

REVIEW SERIES

Oxidative stress in the cardiovascular center has a pivotal role in the sympathetic activation in hypertension

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Activation of the sympathetic nervous system has an important role in the pathogenesis of hypertension. However, the precise mechanisms involved are not fully understood. Oxidative stress may be important in hypertension as well as in other cardiovascular disorders. We investigated the role of oxidative stress, particularly in the rostral ventrolateral medulla (RVLM), which is known as the cardiovascular center in the brainstem, in the activation of the sympathetic nervous system in hypertension. We observed that the reactive oxygen species (ROS) production increases in the RVLM in hypertensive rats, thereby enhancing the central sympathetic outflow, which leads to hypertension. Furthermore, the environmental factors of high salt intake and a high-calorie diet may also increase the ROS production in the RVLM, thereby activating the central sympathetic outflow and increasing the risk of hypertension. The activation of the nicotinamide adenine dinucleotide phosphate oxidase via the angiotensin type 1 (AT1) receptors is suggested to be the major source of ROS production, and an altered downstream signaling pathway is involved in the activation of the RVLM neurons, leading to enhanced central sympathetic outflow and hypertension. Thus, the brain AT1 receptors may be novel therapeutic targets, and, in fact, oral treatment with angiotensin receptor blockers has been found to inhibit the central AT1 receptors, despite the blood–brain barrier.

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INTRODUCTION

Recent studies in animals as well as humans support the importance of the activation of the sympathetic nervous system in the pathogenesis of hypertension, including the initial pathological events, the development of hypertension and the end-organ damage.^{1–5} The activation of the renin–angiotensin system is known to be involved in the cardiovascular continuum.^{2,6}

Although the kidney has an important role in the pathogenesis of hypertension, the kidney function itself is influenced by renal nerves.^{7,8} This is also evidenced in humans with the development of hypertension after renal nerve ablation.^{9,10}

There is accumulating evidence demonstrating that oxidative stress has an important role in the various aspects of hypertension.¹¹ However, the study of the role of brain oxidative stress in the activation of the sympathetic nervous system and hypertension has been limited.^{12–17} We investigated the role of oxidative stress in the brain, particularly in the rostral ventrolateral medulla (RVLM) in the brainstem, which is known as the cardiovascular center, in the pathogenesis of hypertension.^{2,13,16,18–21} The RVLM neurons determine the basal central sympathetic outflow and integrate the inputs

from baroreceptors, chemoreceptors and visceral receptors via the nucleus of the solitary tract (NTS).^{22–27} Importantly, it also receives the inputs from the paraventricular nucleus of the hypothalamus, which is known as a key nucleus of the central cardiovascular regulation.^{22,28,29} Therefore, we focused on the RVLM in our studies. Other investigators have also confirmed our initial observations, and other details have been further investigated. In this review, we describe the importance of oxidative stress in the brainstem, particularly in the RVLM, on the neural regulation of the sympathetic activity and its contribution to the pathophysiology of hypertension based on the findings of our studies and those of others.

ROLE OF THE REACTIVE OXYGEN SPECIES (ROS) IN THE RVLM IN HYPERTENSIVE RATS

We found that oxidative stress in the RVLM is increased and contributes to the neural mechanisms of hypertension in stroke-prone spontaneously hypertensive rats (SHRSPs).¹⁶ Oxidative stress was evaluated by two methods. The levels of the thiobarbituric acid-reactive substances, an end product of lipid peroxidation, were increased in the whole brain, NTS and RVLM of the SHRSP compared

