HIGHLIGHTED TOPIC | Mechanisms of Sympathetic Regulation in Cardiovascular Disease

Brain stem oxidative stress and its associated signaling in the regulation of sympathetic vasomotor tone

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There is now compelling evidence from studies in humans and animals that overexcitation of the sympathetic nervous system plays an important role in the pathogenesis of cardiovascular diseases. An excellent example is neurogenic hypertension, in which central sympathetic overactivation is involved in the development, staging, and progression of the disease, and one of the underlying mechanisms involves oxidative stress in key brain stem sites that are engaged in the regulation of sympathetic vasomotor tone. Using the rostral ventrolateral medulla (RVLM) and nucleus tractus solitarii (NTS) as two illustrative brain stem neural substrates, this article provides an overview of the impact of reactive oxygen species and antioxidants on RVLM and NTS in the pathogenesis of neurogenic hypertension. This is followed by a discussion of the redox-sensitive signaling pathways, including several kinases, ion channels, and transcription factors that underpin the augmentation in sympathetic vasomotor tone. In addition, the emerging view that brain stem oxidative stress is also causally related to a reduction in sympathetic vasomotor tone and hypotension during brain stem death, methamphetamine intoxication, and temporal lobe status epilepticus will be presented, along with the causal contribution of the oxidant peroxynitrite formed by a reaction between nitric oxide synthase II (NOS II)-derived nitric oxide and superoxide. Also discussed as a reasonable future research direction is dissection of the cellular mechanisms and signaling cascades that may underlie the contributory role of nitric oxide generated by different NOS isoforms in the differential effects of oxidative stress in the RVLM or NTS on sympathetic vasomotor tone.

superoxide; rostral ventrolateral medulla; nucleus tractus solitarii; nitric oxide synthase II; peroxynitrite

There is now compelling evidence from studies in humans and animals that overexcitation of the sympathetic nervous system plays an important role in the pathogenesis of cardiovascular diseases, including heart failure, atherosclerosis, and hypertension (41, 47, 50). In neurogenic hypertension, in which chronic elevation of the 24-h average blood pressure (BP) is not caused primarily by a vascular or renal defect, central sympathetic overactivation is involved in the development, staging, and progression of the disease, as well as the end-organ damage associated with the disease (75). It is now well accepted that one of the underlying mechanisms of neurogenic hypertension involves oxidative stress in key brain stem sites that are engaged in the generation, maintenance, and regulation of sympathetic vasomotor tone (58, 75, 126). This article provides an overview of this particular aspect of neurogenic hypertension, together with the redox-sensitive signaling pathways that underpin the augmentation in sympathetic vasomotor tone, with the realization that many of the mechanisms discussed probably hold true for heart failure, essential hypertension, and cardiac autoimmune disorders. Also included will be the emerging view that, under some pathological conditions, brain stem oxidative stress is causally related to a reduction in sympathetic vasomotor tone and hypotension, along with the underlying mechanisms.

Brain stem sites engaged in the generation, maintenance, and regulation of sympathetic vasomotor tone

An extensive neural network, including the rostral ventrolateral medulla (RVLM), nucleus of the solitary tract (NTS), and hypothalamus is involved in the generation, maintenance,
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and regulation of sympathetic nerve activity (38). In addition, cortical, limbic, and midbrain regions modulate ongoing sympathetic nerve activity (51). It is beyond the scope of this review to discuss this extensive neural network, which forms the subject of several recent reviews (58, 68, 78). Instead, brain stem sites engaged in the generation, maintenance, and regulation of sympathetic vasomotor tone will be highlighted to set the stage for subsequent elaborations on how oxidative stress, by acting on those neural substrates, regulates this particular aspect of sympathetic nerve activity.

Considerable experimental evidence suggests that the RVLM plays a crucial role in the generation of sympathetic vasomotor tone, which maintains resting BP. Anatomical (79, 97) and electrophysiological (5, 56, 82) results indicate that tonic sympathetic vasomotor outflow from the RVLM is mediated via direct bulbospinal projections to the intermediolateral autonomic nucleus in the spinal cord. Although RVLM activation increases BP (37, 49, 79, 112), bilateral lesion or inhibition of the RVLM reduces BP to levels that resemble those produced by spinal cord transection (49, 55). The neurotransmitters released at the spinal cord include at least epinephrine (49, 79, 82) and glutamate (3, 6, 60, 81).

