

Angiotensin-II Induced Reactive Oxygen Species: Implications in Neurogenic Hypertension

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Abstract

Reactive oxygen species (ROS) play an important role in the development and maintenance cardio vascular diseases, especially hypertension. The relationship between ROS and hypertension has been demonstrated in several models of experimental hypertension. Accumulating evidence has suggested that the key mechanism through which Angiotensin II (Ang II) influences blood pressure is via its ability to activate ROS signaling pathways. Ang II, by stimulating angiotensin AT1 receptors, involved in the activation of NAD(P)H oxidase, which is a major source of ROS production. Despite many elegant studies that have been accumulated regarding ROS/Ang II signaling, there is still much to be elucidated in the role of ROS in neurogenic hypertension. The present review mainly discussed some recent findings documenting about the signaling mechanisms of Ang II-induced ROS in neurogenic hypertension and therapeutic target.

Keywords: Reactive Oxygen Species (ROS), Angiotensin II, Neurogenic hypertension

Abbreviations: Ang II: Angiotensin II; AT1R: Angiotensin Type 1 Receptor; BDNF: Brain-Derived Neurotrophic Factor; CREB: cAMP Response Element Binding Protein; MAP: Mean Arterial Pressure; O₂^{•-}: Superoxide Anion; ROS: Reactive Oxygen Species; RVLM: Rostral Ventrolateral Medulla; SOD: Superoxide Dismutase; SHR: Spontaneously Hypertensive Rat; TrkB: Tropomyosin receptor kinase B; WKY: Wistar-Kyoto; UCP: Uncoupling Protein; BBB: Blood Brain Barrier; CaMKII: Calmodulin Kinase II; MAPK: Mitogen-Activated Protein Kinase; TF: Transcriptional Factors; NFκB: Nuclear Factor-κB; TH: Tyrosine Hydroxylase; AP-1: Activator Protein-1; 2K1C: Two Kidney One Clip; RVH: Renovascular Hypertension; ESRK: Extracellular Signal-Regulated Kinase; RSK: ribosomal Protein S6 Kinase; COX: Cyclooxygenase; PGE: Prostaglandin E2; IP3: Inositol Triphosphate; SFO: Subfornical Organ; PVN: Paraventricular Nucleus; NTS: Nucleus Tractus Solitaries; ETC: Electron Transport Capacity

Introduction

Hypertension is the most important risk factor for cardio-vascular disease and, thus, remains a global public health challenge. Despite lots of advances in antihypertensive agents, it has been extremely difficult to manage hypertension in 40% of hypertensive patients [1]. A majority of unresponsive patients may have neurogenic hypertension which is associated with a rise in sympathetic outflow and often, an inhibition of parasympathetic drive [2-4].

Many investigators demonstrated that neurogenic hypertension is caused in part by the formation of Ang II-induced ROS [5-11]. However, since Ang II is composed by eight amino acids, which make it incapable of crossing the Blood Brain Barrier (BBB). As a result, locally produced Ang II may play a key role for brain ROS. In central neurons, local angiotensin II (Ang II) activates the Angiotensin II Type 1 Receptor (AT1R) to induce a cascade of intra-neuronal signaling events that ultimately leads to changes in membrane potential and an increase in neuronal firing [6]. Accumulating evidence over the past years has established that Ang II increases levels of ROS, particularly O₂^{•-}, in neurons which contribute to the increase in neuronal activation [7-12].

As we all known, there is a large amount of signaling pathways in

the upstream and downstream of ROS, some of which is familiar to many researchers, some of which is newly discovered, some of which is a protective role. Next, I will give a brief introduction of ROS-mediated signaling pathway.

NADPH-ROS signaling pathway

Basically, it is the most well known signaling pathway, confirmed by many investigators. Ang II is upstream of NADPH oxidase activation, which requires Rac1 [13-15], a regulator of Nox1 and Nox2. Adenoviral-mediated inhibition of Rac1 decreases BP. Abundant evidence suggests that a key mechanism by which Ang II influences blood pressure is via its ability to stimulate the production of ROS, mainly superoxide anion, via activation of NADPH oxidase [16-19]. A recent study demonstrated increased expression of three isoforms of NADPH oxidase (i.e. Nox1, Nox 2 and Nox 4) and enzyme activity in Spontaneously Hypertensive Rat (SHR) [20], but not in normotensive rat. NADPH oxidase, an enzyme composed of two membrane-bound subunits (gp91phox and p22phox), cytoplasmic subunits (p40phox, p47phox and p67phox) and the small G-protein Rac1a [21], is a major source of ROS in hypertension [21,22] and has a critical role in generating ROS in the brain [14,22-25].