with normotensive control Wistar–Kyoto (WKY) rats. Another method used the electron spin resonance spectroscopy with the nitroxide radical 4-hydroxy-2,2,6,6-tetramethyl-piperidine-*N*-oxyl as a spin probe. The intensity of the electron spin resonance signals decreased more rapidly in the RVLM of the SHRSP than in the WKY rats. Functionally, we performed a microinjection of tempol, a membrane-permeable superoxide dismutase (SOD) mimetic, into the RVLM and measured blood pressure and heart rate. Microinjection of tempol into the RVLM decreased the blood pressure and heart rate in the SHRSP, but not in the WKY rats. To confirm the role of ROS production in the RVLM in hypertension in the awake state, we transfected adenovirus vectors encoding the *Mn-SOD* gene into the bilateral RVLM in SHRSP. MnSOD overexpression in the RVLM decreased blood pressure, heart rate and urinary norepinephrine excretion in the SHRSP, but not in WKY rats. We also found reduced SOD activity in the RVLM of the SHRSP compared with the WKY rats, which led to a decreased capability of scavenging superoxide anions. Together, these findings indicated that oxidative stress in the RVLM increased the blood pressure, which may have occurred via an increase in sympathetic nerve activity, and this mechanism was involved in the neural pathophysiology of hypertension in the SHRSP. Consistent with our observations, it was reported that increased superoxide anion in the RVLM contributed to hypertension in spontaneously hypertensive rats (SHRs).¹⁷ There was an increase in the intensities and numbers of cells in the RVLM with red fluorescent ethidium bromide, which reflected the superoxide level, in SHR compared with WKY rats. Microinjection of a membrane-permeable SOD mimetic, Mn(III)-tetrakis-(4-benzoic acid) porphyrin, into the RVLM elicited an enhanced depressor response in the SHR compared with the WKY rats. The expression of Mn-SOD, but not Cu/Zn-SOD, in the RVLM was reduced in the SHR. Chan *et al.*³⁰ demonstrated that the Cu/Zn-SOD, Mn-SOD and catalase expression levels (both protein and mRNA) and their activities in the RVLM were reduced in the SHR compared with the WKY rats, and this was also associated with the increased superoxide and hydrogen peroxide production in the SHR. In fact, overexpression of Cu/ZnSOD, MnSOD or catalase into the RVLM reduced blood pressure and heart rate in the SHR. It is interesting to note that every intervention tested led to a similar extent of reduction of blood pressure and superoxide production in the SHR, although it remains unknown whether the reduced expression and activity of hydrogen peroxide is a major factor for stimulating ROS production by a feed-forward mechanism. In addition, recent studies suggest that hypertension and sympathoexcitation in renovascular (two-kidney one-clip) hypertensive rats are associated with oxidative stress in the RVLM and paraventricular nucleus of the hypothalamus and with systemic oxidative stress.^{31,32} Because the two-kidney and one-clip model is an angiotensin II-dependent hypertension model, it is not surprising that oxidative stress was increased in this model. However, it is important that the increased oxidative stress in the autonomic brain regions, such as the RVLM and paraventricular nucleus of the hypothalamus, was involved in the activation of the sympathetic nervous system as one of the mechanisms of hypertension in this model. The nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase subunits and angiotensin type 1 (AT1) receptor mRNA expression levels in the RVLM were increased in this model, and the microinjection of tempol or vitamin C into the RVLM reduced the blood pressure and renal sympathetic nerve activity, supporting this notion. Importantly, a recent study found that ROS signaling in the RVLM had a major role in the enhanced central sympathetic outflow in the two-kidney one-clip hypertensive rats.³³ In that study, transfection of an adenovirus vector encoding

the *Cu/ZnSOD* gene into the RVLM neurons was confirmed by costaining with tyrosine hydroxylase and neuronal nuclei. Regarding angiotensin II, the peripheral slow-prior dose of angiotensin II (600 ng kg⁻¹ min⁻¹ for 2 weeks) in mice led to a gradual development of hypertension that was correlated with marked elevations in superoxide production.³⁴ In this case, the authors emphasized the importance of the sub-fornical organ, in which the blood–brain barrier is lacking and the AT1 receptors are rich. Angiotensinergic inputs in the sub-fornical organ are delivered to the paraventricular nucleus of the hypothalamus, and then it sends the neural information to the RVLM neurons. Thus, the functional responses of the sub-fornical organ to angiotensin II are the increases in drinking behavior and blood pressure via the activation of the sympathetic nervous system and vasopressin release.

