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Angiotensin-II-induced reactive oxygen species along the SFO-PVN-RVLM pathway: implications in neurogenic hypertension

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Abstract

Neurogenic hypertension has been the subject of extensive research worldwide. This review is based on the premise that some forms of neurogenic hypertension are caused in part by the formation of angiotensin-II (Ang-II)-induced reactive oxygen species along the subfornical organ-paraventricular nucleus of the hypothalamus-rostral ventrolateral medulla pathway (SFO-PVN-RVLM pathway). We will discuss the recent contribution of our laboratory and others regarding the mechanisms by which neurons in the SFO (an important circumventricular organ) are activated by Ang-II, how the SFO communicates with two other important areas involved in sympathetic activity regulation (PVN and RVLM) and how Ang-II-induced reactive oxygen species participate along the SFO-PVN-RVLM pathway in the pathogenesis of neurogenic hypertension.

Key words: Superoxide; Sympathetic; Angiotensin; Subfornical organ; Paraventricular nucleus of the hypothalamus; Rostral ventrolateral medulla

Introduction

The mechanisms underlying neurogenic hypertension have been the subject of extensive research worldwide. This review is based on the premise that neurogenic hypertension is caused in part by the formation of angiotensin-II (Ang-II)-induced reactive oxygen species (ROS) along the subfornical organ-paraventricular nucleus of the hypothalamus-rostral ventrolateral medulla pathway (SFO-PVN-RVLM pathway).

Abundant evidence now points to oxidative stress as a key mechanism in Ang-II-dependent neurogenic hypertension (1). Furthermore, it has become evident that ROS are important in the increase in blood pressure elicited by Ang-II administered directly to the central nervous system (2). However, since Ang-II consists of eight amino acids, which make it incapable of crossing the blood brain barrier, the mechanisms underlying how circulating Ang-II acts within the brain to eventually modulate sympathetic activity and induce hypertension remain intriguing. The most accepted hypothesis is that Ang-II, as any other circulating lipophobic substance, acts on neurons in specialized re-

gions of the brain known as the circumventricular organs (CVOs), which lack the normal blood brain barrier, in order to alter the function of other brain regions protected by this important barrier that "filters" what enters the central nervous system. As a result, activation of the CVOs triggers the local production of Ang-II in brain areas protected by the blood brain barrier. Local Ang-II production within the brain is supported by neuron-specific renin and glial- and neuronal-specific angiotensinogen expression as well as by angiotensin type 1 and type 2 (AT1 and AT2) receptor expression in cardiovascular regulatory areas including the SFO, the PVN and the RVLM. The mechanisms involving local Ang-II production and the expression of its receptors in the brain have been described elsewhere (3-6). Here we will discuss the recent advances regarding the mechanisms by which neurons in the SFO (the most important among the CVOs) are activated by Ang-II, how the SFO communicates with two other important areas involved in sympathetic activity regulation (PVN and RVLM) and how Ang-II-induced ROS participate along the SFO-PVN-RVLM

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pathway in the context of neurogenic hypertension.

Reactive oxygen species in the subfornical organ

The SFO is a highly vascularized structure located in the roof of the third ventricle. It is composed of two distinct regions: a core region and a peripheral outer zone, each region with different neuronal projections and ligand binding abilities (7). The majority of anatomical data suggest that SFO neurons have compact dendritic trees and do not receive extensive neural inputs, supporting the suggested principal role of this region in receiving afferent information from the peripheral circulation (8,9). Furthermore, SFO neurons synapse directly (monosynaptic) and indirectly (polysynaptic) to the hypothalamic areas, such as the PVN and supraoptic nucleus (10,11). Excitatory projections have been found in the parvocellular region of the PVN, which, in turn project either to the RVLM or the spinal cord in order to modulate sympathetic activity (12,13).

The SFO has long been known to be an important cardiovascular control region regulating fluid homeostasis in response to Ang-II (5,14). Systemic Ang-II causes drinking that is mediated by the SFO, which involves angiotensinergic connections between the SFO and hypothalamic nuclei that control drinking (15,16). In addition, hypertension elicited by chronic infusion of Ang-II alters AT1 receptor mRNA expression in the SFO (6).