The arterial baroreflex is a fundamental homeostatic mechanism that provides a rapid negative feedback to dampen fluctuations in cardiovascular parameters induced by environmental insults. As part of the baroreflex circuit, the sympathetic vasomotor efferents from the RVLM are also modulated by inputs from the NTS (38, 103). For example, a rise in arterial transmural pressure increases the discharge rate of high-pressure mechanoreceptors in the carotid sinus and aortic arch that are sensitive to changes in vessel wall distention. The carotid sinus and aortic depressor nerves convey primary baroreceptor afferent information to partly overlapping regions of the NTS (36). Outputs from the NTS mediate reflex adjustment of sympathetic vasomotor outflow by inhibiting the reticulospinal neurons (RVLM, 38, 57), resulting in peripheral vasodilation (103). This is executed by enhancing the excitatory input from the NTS to a group of GABAergic neurons located in an area immediately caudal to the RVLM, and which inhibit the premotor sympathetic RVLM neurons (100).

REACTIVE OXYGEN SPECIES AND ANTIOXIDANTS

Virtually all aerobic cells produce reactive oxygen species (ROS), which are among the newest additions to the family of second-messenger molecules. Many ROS, including molecules such as nitric oxide (NO), superoxide (O2−) and hydroxyl radical (OH), possess unpaired electrons and thus are free radicals. Other ROS, such as hydrogen peroxide (H2O2) and peroxynitrite (ONOO−) are not free radicals per se, but they exert oxidizing effects. ROS avidly interact with a large number of molecules, including small inorganic molecules, proteins, lipids, carbohydrates, and nucleic acids. Through such interactions, ROS may irreversibly destroy or alter the function of the target molecules (7). The metabolism of ROS is tightly controlled in the cells. This rigid regulation of both production and removal of ROS makes fluctuations in their levels within cells transient and minimal under physiological conditions. Nonetheless, under various pathological conditions, excessive production of ROS may exceed endogenous antioxidant defense mechanisms for degradation of ROS, leading to conditions that are referred to as oxidative stress. An increasing body of evidence suggests that oxidative stress is involved in the pathogenesis of many cardiovascular diseases (2, 4, 69, 99).

Mitochondria and NADPH oxidase are the two major sources of ROS (31, 52–54, 80). The primary function of the mitochondrion is cellular bioenergetics and the control over energy demands. Cellular energy is stored in the mitochondrion as high-energy phosphate bonds in ATP. Within the neuron, ATP is derived from glucose primarily through aerobic metabolism. The free energy necessary to generate ATP is extracted from the oxidation of nicotinamide adenine dinucleotide by the protein complexes of the electron transport chain (ETC) located in the inner membrane of the mitochondrion. Oxidation and phosphorylation are tightly coupled in the mitochondrial under physiological conditions so that electron transport occurs only if ADP is being phosphorylated. However, a small quantity of oxygen may be incompletely reduced, as electrons leak from the ETC can cause the reduction of oxygen to O2−. In view of the indispensable dependence of neurons on ATP supply via aerobic metabolism, the mitochondrion is therefore the major cellular site of O2− production in the central nervous system (31). In addition to the mitochondrion, O2− and H2O2 can be derived from xanthine oxidase (XaO), cyclooxygenase, lipoygenase, nitric oxide synthase (NOS), heme oxygenase, peroxidases, and NADPH oxidase (80). Among them, the membrane-associated NADPH oxidases are the primary physiological producers of ROS (52–54, 80) because they transport electrons across biological membranes to reduce oxygen to O2−. Seven isoforms of the catalytic, membrane-spanning NADPH subunits exist in the NOX family of oxidases, each encoded by a separate gene (i.e., NOX1–5, and Duox1 and Duox2). They differ in molecular composition, subcellular localization, tissue distribution, and expression (9, 86). Activation of NADPH oxidase is a multistep process that is initiated by serine phosphorylation of the cytosolic regulatory p47phox subunit, although this step is not required by all NADPH oxidase complexes. Following translocation to the membrane, the activated p47phox subunit is associated with the membrane-bound gp91phox (Nox2) and p22phox subunits to bring forth enzymatic activity. Once assembled, the complex is active and generates O2− by transferring an electron from NADPH in the cytosol to oxygen in the luminal or extracellular space. Another source of O2− and H2O2 is XaO, which is localized in the outer surface of the cellular membrane, peroxisomes, lysosomes, and cytosol. Cytosolic phospholipase A2 is associated with the lipid layer of the cellular membrane and membranes of subcellular organelles, and releases O2− to the cytosol. Secretory phospholipase A2 is localized in the extracellular space where it produces O2−. Cytochrome P450 is localized in the cell membrane and endoplasmic reticular membrane and releases O2− to the cytosol (116). A majority of the evidence on generation of ROS in the brain is derived from studies in which angiotensin II (Ang II) is used to activate the system. Nonetheless, it is noteworthy that ROS can also be produced in an Ang II-independent manner. For example, activation of the glutamate receptors and the subsequent calcium-dependent enzymes, phospholipase A2 and XaO, contribute to O2− production in response to glucose deprivation (89). In addition, intermittent hypoxia upregulates Nox2 expres-
Brain Stem Oxidative Stress and Sympathetic Vasomotor Tone