The general process of this signaling pathway: Firstly, Ang II activates the AT1 receptors, then, the cytoplasmic subunits bind to the membrane subunits and activate the enzyme, resulting in the intracellular production of superoxide (converts the intracellular nitric oxide to peroxynitrite). The accumulation of superoxide leads to changes in ion channels, particularly calcium and potassium channels, altering neuronal firing properties in areas of the brain such as the

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RVLM, resulting in increased sympathetic nerve activity and increased blood pressure [26] (Figure 1).

Yin et al. [10], recently reported that Ang II increases mitochondrial $O_2^{\cdot-}$ levels in catecholaminergic (CATH.a) neurons and that this increase in mitochondrial $O_2^{\cdot-}$ mediates the Ang II-induced activation of a redox-sensitive kinase, calcium/calmodulin kinase II (CaMKII), and the Ang II-induced inhibition of neuronal potassium current (Ik). In addition, increase in concentration of intracellular calcium also result in activation of the CaMKII complex. CaMKII act on ion channels in the cell membrane changing neuronal firing and induced hypertension.

MAPK-AP1/ NFκB pathway

Ang-II also activates the Mitogen-Activated Protein Kinase (MAPK) pathway. ROS directly activate the MAPK, which increases the NADPH oxidase activity leading to more ROS. MAPK also activates nuclear Transcriptional Factors (TF), such as NFκB and AP-1, which modulate the expression of Tyrosine Hydroxylase (TH) and AT1 receptors (Figure 2).

In hypertension, angiotensin II (Ang II) and ROS can increase the activity of the transcription factor Nuclear Factor-κB (NFκB), which, in turn, can further increase ROS expression in a positive feed-forward way [27-31], and modulate the expression of Tyrosine Hydroxylase (TH) and AT1 receptors. Along with Ang II, NFκB acts to increase the presence of ROS, such as $O_2^{\cdot-}$, which subsequently affects the present NO levels, thereby effecting neuronal activity/function and NE release. Blockade of NFκB at two separate locations in its activation pathway prevents these changes, restores the RAS balance, and promotes the antihypertensive RAS arm, including ACE2 and the MasR. This blockage also reduces ROS expression and elevates nNOS, all of which contribute to a reduction in sympathetic outflow and an improvement in the Ang II-induced hypertensive state [32].

The other downstream mechanisms by which ROS affect the PVN neurons is by modulating activator protein-1 (AP-1) activity [33]. AP-1 activity can be longitudinally monitored in vivo by bioluminescence imaging in 2K1C or sham mice model that had undergone PVN-targeted microinjections of an adenovirus encoding the firefly luciferase (Luc) gene downstream of AP-1 response elements (AdAP-1Luc) [34]. In 2K1C-induced Renovascular Hypertension (RVH) model in mice, Burmeister et al. [33] found that AP-1 is robustly activated in the PVN with a time-course that is consistent with its involvement in the development of hypertension, and its inhibition by $O_2^{\cdot-}$ scavenging parallels the inhibition of hypertension. And they also found that dominant-negative inhibition of AP-1 transcriptional activity in the PVN prevents RVH. Therefore they concluded that RVH is mediated by oxidative stress-induced AP-1 activation in the PVN.

The ERK1/2-RSK-nNOS Signaling Pathway

Previous study showed that eNOS might play a critical role in NTS cardiovascular function via the adenosine- extracellular signal-regulated kinase (ERK)1/2- eNOS signaling pathway [35]. But recently, Wen et al. [36] found nNOS increased after inhibition of Ang II downstream of AT₁R or depletion of ROS in the NTS of SHR_s which suggests nNOS might be one of the downstream targets of Ang II that is involved in NO production and that modulates blood pressure function in the NTS of SHR_s. They also observed that ROS accumulation inhibited ERK1/2 activation in the NTS and induced hypertension. Wu et al. [37] demonstrated that ROS can enhance protein tyrosine kinase activity to

regulate extracellular signal-regulated kinase (ERK1/2 phosphorylation in neuroblastoma in vitro). Chen et al. [38] also reported that activation of protein phosphatases (e.g. PP2A or PP5) can inhibit hydrogen peroxide-induced ERK1/2 phosphorylation in neuronal cells. ERK1/2 is involved in cellular differentiation, proliferation and development in neuronal cells. Thus, ERK1/2 not only regulates neuronal development but also play regulatory roles in neurons in response to various physiological stimuli.