Abundant research now suggests that a key mechanism by which Ang-II influences blood pressure is via its ability to activate ROS signaling pathways. The pathways for ROS production in mammalian cells have been described elsewhere (17). The first evidence that Ang-II activates an NADPH oxidase in vascular smooth muscle cells in order to produce ROS was presented by Griendling et al. (18). More recently, accumulating evidence from our laboratory and others suggests that, like vascular cells, neurons also require ROS to carry out crucial functions related to the central control of blood pressure (2,19-23).

There is compelling evidence that superoxide anion is necessary to elicit the vasopressor, bradycardic, and dipsogenic responses produced by intracerebroventricular (*icv*) administration of Ang-II in conscious mice (2). It has also been shown that Ang-II causes robust increases in superoxide production in cultured SFO neurons. In addition, adenoviral-mediated delivery of cytoplasmically targeted superoxide dismutase (SOD) selectively to the SFO abolishes the cardiovascular and dipsogenic actions of Ang-II in normotensive mice and prevents the hypertension in chronic peripheral Ang-II-infused mice (1,2). In addition, adenoviral vectors encoding small interfering RNA to selectively silence Nox2 or Nox4 (two isoforms of NADPH oxidase) expression in the SFO demonstrate that both Nox2 and Nox4 are required for the full vasopressor effects of brain Ang-II (24). One possible downstream mechanism of

the activation of AT1 receptors in the SFO and subsequent production of ROS is the superoxide-mediated intracellular calcium influx observed in neuroblastoma Neuro-2A cells, which is inhibited by the adenoviral-mediated expression of a dominant-negative isoform of Rac1 (AdN17Rac1), a critical component for NADPH oxidase activation and superoxide production. This evidence suggests that increased intracellular superoxide production in the SFO is critical for the development of neurogenic hypertension.

Reactive oxygen species in the paraventricular nucleus of the hypothalamus

The PVN is located adjacent to the third ventricle in the forebrain and is an important integrative site involved in endocrine and autonomic control. The PVN is composed of different neuronal subgroups. Magnocellular neurons project to the posterior pituitary and release vasopressin or oxytocin. Parvocellular neurons, which can be subdivided further into different subgroups based on their firing pattern, projections, location within the PVN, and morphology, project intrahypothalamically as well as to extrahypothalamic regions in the forebrain, midbrain, brainstem, and spinal cord (25-27).

Some parvocellular neurons in the PVN project to regions of the spinal cord where sympathetic preganglionic motor neurons are located and thereby directly influence sympathetic activity (28,29). Other parvocellular subgroups project to the pressor region of the RVLM and influence sympathetic nerve activity in an indirect fashion (29).

As mentioned earlier, projections from the SFO interact with centers in the hypothalamus, more precisely in the PVN. There is now compelling evidence of functional synaptic release of Ang-II in the PVN. For example, push-pull perfusion experiments have demonstrated physiologically relevant endogenous release of Ang-II in the PVN (30,31). Activation of putative angiotensinergic input to PVN neurons has also been shown to be abolished by specific AT1 receptor antagonists (32). Finally, exogenous administration of Ang-II into the PVN mimics the effects of electrical or chemical stimulation of presumed angiotensinergic cell groups in the SFO (33). Of note, oxidant signaling has been shown to be important in at least some of these Ang-II-mediated effects in the PVN. For example, the cardiac sympathetic afferent reflex involves Ang-II-mediated ROS formation in the PVN (34).

There is new convincing evidence that ROS derived from NADPH oxidase enhances nerve traffic in the SFO-PVN axis. Erdos et al. (35) have shown that *icv* injection of Ang-II increases NADPH oxidase-mediated superoxide production not only in the SFO but also in the PVN. These effects were blocked by the NADPH oxidase inhibitor apocynin as were the hemodynamic effects of centrally administered Ang-II. Interestingly, *icv* infusion of aldosterone has been shown to increase expression of AT1

receptor and angiotensin-converting enzyme (ACE) mRNA in the PVN, which were minimized or prevented by concomitant infusion of tempol, an SOD mimetic (36). In the same study, immunohistochemistry revealed aldosterone-induced increases in dihydroethidium staining (indicating oxidative stress) and Fra-like activity (indicating neuronal excitation) in PVN neurons, which were also attenuated by tempol and losartan.