SOURCES OF ROS PRODUCTION IN THE RVLM IN HYPERTENSION

For the sources of ROS production, there are several candidates, such as NAD(P)H oxidase, xanthine oxidase, uncoupled nitric oxide synthase and mitochondria. Among them, we demonstrated that the activation of the NAD(P)H oxidase through AT1 receptors had a major role in the ROS production in the brainstem including the RVLM of the SHRSP (Figure 1).¹⁹ Regional expression of the NAD(P)H oxidase and SOD has been demonstrated in the brain including the NTS and RVLM.^{35–37} In addition, we showed that Rac1 activation occurs in this process. Rac1 is a small G protein involved in integrating the intracellular transduction pathways toward NAD(P)H activation and requires lipid modifications to migrate from the cytosol to the cell membrane. The inhibition of Rac1 caused by the transfection of the adenovirus vectors encoding a dominant-negative Rac1 into the RVLM or NTS decreased the blood pressure, heart rate and urinary norepinephrine excretion in the SHRSP, but not in the WKY rats.^{12,38} It was demonstrated that Nox2-containing NAD(P)H oxidase followed by an influx of Ca²⁺ via the L-type Ca channels was the source of the angiotensin II-induced ROS production in the NTS neurons that were anterograde labeled from the vagal afferents using DiA.^{39,40} Because azelnidipine could reduce oxidative stress in the RVLM of SHRSP associated with sympathoinhibition, the inhibition of the Ca²⁺ channel in the RVLM may have reduced oxidative stress.²¹ Inhibition of Rac1 also reduced the NAD(P)H oxidase activity and ROS production in the brainstem of SHRSP. Thus, our findings indicate that the activation of the Rac1 in the

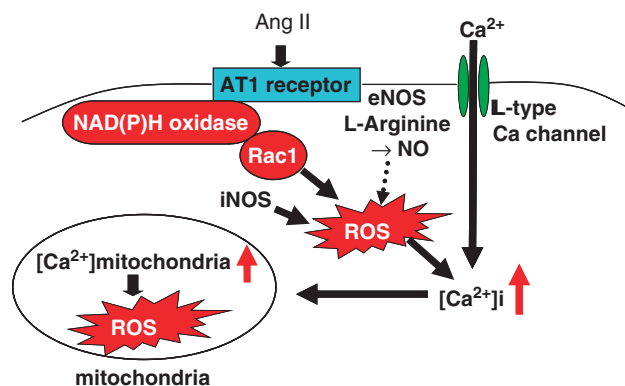


Figure 1 Possible mechanisms involved in the ROS production in the RVLM neurons of the brainstem in hypertension (modified from Nozoe *et al.*,¹⁹ with permission). Ang, angiotensin; eNOS, endothelial NOS.

RVLM or NTS produces ROS via the NAD(P)H oxidase in SHRSP. In fact, it was demonstrated that the activation of the Rac1/NAD(P)H oxidase was required in the pressor and dipsogenic actions of angiotensin II in the brain.⁴¹

The brain renin-angiotensin system is upregulated in chronic disease states such as hypertension and heart failure with enhanced central sympathetic outflow.^{42–47} We also determined whether mitochondria-derived ROS mediates sympathoexcitation induced by angiotensin II in the RVLM.¹⁹ It is well established that exogenously administered angiotensin II into the RVLM elicits the pressor response via the activation of the sympathetic nervous system.^{48,49} In contrast, the inhibition of AT1 receptors in the RVLM by an AT1 receptor blocker does not reduce blood pressure in normotensive rats.^{48,49} However, it reduces blood pressure with sympathoinhibition in hypertensive rats such as SHR.^{18,48} We found that the overexpression of MnSOD attenuated the angiotensin II-induced pressor response and also suppressed the angiotensin II-induced ROS production in the RVLM.¹⁹ In that study, we showed that angiotensin II increased mitochondrial ROS production *in vitro*. Overexpression of MnSOD and rotenone, a mitochondrial respiratory complex I inhibitor, suppressed the angiotensin II-induced ROS production. The depletion of extracellular Ca²⁺ with ethylene glycol bis-*N,N,N',N'*-tetraacetate and the administration of *p*-trifluoromethoxycarbonylcyanide phenylhydrazide, which prevents Ca²⁺ uptake into the mitochondria, blocked the angiotensin II-elicited mitochondrial ROS production. Together, our findings indicate that angiotensin II increases mitochondrial ROS production in the RVLM, leading to sympathoexcitation. Furthermore, NAD(P)H oxidase-derived ROS may trigger a Ca²⁺ influx, and the mitochondrial Ca²⁺ accumulation would lead to mitochondrial ROS production. A recent study also demonstrated that the ROS-induced impairment of the mitochondrial electron transport chain complexes in the RVLM contribute to further chronic oxidative stress, thereby leading to augmented central sympathetic outflow and hypertension.⁵⁰ In addition, it was demonstrated that superoxide mediates the angiotensin II-induced influx of extracellular Ca²⁺ in cultured neural cells.⁵¹ It should be noted that angiotensin II regulates neuronal activity by reducing the potassium current, and the increased superoxide production in neurons closes the potassium channels to inhibit a delayed rectifying potassium current resulting in membrane depolarization.⁵²