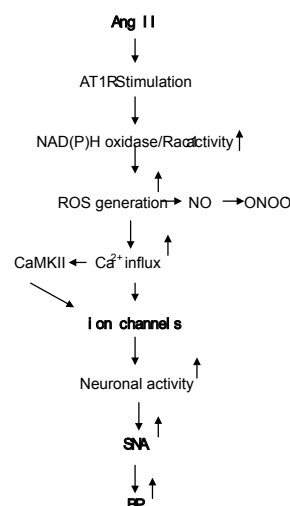


Figure 1: NADPH-ROS Signaling Pathway

The general process of this signaling pathway: firstly, Ang II activates the AT1 receptors, then, the cytoplasmic subunits bind to the membrane subunits and activate the enzyme, resulting in the intracellular production of superoxide (converts the intracellular nitric oxide to peroxynitrite). The accumulation of superoxide leads to changes in ion channels, particularly calcium and potassium channels, altering neuronal firing properties in areas of the brain such as the RVLM, resulting in increased sympathetic nerve activity and increased blood pressure [27]. In addition, the intracellular calcium concentration increased also result in activation of the CaMKII complex. CaMKII act on ion channels in the cell membrane changing neuronal firing and induced hypertension.

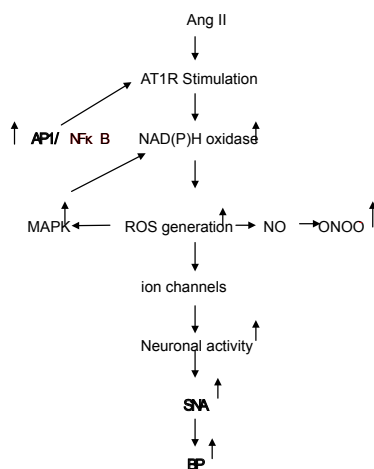


Figure 2: MAPK-AP1/ NFκB pathway

Ang-II activates the Mitogen-Activated Protein Kinase (MAPK) pathway. ROS directly activate the MAPK, which increases the NADPH oxidase activity leading to more ROS. MAPK also activates nuclear Transcriptional Factors (TF), such as NFκB and AP-1, which modulate the expression of Tyrosine Hydroxylase (TH) and AT1 receptors.

In conclusion, Ang II binds to the AT1R, leading to superoxide production. Superoxide accumulation may inhibit ERK1/2 activity. Moreover, Ribosomal protein S6 Kinase (RSK) and nNOS cascade activity is also inhibited. This ultimately leads to decreased NO concentrations in the NTS and increased blood pressure. Ang II may inhibit ERK1/2-RSK-nNOS signaling, (Figure 3) not only through ROS but also through other signaling molecules, to modulate blood pressure in the NTS of SHRs.

COX-1-derived PGE₂ and EP₁R signal pathway

Cyclooxygenase (COX)-derived prostanoid signaling has long been implicated in the pathogenesis of Ang II-dependent hypertension. Ang II-evoked ROS formation in dissociated SFO cells is prevented by inhibition of COX-1 but not COX-2. A key finding of the present study demonstrates that COX-1-derived prostaglandin E₂ (PGE₂) and its receptor EP₁ (EP1R) are required for this Ang II-evoked ROS formation in the SFO in vitro and in vivo [39]. And both in vitro and in vivo inhibition of EP1R prevent Ang II-induced ROS accumulation in the SFO, a response that is known to have a causative role in slow-pressor Ang II hypertension [12,40]. Virally mediated reconstitution of EP1R selectively in the SFO of EP1R-null mice restores hypertension and SFO ROS formation in response to slow pressor Ang II infusions. It is the first evidence that COX-1-derived PGE₂/EP1R signaling in the SFO is required for the ROS-mediated hypertension elicited in the slow-pressor Ang II model. It is a key role for PGE₂/EP1R signaling in the CNS, particularly the SFO, in mediating systemic Ang II-dependent hypertension [39].

NADPH oxidase mediates PGE₂/EP1R-mediated ROS formation in the SFO may be through Ca²⁺ signaling. Because activation of EP1R results in inositol triphosphate (IP₃)-mediated release of intracellular Ca²⁺ and reduced Ca²⁺ efflux through the Na⁺/Ca²⁺ exchanger [41].