In the context of hypertension, Ang-II microinjected into the PVN caused larger responses of the cardiac sympathetic activity reflex, baseline renal sympathetic nerve activity and mean arterial pressure in 2-kidney-1-clip (2K1C) rats than in sham rats and these effects were abolished by pretreatment with tempol or apocynin (37). In addition, gene expression of the NAD(P)H oxidase subunits p47phox and gp91phox as well as AT1 receptors is considerably increased in the PVN of rats with renovascular hypertension (38). Furthermore, microinjection of tempol, an SOD mimetic, or vitamin C into the PVN reduces the high blood pressure and sympathetic activity of renovascular hypertensive rats (38,39).

Recently, we have shown that one of the downstream mechanisms by which ROS affect the PVN neurons is by modulating activator protein-1 (AP-1) activity (40). For that purpose, AP-1 activity was longitudinally monitored *in vivo* by bioluminescence imaging in 2K1C or sham mice that had undergone PVN-targeted microinjections of an adenovirus encoding the firefly luciferase (Luc) gene downstream of AP-1 response elements (AdAP-1Luc), as we described earlier (41). Our findings demonstrated that 2K1C-evoked chronic hypertension increases superoxide production in the PVN. In addition, viral delivery of CuZnSOD to the PVN not only prevented the elevation in superoxide, but also abolished the hypertension. Renovascular hypertension also caused a surge in AP-1 activity in the PVN, which paralleled the rise in superoxide production in the PVN, and this was prevented by treatment with AdCuZnSOD. Finally, we demonstrated that adenovirus-mediated expression of a dominant-negative inhibitor of AP-1 activity in the PVN prevented 2K1C-evoked hypertension. These studies bring new insights into the mechanisms underlying ROS in the PVN in the pathogenesis of neurogenic hypertension.

Reactive oxygen species in the rostral ventrolateral medulla

The RVLM is a brainstem region that contains bulbospinal neurons providing a major input to the preganglionic neurons of the sympathetic nervous system (42,43). The importance of the RVLM for maintaining blood pressure in anesthetized animals was first documented 130 years ago (44). However, only after the pioneering studies performed by Guertzenstein et al. (45,46) suggesting that supraspinal sympathetic vasomotor drive originates from

the RVLM was the attention of researcher truly brought to this brainstem area.

The RVLM receives inputs from the SFO and the PVN as discussed earlier in this review, forming the so-called SFO-PVN-RVLM pathway, where Ang-II seems to be the key neurotransmitter. For instance, immunohistochemistry studies showing Ang-II-like immunoreactive neurons in the PVN and terminals in the RVLM support the concept that angiotensinergic neurons in the PVN innervate the RVLM (47). Of note, angiotensin receptors, mainly the AT1 receptor subtype, are also present in the RVLM (48) and play an important role in altering the activity of RVLM neurons (49). For example, injection of Ang-II into the RVLM of the cat produces pressor response (50). In addition, pharmacological blockade of AT1 receptors attenuates the pressor response to Ang-II microinjection in the RVLM of rabbits (51). Furthermore, microinjection of losartan in the RVLM attenuates the pressor response produced by peripheral chemoreflex activation (21).

In experimental models of hypertension the action of Ang-II is enhanced, probably due to tonic AT1 receptor stimulation. In spontaneously hypertensive rats (SHR), for example, it has been shown that injection of Ang-II into the RVLM produces a significantly greater increase in blood pressure when compared to their normotensive controls (52). In addition, candesartan and valsartan, AT1 receptor antagonists, injected into the RVLM decreased blood pressure in SHR to normotensive levels but had no effect in normotensive rats (52).