When discussing the role of ROS in the regulation of sympathetic activity, we need to consider the role of nitric oxide (NO) because NO and ROS interact. In general, NO in the brain, including the RVLM, inhibits sympathetic activity.^{53,54} In fact, we found that an increase in NO in the RVLM reduces blood pressure, heart rate, and sympathetic activity in the WKY rats and SHRSP.⁵⁵ Moreover, the magnitude of the decreases in these variables were greater in the SHRSP than in the WKY rats, suggesting a deficiency in the NO bioavailability in SHRSP. Our important finding was that overexpression of the inducible NO synthase (iNOS) in the RVLM elicited blood pressure elevation and sympathoexcitation in the WKY rats and that this was caused by an increase in oxidative stress.⁵⁶ This may have been caused by the so-called uncoupling of the NO synthase function because of the deficiency of the precursor of L-arginine and/or the cofactor tetrahydrobiopterin. We found that the microinjection of the L-arginine reversed the blood pressure elevation elicited by the overexpression of iNOS in the RVLM. Importantly, we have found that the iNOS expression in the RVLM is enhanced in the SHR compared with the WKY rats, and the microinjection of the iNOS blockers into the RVLM reduced the blood pressure only in the SHR.⁵⁷ A recent study also suggested that the upregulation of AT1 receptors and iNOS in the RVLM was important

for the maintenance of high blood pressure and renal sympathetic activation in the two-kidney one-clip hypertensive rats.⁵⁸

SIGNALING PATHWAYS OF AT1 RECEPTOR ACTIVATION AND ROS PRODUCTION IN THE BRAIN

Recently, we found that the AT1 receptor activates caspase-3 through the Ras/mitogen-activated protein kinase/extracellular signal-regulated kinase (ERK) in the rostral ventrolateral medulla, which is involved in the sympathoexcitation in SHRSP.²⁰ The activities of Ras, p38 mitogen-activated protein kinase, ERK and caspase-3 in the RVLM were elevated in the SHRSP compared with those in the WKY rats. The phosphorylation of the proapoptotic protein Bax and Bad, which releases cytochrome *c* in the mitochondria, leads to caspase-3 activation.²⁰ In contrast, the phosphorylation of the antiapoptotic protein Bcl-2 inhibits the caspase-3 activation. Intracerebroventricular infusion of a caspase-3 inhibitor reduces blood pressure, heart rate and sympathetic activity in the SHRSP, but not in the WKY rats. Intracerebroventricular infusion of an AT1 receptor blocker also reduced the blood pressure, heart rate, and sympathetic activity and also reduced the activities of Ras, p38 mitogen-activated protein kinase, ERK and caspase-3 in the RVLM of SHRSP, suggesting that these pathways exist downstream to the AT1 receptor activation in the RVLM of SHRSP and are related to blood pressure elevation and sympathoexcitation of SHRSP. In that study, we did not examine the effect of ROS reduction in the RVLM on caspase-3 activity in SHRSP. In support of our findings, it was reported that the NAD(P)H oxidase-derived superoxide anion mediates the activation of p38 mitogen-activated protein kinase/Jun N-terminal kinase by angiotensin II in the RVLM and pressor response.⁵⁹ Furthermore, in a later study, the authors suggested that the activation of the NAD(P)H oxidase/ERK in the RVLM induced the phosphorylation of the transcriptional factor cyclic adenosine monophosphate response element-binding protein and *c-fos* induction, thereby contributing to the long-term pressor response triggered by angiotensin II.⁶⁰ It is also important to note that the activation of the activator protein 1 and the Jun N-terminal kinase may occur in rabbits with rapid pacing-induced heart failure.⁶¹ Thus, the signaling pathway followed by the activation of the ROS production may differ between hypertension and heart failure because of the advancement of the disease state. Further studies are required to clarify the mechanisms involved.