BDNF/TrkB-UCP2 signaling pathway (protective pathway)

One of the well-recognized protective mechanisms against stressful insults in brain is expression of neurotrophic factors, in particular Brain-Derived Neurotrophic Factor (BDNF) [42]. BDNF engaged in oxidative stress-associated neurogenic hypertension via negative-feedback regulation of tissue O₂⁻ levels in RVLM, protects striatal neurons from cell death as an antioxidant [42]. Ang II induces O₂⁻ dependent upregulation of BDNF in RVLM via phosphorylation of cAMP response element binding protein (CREB). The Ang II-activated BDNF/TrkB signaling, in turn, exerts negative-feedback regulation on tissue O₂⁻ level in RVLM [43]. BDNF and the Tropomyosin receptor kinase (Trk) B are distributed in brainstem nuclei that subserve neural regulation of arterial pressure [44,45].

Recently, Chan et al. [46] suggested that transcriptional up-regulation of mitochondrial uncoupling protein 2 (UCP2) in response to an increase in superoxide plays an active role in the feedback regulation of ROS production in the RVLM. BDNF restores the reduced mitochondrial electron transport capacity (ETC) and upregulates mitochondrial UCP2 expression in RVLM. UCP2 is a homolog of UCP protein family of mitochondrial anion transporters that uncouple ATP synthesis from oxidative phosphorylation by causing proton leakage across the mitochondrial inner membrane, leading to a decrease in proton electrochemical gradient across the inner mitochondrial membrane and the resultant mitigation in the production of mitochondria-derived ROS, in particular O₂⁻. Through inhibition of p47 phosphorylation, preservation of mitochondrial electron transport capacity, and upregulation of mitochondrial UCP2,

result in protection against Ang II-induced hypertension and oxidative stress. Besides, stimulation of PPAR- γ also results in the upregulation of UCP2, thereby ameliorates Ang II-induced chronic oxidative stress and long-term pressor response (Figure 4).

Other signaling pathway

Except the signaling pathway above, there is another pathway may involve in the Ang II induced hypertension. Such as Kirabo A et al. [47] found that, vascular smooth muscle cells (VSMCs) Jak2 expression is involved in the pathogenesis of Ang II-dependent hypertension due to the increased presence of reactive oxygen species (ROS). Chan et al. [48] reported that, in neurons, damaged ETC complexes are a source of mitochondrial-produced ROS (Figure 5). Though there have been many signaling pathway were found, additional studies are still required to examine the mechanism(s) by which ROS increase sympathetic tone and drive the development of hypertension.

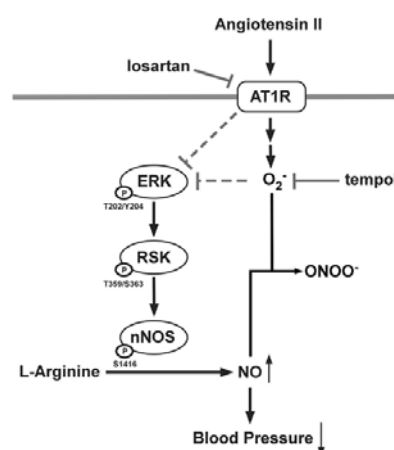


Figure 3: The ERK1/2-RSK-nNOS Signaling Pathway
Cheng WH, Lu PJ, et al [39] Angiotensin II Inhibits Neuronal Nitric Oxide Synthase Activation through the ERK1/2-RSK Signaling Pathway to Modulate Central Control of Blood Pressure.

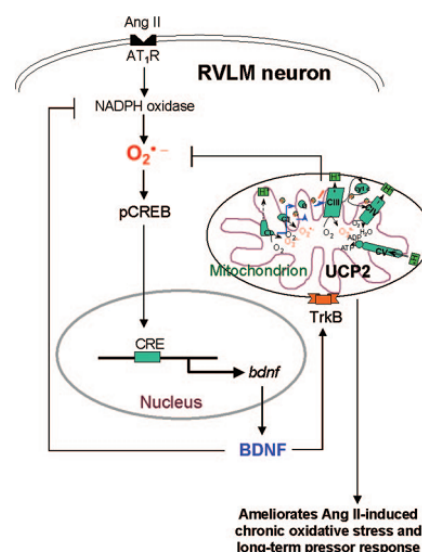


Figure 4: BDNF/TrkB-UCP2 signaling pathway
Samuel H.H. Chan, Chih-Wei J et al. [46] Transcriptional Upregulation of Brain-Derived Neurotrophic Factor in Rostral Ventrolateral Medulla by Angiotensin II.