Regarding ROS in the RVLM, our laboratory and others have shown that accumulation of Ang-II-induced superoxide anions in the RVLM is critical for the pathogenesis of neurogenic hypertension (20,38,39,53,54). To date, we have shown that chronic peripheral Ang-II infusion in mice produces a slow developing hypertension, which is accompanied by superoxide accumulation in the RVLM and increased sympathetic activity (53). Similar results have been obtained with 2K1C rats (54). Interestingly, superoxide scavenging by adenovirus-mediated overexpression of copper/zinc superoxide dismutase (CuZnSOD) in the RVLM prevents both the accumulation of superoxide and the increase in sympathetic activity. Of note, we demonstrated in rats that the association of dietary salt with Ang-II infusion potentiates the superoxide accumulation in the RVLM and the increase in sympathetic activity caused by Ang-II alone (20). Furthermore, we demonstrated that selective ablation of the AT1 receptors in the RVLM using the loxP-Cre recombinase technique also prevented hypertension and superoxide accumulation in the RVLM of Ang-II-infused mice (53).

In addition to superoxide accumulation in the RVLM, it has been documented that AT-1 mRNA expression is higher and NAD(P)H oxidase subunits are increased in the RVLM and PVN of 2K1C rats compared to their sham control group, while CuZnSOD expression remains unchanged.