INFLUENCE OF SALT AND OBESITY ON OXIDATIVE STRESS IN THE BRAIN AND SYMPATHETIC FUNCTION IN HYPERTENSION

A high salt intake is an important environmental factor for the development of hypertension.⁶² Increasing evidence suggests that central nervous system mechanisms are involved in salt-induced hypertension, although the kidney also has a key role in salt-induced hypertension.^{63–66} We sought to determine whether high salt intake increases hypertension in the SHR and whether the increased ROS production in the RVLM contributes to this mechanism.¹⁸ High salt intake augmented the development of hypertension in the SHR beginning at the age of 6–12 weeks. There was greater ROS production in the RVLM of the SHR with high salt intake than in those with a regular salt intake. The scavenging of the produced ROS by tempol injected into the RVLM elicited a greater reduction of blood pressure in the SHR with a high salt intake than in those with a regular salt intake. We suggested that the increased AT1 receptors and NAD(P)H oxidase expression levels in the RVLM were responsible for the increased ROS production and blood pressure in SHR with a high salt intake

compared with those with a regular salt intake. The enhanced depressor response to an AT1 receptor supported this suggestion. Consistent with our findings, increased oxidative stress was involved in the blood pressure elevation through an enhanced central sympathetic outflow in Dahl salt-sensitive rats.⁶⁷ In that study, the activation of the NAD(P)H oxidase in the brain, particularly in the hypothalamus, was also suggested to be a source of ROS production. It is possible that the enhanced neuronal activity in the hypothalamus, such as the paraventricular nucleus, conveyed the input to the RVLM, thereby augmenting the RVLM neuronal activity. It is interesting that the RVLM neuronal activity was enhanced in rats with a high-salt diet. However, on the basis of our findings, oxidative stress in the RVLM itself causes the increased central sympathetic outflow, thereby contributing to the development of hypertension with a high-salt diet.

It has also been demonstrated that the sympathetic activation has an important role in obesity-related hypertension, including the metabolic syndrome.^{68–73} Insulin or leptin increases the sympathetic activity, thereby causing increased blood pressure; however, with obesity or metabolic syndrome, there is insulin or leptin resistance. In addition, it has been demonstrated that the RVLM neurons are activated in obesity-induced rats.⁷³ A recent study suggested that oxidative stress, particularly in the hypothalamus, was involved in the activation of the sympathetic nervous system in obesity-induced rats.⁷⁴ The importance of the RVLM is also suggested in this regard.⁷⁵