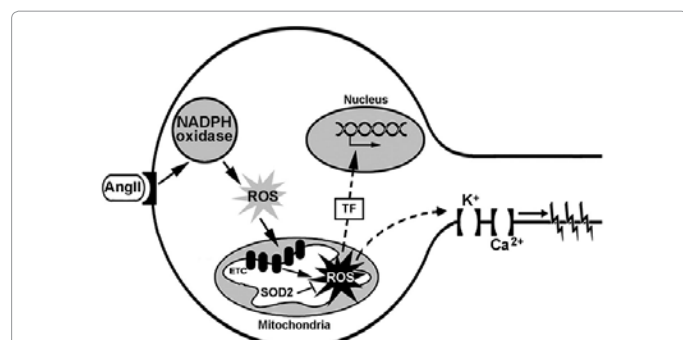


Figure 5: Ang II stimulation of neurons in the RVLM increases NADPH oxidase-derived ROS, which, in turn, damage mitochondrial ETC complexes leading to an increase in mitochondrial-produced ROS.

The Novel Therapeutic Targets of Neurogenic Hypertension

ROS have been shown as important signaling factors for enhancement of the neurogenic hypertensive response through Ang II mechanisms [12,30,31]. Thus, potentially inhibiting one of the mechanistic pathways by which the hypertensive response and sympathoexcitation, is modulated. These conclusions are confirmed by previously that ICV administration of the ROS scavenger, Tempol-an SOD mimetic, reduced renal sympathetic nerve activity in Ang II-treated rats. Similarly, acute inhibition of the NADPH oxidase with apocynin or scavenging of superoxide with tiron, a superoxide dismutase mimetic, improves the baroreflex sensitivity in spontaneously hypertensive rats [49]. In addition, microinjection of vitamin C into the PVN also reduces the high blood pressure and sympathetic activity of renovascular hypertensive rats [50,51].

Of note growing evidence now suggests that hypertension and sympathoexcitation can be prevented /attenuated by selective genetic or pharmacological manipulation of AT1 mRNA expression specifically in the SFO or RVLM. In support of that concept, Zimmerman et al. [12] have shown that intracerebroventricular injection of losartan, an AT1 receptor antagonist targeting the SFO, prevents the hypertension induced by chronic peripheral ang II infusion in mice. Administration of losartan reduces ROS accumulation in the RVLM and improves baroreflex sensitivity.

Considering the central position that MAPK plays in regulating the neurogenic component of hypertension, choline exerts significant anti-hypertensive activity through inhibition of ROS-mediated p38 MAPK activation as well as regulation of Ca^{2+} -mediated calcineurin cell signal transduction pathway. Rosiglitazone also be able to inhibit Ang II-induced CRP generation in HAECs by regulating AT(1)-ROS-MAPK signal pathway. And bilateral NF κ B (the downstream of MAPK) blockade can reduce the ROS reactions typically associated with Ang II-induced hypertension [32].

In addition, EP1R, as part of signaling pathway, is a novel target for the treatment of hypertension. Pharmacological inhibition of EP1R selectively in the CNS prevents slow-pressor Ang II hypertension, and central Ang II-driven sympathetic and dipsogenic responses are also mediated by brain EP1R [39].

Direct RVLM administration of coenzyme Q10, a mitochondrial electron transporter and antioxidant, restored electron transport capacity, decreased mitochondrial- localized ROS production, and significantly reduced mean systemic arterial pressure and sympathetic

neurogenic vasomotor tone in SHRs [52]. Therefore, Coenzyme Q10 can be used as a means of treatment of hypertension.

Intra-neuronal signaling and the downstream redox-sensitive proteins involved in controlling the neuronal discharge rate, the sympathetic outflow. Thus, the role of elevated sympathetic nervous system in neurogenic hypertension becomes an important therapeutic target, and corroborated by the recent successes in its treatment by renal sympathetic denervation in humans [53-56]. In our laboratory we found that [56], in mongrel neurogenic hypertensive dogs, renal Sympathetic Denervation causes substantial and sustained blood pressure reduction, along with reducing the level of angiotensin II and increasing the expression of ACE2. Moreover, in our clinical trials of resistant hypertension, we also found the similar results.

Future studies are needed to identify new redox-based therapeutics. Upregulation of endogenous antioxidants in the regulation of ROS homeostasis and renal sympathetic denervation are both potential therapeutic targets.

Summary and Conclusion

In summary, abundant research now suggests that a key mechanism by which Ang-II influences blood pressure is via its ability to activate ROS signaling pathways. Upstream and downstream consequences of the precise mechanisms are discussed. Several Questions remain, however, because the ROS signaling pathway are complex. Further studies are required to gain a better understanding of the role of brain ROS in autonomic cardiovascular regulation and potential therapeutic targets.

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