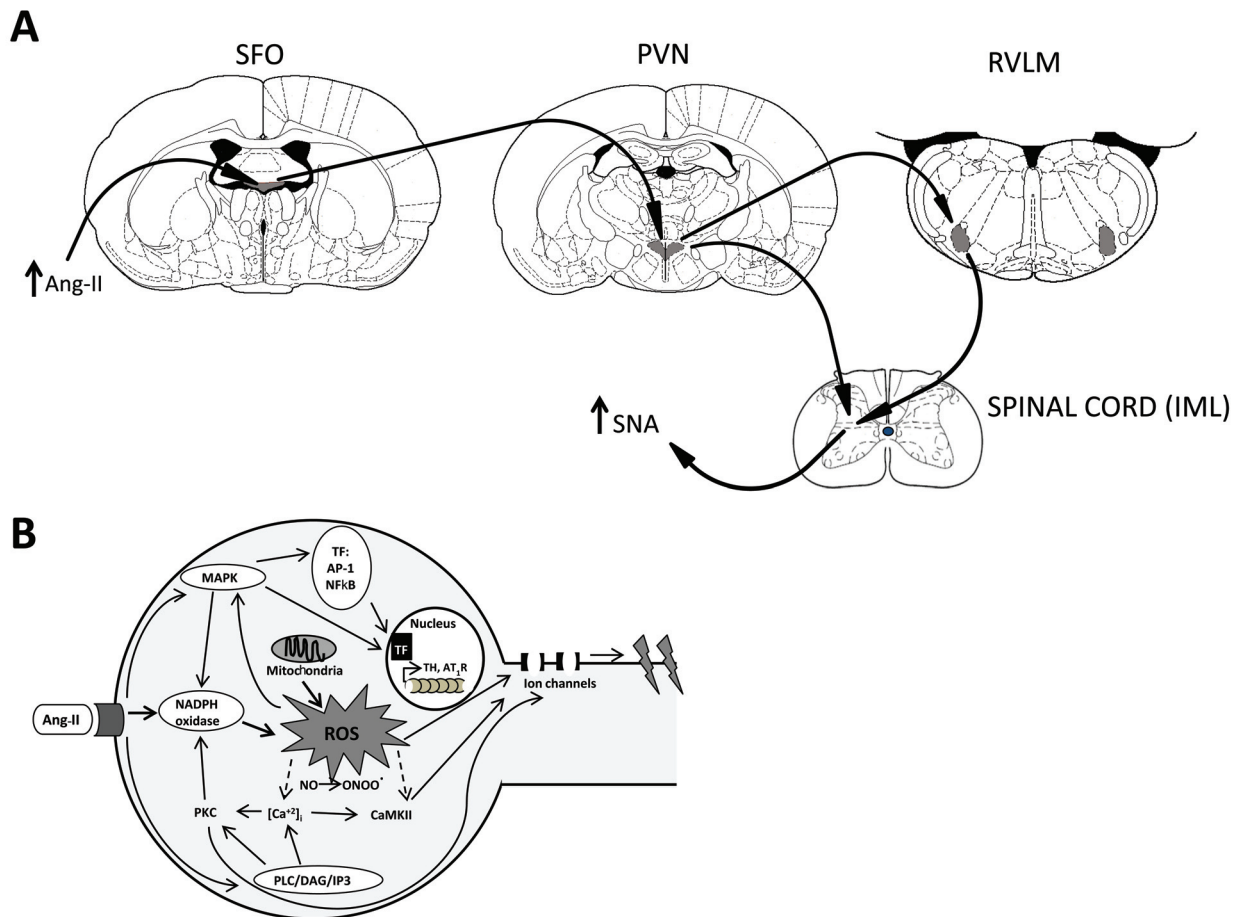


Figure 1. A, The SFO-PVN-RVLM pathway. Diagram showing four coronal sections where the subfornical organ (SFO), the paraventricular nucleus of the hypothalamus (PVN), and the rostral ventrolateral medulla (RVLM) have been highlighted in gray. Note that both PVN and RVLM neurons project to the intermediolateral column (IML) of the spinal cord. B, Mechanisms involving angiotensin-II (Ang-II)-induced reactive oxygen species (ROS) that affect neuronal firing. Of note, the SFO neurons are affected by both blood-borne and brain-borne Ang-II (local production within the SFO) while the PVN and RVLM neurons are affected only by the brain-borne Ang-II. B, Ang-II binds to its AT₁ receptor (AT₁R) resulting in activation of NADPH oxidase, which in turn increases ROS within the neuron. The increase in ROS converts the intracellular nitric oxide to peroxynitrite. Ang-II also activates the mitogen-activated protein kinase (MAPK) and the PLC/DAG/IP₃ pathway. ROS can directly activate the MAPK, which increases the NADPH oxidase activity leading to more ROS. MAPK also activates nuclear transcription factors (NF) such as AP-1 and NFκB, which modulate the expression of tyrosine hydroxylase (TH) and AT₁ receptors. The PLC/DAG/IP₃ pathway activates protein kinase (PKC), contributing to the activation of NADPH oxidase. Moreover, the PLC/DAG/IP₃ pathway increases the intracellular calcium concentration resulting in activation of the CaMKII complex. Finally, PKC, CaMKII, and ROS act on ion channels in the cell membrane changing neuronal firing. PLC = phospholipase C; DAG = diacylglycerol; IP₃ = inositol 1,4,5-trisphosphate; AP-1 = activator protein 1; NFκB = nuclear factor kappa-light-chain-enhancer of activated B cells; CaMKII = calcium/calmodulin-dependent protein kinase II.

Furthermore, injection of tempol into the RVLM reduced blood pressure and renal sympathetic activity in 2K1C but not in sham-operated rats (55).

Oxidative stress in the RVLM has also been associated with the pathogenesis of the hypertension observed in chronic renal failure. For instance, rats with chronic renal failure show increased p47phox and gp91phox mRNA expression in the RVLM associated with a reduction of AT₁ mRNA in the brainstem compared to their controls (56).

Perspectives

It is now clear that the brain possesses all the elements for producing Ang-II locally. Therefore, neurogenic hypertension caused by increased circulating levels of Ang-II uses the SFO as an entry door to stimulate the local production of Ang-II in other brain areas protected by the blood brain barrier. In this scenario, the SFO-PVN-RVLM pathway has been gaining more and more attention (see Figure 1). Inter-

estingly, as we have presented here, scavenging superoxide at any point of this pathway reduces hypertension.

Although basic research using laboratory animals has contributed considerably to identifying the mechanisms underlying the role of ROS in the pathogenesis of hypertension, its translation into human benefits is still the subject of debate, mainly because some clinical trials have failed to document the benefits of antioxidant therapies (57). Therefore, the challenge for the next decades will be finding

a path to safely interfere with ROS inside the human brain, especially in hypertensive patients, in order to prove the benefits of local antioxidant therapy as a reliable treatment for neurogenic hypertension.

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