A role for brain oxidative stress in sympathetic regulation of BP has been suggested in several types of hypertensive animal models. Indeed, the ROS level is increased in brain stem sites that subserve the generation, maintenance, and regulation of sympathetic vasomotor tone, including the RVLM and NTS, in spontaneously hypertensive rats (SHR) or stroke-prone SHRs (23, 65, 66, 84, 110). We present below the impact of oxidative stress on these two representative brain stem sites, which leads to augmented sympathetic vasomotor and hypertension, based primarily on investigations using Ang II as the inducer of ROS, particularly $O_2^-$, via activation of the angiotensin receptor subtype 1 (AT1R).

_Rostral ventrolateral medulla._ An augmented sympathetic vasomotor outflow from the RVLM is a major contributor to neurogenic hypertension (39, 87). Ample evidence implicates that this augmented sympathetic vasomotor tone results from oxidative stress in the RVLM via dysfunctions of mitochondria, activation of NADPH oxidase, or reduction in antioxidant capacity. Ang II increases the mitochondrial $H_2O_2$ level in the RVLM by reducing the enzyme activity of Complexes I, II, and III in the mitochondrial ETC, along with suppression of the electron transport capacity between Complexes I and III and Complexes II and III (28). On the other hand, preservation of the mitochondrial electron transport capacity in the RVLM with a highly mobile electron carrier, coenzyme $Q_{10}$, reduces arterial pressure in SHR and attenuates the pressor response of normotensive Wistar Kyoto rats to Ang II infusion (28). Long-term treatment with the AT1R blocker losartan prevents hypertension and improves mitochondrial ETC function and coenzyme $Q$ content in brain mitochondria (104).

Ang II also induces upregulation of NADPH oxidase subunits and enhances their enzymatic activity through activation of Ras-related C3 botulinum toxin substrate 1 (Rac1; Ref. 119) or phosphorylation of $p47^{phox}$ (22), leading to generation of ROS in the RVLM (22, 25, 29, 44, 45, 90, 122). Furthermore, pharmacological blockade of Ang II-activated NADPH oxidase in the RVLM (22, 77, 85) attenuates ROS production, hypertension, sympathoexcitation associated with chronic heart failure, and baroreflex impairment. Gene knockdown with antisense (22, 27, 29) or dominant-negative mutants of the NADPH oxidase subunits (122) and genetically modified mice that lack components of the NADPH oxidase (46, 70, 76) further substantiate a causal role for NADPH oxidase-derived ROS at the RVLM in the pathogenesis of hypertension.

Overexpression of SOD2 in the RVLM blunts the cardiovascular responses to microinjection of Ang II to this nucleus (23, 83) or reduces sympathoexcitation and hypertension in SHR (23). Ang II-induced hypertension is also attenuated in transgenic mice overexpressing mitochondrial SOD2 (40); administration of catalase into the RVLM reduces the elevated ROS level in stroke-prone SHR (65). In addition, the expression of catalase in the RVLM of SHR is decreased, and transfection of adeno viral vectors expressing SOD1, SOD2, or catalase results in prolonged antihypertension of comparable degrees (23), implicating a role for $H_2O_2$. These comparable degrees of antihypertension are attributed to a reduction of the augmented tissue levels of both $O_2^-$ and $H_2O_2$ in the RVLM by the catalase transgenes (23). In view that SOD scavenges $O_2^-$ and produces $H_2O_2$ while catalase reduces both $O_2^-$ and $H_2O_2$, it is not surprising that the authors also found that the duration of the antihypertensive action of the catalase transgene is longer than that of SOD1 or SOD2 transgenes (23). Finally, an $O_2^-$- and $H_2O_2$-dependent feed-forward impairment of mitochondrial ETC complexes because of a predisposed reduction in synthesis or the activity of SOD or catalase (28) and a cross-talk between NADPH oxidase-derived $O_2^-$ and mitochondrial ETC enzymes (28) that contribute to chronic oxidative stress in the RVLM of SHR have recently been put forth as mechanisms that underpin the sustained augmentation in sympathetic vasomotor tone and maintenance of hypertension.

