitric oxide production in the NTS facilitates glutaminergic signaling in baroreceptor and cardiopulmonary reflexes.

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Blockade of eNOS activity using adenoviral-mediated gene transfer increased cardiac baroreceptor reflex sensitivity in both normotensive and hypertensive rats, but decreased arterial pressure in the hypertensive group. This suggests that basal levels of nitric oxide contribute constitutively to reflex control, and may explain how increased nitric oxide generation may desensitize baroreflexes and contribute to hypertension.

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Nitric oxide presynaptically modulates neurotransmitter release in NTS neurons. Low concentrations of nitric oxide facilitate glutaminergic transmission in NTS neurons, whereas higher concentrations inhibit transmission.

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Using electron-paramagnetic resonance spectroscopy, these investigators demonstrated that AII microinjection into the NTS stimulates local nitric oxide release, blunting baroreflex gain. This suggests that AII acts via a nitric oxide-dependent pathway in the NTS, and supports that hypothesis that AII modulates baroreceptor function via endothelial cell release of nitric oxide in the area of the NTS. This hypothesis is further explained in reference 46.