EFFECTS OF AT1 RECEPTOR BLOCKERS ON OXIDATIVE STRESS IN THE BRAIN AND SYMPATHETIC FUNCTION IN HYPERTENSION

As described above, the upregulation of AT1 receptors in the brain has an important role in the pathophysiology of hypertension.^{22,24,42} It is interesting to note that AT1 receptors are rich in the specific brain nuclei that regulate the sympathetic activity such as the anteroventral third ventricle, paraventricular nucleus of the hypothalamus, NTS and the RVLM in the brainstem.^{46,47,76} It has been demonstrated that the peripheral administration of the AT1 receptor blockers penetrate the blood–brain barrier and blocks the AT1 receptors within the brain as well as outside of the brain, although the extent of the blocking action within the brain varies among the AT1 receptor blockers when they are administered peripherally.⁷⁷ There are two important observations in this regard. First, the peripheral treatment with AT1 blockers attenuates or nearly blocks the pressor response to centrally administered angiotensin II.^{78–80} This is also observed with the microinjection of angiotensin II into the RVLM in SHR that are orally treated with olmesartan.⁸¹ Second, the central nervous system blockade by the peripheral administration of AT1 receptor blockers has been documented by autoradiographic binding studies.⁷⁷ It should be noted that the high density of AT1 receptors is present in brain regions that are involved in the regulation of the autonomic nervous system such as the circumventricular organs (for example, the sub-fornical organ, the organum vasculosum laminae terminalis and area postrema) outside of the blood–brain barrier where peripherally administered AT1 receptor blockers are able to access without considering the existence of the blood–brain barrier as well as inside of the blood–brain barrier (paraventricular nucleus of the hypothalamus, NTS and the RVLM).⁷⁶ Recent studies suggest that the systemic administration of the AT1 receptor blockers also act on the AT1 receptors within the brain, thereby reducing blood pressure in hypertensive rats.^{80–82} The extent of the actions of the AT1 receptor blockers within the brain depends partly on the lipophilicity and pharmacokinetics.^{77,78} However, even the hydrophilic AT1 receptor blockers are able to block the AT1 receptors within the blood–brain barrier.⁷⁹ The mechanism(s)

involved remain unknown. It is an important question because the brain AT1 receptors are now considered to be novel and pleiotropic therapeutic targets for hypertension and related cardiovascular diseases. Thus, it is conceivable that oral treatment with an angiotensin receptor blocker may block the AT1 receptors in the brain, particularly in the RVLM, thereby reducing the ROS production and reducing the blood pressure via inhibiting the sympathetic activity. Treatment with telmisartan orally reduced the blood pressure and urinary norepinephrine excretion in SHRSP, and it was associated with a reduction of ROS production in the brainstem including the RVLM.¹² The reduction of oxidative stress was evaluated with thiobarbituric acid-reactive substance levels, electron spin resonance spectroscopy and 2,3-dihydroxybenzoic acid measurements. In another study, treatment with olmesartan was carried out in SHRSP, and brain oxidative stress was evaluated by using the *in vivo* electron spin resonance spectroscopy method.⁸³ It did not induce reflex-mediated sympathoexcitation, despite the fact that blood pressure reduction was > 50 mm Hg. In contrast, treatment with hydralazine or a combination of hydralazine and hydrochlorothiazide elicited a reflex-mediated sympathoexcitation and a blood pressure reduction that was similar in extent to the reduction observed with the treatment with telmisartan or olmesartan. Importantly, the antihypertensive treatment with hydralazine or a combination of hydralazine and hydrochlorothiazide did not reduce oxidative stress in the brain including the RVLM.^{12,83}

PERSPECTIVES

Considering the importance of the AT1 receptors in the brain, particularly in the autonomic regulatory regions, such as the RVLM, NTS and paraventricular nucleus of the hypothalamus, it should be noted that these areas contain a high density of AT1 receptors in human.⁸⁴ The AT1 receptor blockers are widely used in the treatment of hypertension.⁸⁵ The inhibition of the brain AT1 receptors may have a significant role in the sympathoinhibitory effect via the reduction of oxidative stress in humans. It is also suggested that AT1 blockers may have neuroprotective effects, reducing the incidence of stroke and improving cognition function.^{85,86} In addition, renal afferent nerves may also contribute to the blood pressure elevation according to the recent findings of the renal nerve ablation in patients with resistant hypertension.^{8,10} Renal afferent nerves project directly to many areas in the central nervous system controlling the sympathetic nervous system activity such as the NTS and hypothalamus.^{87–89} It is demonstrated that oxidative stress mediates the stimulation of the sympathetic nerve activity in the phenol renal injury model of hypertension in which the renal afferent nerves are stimulated.⁹⁰ In this model, the brain AT1 receptor and NAD(P)H oxidase are activated. It is suggested that the increased ROS production and reduced neuronal NOS expression may be involved in this mechanism(s), which leads to the alteration of cytokines in the brain.^{90,91} It is interesting and important to consider AT1 receptors and the related ROS production in the brain as novel therapeutic targets for the treatment of hypertension, which are focused on the aspects of sympathetic activation.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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