_Nucleus tractus solitarii._ By elevating sympathetic vasomotor tone indirectly via an augmentation of the activity of the barsensitive sympathetic efferent neurons in the RVLM, depression of the baroreflex is a hallmark of neurogenic hypertension. Studies devoted to this aspect have suggested a role for oxidative stress in the NTS, again based primarily on experiments using Ang II as the input. Microinjection of Ang II into the NTS promotes hypertension (33, 63, 91, 107, 117) and inhibits baroreflex (74, 117) via activation of the AT1R (74, 113). The documented source of $O_2^-$ is activation of NADPH oxidase (48, 107, 113, 114), including at least the Nox2 (113, 114) and $p47^{phox}$ (48) subunits. Also reported is the engagement of Rac1, a small G protein that serves as an important signaling molecule involved in integrating intracellular transduction pathways for NADPH oxidase activation (62). Rac1 inhibition by transfection of adenovirus vectors encoding dominant-negative hemagglutinin-tagged Rac1 into the NTS reduces NADPH oxidase activity and $O_2^-$ production in this brain stem site (84). The same treatment also reduces BP in stroke-prone SHR but not in Wistar Kyoto rats, as does overexpression of SOD1 in the NTS (65, 84). In addition, Ang II induces ROS production in the NTS of SHR, and ROS accumulation exerts a negative regulation on NO production in the NTS, resulting in manifestation of hypertension (34).
BRAIN OXIDATIVE STRESS–ASSOCIATED SIGNALING AND AUGMENTED SYMPATHETIC VASOMOTOR TONE

There are indications that cellular signals associated with augmented sympathetic vasomotor tone generated by brain oxidative stress at least include mitogen-activated protein kinase (MAPK), ion channels, and transcription factors. These oxidative stress–associated signaling pathways will be illustrated using work done on the RVLM or NTS.

Mitogen-activated protein kinase. As a major class of redox-regulated signaling molecules, the activities of the MAPK family in the RVLM, in particular p38 MAPK and extracellular signal-regulated kinase (ERK), are potentiated by Ang II via an AT1R-dependent signaling cascade that includes sequential activation of protein kinase C (PKC) and NADPH oxidase, and ROS production (22, 25). The AT1R-Ras-ROS-p38 MAPK/ERK pathway in the RVLM is also activated in stroke-prone SHR (66). Of interest is that redox activation of p38 MAPK and ERK in the RVLM plays a differential role in Ang II-induced hypertension. Whereas ROS-p38 MAPK signaling mediates the short-term pressor response of Ang II by enhancing glutamatergic neurotransmission in the RVLM (22), O₂⁻-dependent ERK phosphorylation leads to a long-term pressor response to Ang II via transcriptional upregulation of AT1R mRNA expression (25). In addition, both mRNA and protein of major NADPH oxidase subunits, including Nox2, p67 phosphate, p47 phosphate, and p40 phosphate in the RVLM or NTS are upregulated by Ang II (22, 44, 85, 90, 126) via PKC (22, 44, 114) pathways. Mediation of Ang II-induced NADPH oxidase by PKC activation in the NTS has also been reported (113), with phosphatidylinositol 3-kinase (PI3K) acting as the interposing signal (107).

Ion channels. Several classes of ion channels in the RVLM or NTS have been linked to Ang II-induced oxidative stress and the associated, augmented sympathetic vasomotor tone. Ang II increases neuronal discharges in the RVLM by increasing voltage-gated Ca²⁺ current (113, 123) or inhibiting K⁺ current, in particular the delayed rectifier K⁺ current (105, 106). Blockade of voltage-gated K⁺ channels in the RVLM elicits sympathoexcitation and hypertension (43); a mitochondrial ATP-sensitive K⁺ channel blocker reverses Ang II-induced ROS production (96). Ang II also activates I-type Ca²⁺ current in isolated NTS neurons (113). Whether ROS modulate channel activity by direct oxidation of amino acid residues in channel proteins or indirect modulation of protein kinases that control gating property of the channels remains to be determined.

Transcription factor. Redox-sensitive upregulation of AP-1, c-Jun, or c-fos is observed in the RVLM of animals with hypertension (25), along with an increase in DNA binding activity of AP-1 (126). Of note is that in the RVLM, transcriptional upregulation of AT1R mRNA expression because of c-fos activation by Ang II-NADPH oxidase-dependent ERK phosphorylation leads to a long-term pressor response to Ang II (8). Interestingly, an augmented basal level of c-fos in the NTS underlies the genesis of hypertension by exerting a tonic inhibitory modulation on baroreflex (14, 16, 21, 102) via sustaining the expression of angiotensin AT1R in the NTS (15, 18, 115).

BRAIN OXIDATIVE STRESS AND REDUCED SYMPATHETIC VASOMOTOR TONE

There are recent reports that an elevation in O₂⁻ level in the RVLM is also causally related to the reduction in baroreflex-mediated sympathetic vasomotor tone and hypotension in animal models of brain stem death (30, 32, 35, 101, 119, 120), methamphetamine intoxication (72, 73), and temporal lobe status epilepticus (111). The source of this increase in O₂⁻ includes reduced SOD2 activity (30), dysfunction of mitochondrial ETC complexes (32, 35, 72, 101, 119, 120), and NADPH oxidase (101, 111). These findings seemingly contradict the general notion that oxidative stress in the RVLM is associated with the hypertensive state. A potential solution to this paradox arises from several observations. First, NOS II is tonically active in the RVLM (26), although the expression and activity of this NOS isozyme are significantly less in the RVLM of SHR (17, 20). Second, a significant elevation in the molecular synthesis and functional expression of NOS II is associated with experimental brain stem death (12, 13, 30, 32, 35, 101, 119, 120) and temporal lobe status epilepticus (111). Third, formation of the oxidant peroxynitrite by a reaction between NOS II-derived NO⁻ and O₂⁻ in the RVLM results in progressive hypertension and a reduction in baroreflex-mediated sympathetic vasomotor tone observed in these animal models (13, 24, 111). It follows that it is the oxidative stress exerted by peroxynitrite, which induces apoptosis in the RVLM (30), that underpins the reduction in baroreflex-mediated sympathetic vasomotor tone, leading to hypotension. On the other hand, a hypertensive state associated with augmented O₂⁻ in the RVLM will prevail because of the significantly less NO² expression and activity (17, 20). In the NTS, H₂O₂ evokes hypotension that is antagonized by an antioxidant L-ascorbate (10).

PEROXYNITRITE-ASSOCIATED SIGNALINGS AND REDUCED SYMPATHETIC VASOMOTOR TONE

Several mechanisms may account for the cardiovascular depression of peroxynitrite in the RVLM. Peroxynitrite has been reported to oxidize protein and nonprotein sulfhydryls (93) and induce membrane lipid peroxidation (94, 98). It also shuts down cellular energy production by inhibiting mitochondrial ETC (11, 88) or inactivating mitochondrial respiratory enzymes (92, 95). Activation of poly(ADP)ribosyltransferase contributes to peroxynitrite toxicity by promoting excessive ADP ribosylation of nuclear protein (108, 109, 124), leading to ATP depletion and cellular injury (108, 124). In the RVLM, peroxynitrite acts presynaptically to elicit a depression of RVLM neuronal activity through inhibition of presynaptic N-type Ca²⁺ channel activity, leading to reduced glutamate release (12, 13, 24, 30, 61, 71). Overproduction of NO via an activation of NOS II (19) also downregulates both synthesis and activity of AT1R in the RVLM alongside a reduction in sympathetic vasomotor tone and hypotension.

CONCLUSION AND FUTURE RESEARCH DIRECTIONS

As presented in this review, there is now compelling evidence to substantiate the notion that oxidative stress and its associated signaling in the RVLM and NTS are actively engaged in the regulation of sympathetic vasomotor tone (Fig. 1).
One common pitfall in contemporary scientific research is to inadvertently assume the universal applicability of an identified physiological phenomenon or cellular mechanism. As presented in this minireview, this assumption requires modification. It is commonly stipulated that oxidative stress in the RVLM or NTS results in an augmentation of sympathetic vasomotor tone, depression of baroreflex, and hypertension. Not to be ignored, however, is that formation of peroxynitrite by a reaction between NOS II-derived NO· and O2·- or H2O2 in the RVLM results in progressive hypotension and a reduction in baroreflex-mediated sympathetic vasomotor tone (Fig. 1). It follows that a reasonable direction of future research would be dissection of the cellular mechanisms and signaling cascades that may underlie the contributory role of NO· generated by different NOS isoforms in the differential effects of oxidative stress in the RVLM or NTS on sympathetic vasomotor tone. This suggestion is concordant with the views of Hirooka et al. (59) that an imbalance exists between NO· and ROS in brain stem in the regulation of sympathetic activity, although those investigators proposed that formation of peroxynitrite in the RVLM causes hypertension and sympathoexcitation (64).

Recent advances in our understanding of the contributions of the angiotensin-converting enzyme 2/Angiotensin-(1–7)/Mas receptor axis (118), autonomic-immune-vascular interaction (125), or autonomic neural regulation of the immune system (1) to neurogenic hypertension offer another direction for future research. Mechanistic delineation of the interplay between oxidative stress and these cellular mechanisms in key brain stem sites involved in the regulation of sympathetic vasomotor tone will certainly offer new insights into the intricate schemes of cardiovascular control.

Finally, it must be recalled that sympathetic vasomotor outflow from the RVLM is mediated via its spinal projection to the intermediolateral autonomic nucleus in the thoracic spinal cord. It is therefore conceivable that oxidative stress may also affect sympathetic vasomotor tone by modulating the activity of the sympathetic preganglionic neurons. Future attention should be paid to this often overlooked role of oxidative stress in spinal cord actions.

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REFERENCES


Superoxide mediates sympathoexcitation in heart failure: roles of angiotension II and NAD(P)H oxidase. 

Sympathoexcitation by central ANG II: roles for AT1 receptor upregulation and NAD(P)H oxidase in RVLM. 

Decreased blood pressure in NOX1-deficient mice. 

47. Giannoglou GD, Chatzizisis YS, Zamboulis C, Parcharidis GE, 
Mikhalidipalis DP, Louridas GE. Elevated heart rate and atherosclerosis: an overview of the pathogenetic mechanisms. 

Krause KH. 
Oxidative stress in the cardiovascular center has a pivotal role in the development of cardiovascular disease. 

49. Gray MA, Rylander K, Harrison NA, Wallin BG, Critchley HD. 
Following one’s heart: cardiac rhythms gate central initiation of sympathetic reflexes. 

50. Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW. 
Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. 

51. Griendling KK, Sorescu D, Lassègue B, Ushio-Fukai M. 
Modulation of protein kinase activity and gene expression by reactive oxygen species and their role in vascular physiology and pathophysiology. 

52. Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW. 
Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. 

53. Griendling KK, Sorescu D, Lassègue B, Ushio-Fukai M. 
Modulation of protein kinase activity and gene expression by reactive oxygen species and their role in vascular physiology and pathophysiology. 

54. Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW. 
Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. 


56. Guertzenstein PG, Silver A. Fall in blood pressure produced from discrete regions of the ventral surface of the medulla by glycine and glutamate, NMDA and NMDA receptor antagonists: cardiovascular effects of intrahypothalamic activation in the rat. 


58. Hirooka Y, Ohtsuka K, Sakai K, Takeshita A, Sunagawa K. 
Impulse of central nitric oxide and reactive oxygen species in the regulation of sympathetic activity and neural mechanisms of hypertension. 

59. Hirooka Y, Ohtsuka K, Sakai K, Takeshita A, Sunagawa K. 
Impulse of central nitric oxide and reactive oxygen species in the regulation of sympathetic activity and neural mechanisms of hypertension. 

60. Hirooka Y, Ohtsuka K, Sakai K, Takeshita A, Sunagawa K. 
Impulse of central nitric oxide and reactive oxygen species in the regulation of sympathetic activity and neural mechanisms of hypertension. 

Impulse of central nitric oxide and reactive oxygen species in the regulation of sympathetic activity and neural mechanisms of hypertension. 

Impulse of central nitric oxide and reactive oxygen species in the regulation of sympathetic activity and neural mechanisms of hypertension. 

63. Hirooka Y, Ohtsuka K, Sakai K, Takeshita A, Sunagawa K. 
Impulse of central nitric oxide and reactive oxygen species in the regulation of sympathetic activity and neural mechanisms of hypertension. 

64. Hirooka Y, Ohtsuka K, Sakai K, Takeshita A, Sunagawa K. 
Impulse of central nitric oxide and reactive oxygen species in the regulation of sympathetic activity and neural mechanisms of hypertension. 


