MINIREVIEW

Role of Nitric Oxide in Central Sympathetic Outflow

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The gaseous molecule nitric oxide (NO) plays an important role in cardiovascular homeostasis. It plays this role by its action on both the central and peripheral autonomic nervous systems. In this review, the central role of NO in the regulation of sympathetic outflow and subsequent cardiovascular control is examined. After a brief introduction concerning the location of NO synthase (NOS) containing neurons in the central nervous system (CNS), studies that demonstrate the central effect of NO by systemic administration of NO modulators will be presented. The central effects of NO as assessed by intracerebroventricular, intracisternal, or direct injection within the specific central areas is also discussed. Our studies demonstrating specific medullary and hypothalamic sites involved in sympathetic outflow are summarized. The review will be concluded with a discussion of the role of central NO mechanisms in the altered sympathetic outflow in disease states such as hypertension and heart failure. [Exp Biol Med Vol. 226(9):814–824, 2001]

Key words: nitric oxide; central nervous system; sympathetic outflow

Originally identified primarily as a mediator of the endothelial control of vascular smooth muscle, nitric oxide (NO) is an important mediator of intracellular signaling in various tissues, including the nervous system (Fig. 1). NO acts via the second messenger cGMP. It is synthesized from its precursor L-arginine by the enzyme NO synthase (NOS). At least three distinct NOS isoforms have been identified in mammalian cells: the constitutive calcium-dependent enzymes neuronal NOS (nNOS: originally identified in neural tissue; Type I) and endothelial NOS (eNOS; originally identified in vascular endothelium; Type III), and the calcium-independent cytokine-induced isoform, inducible NOS (iNOS; identified in macrophages; Type II). NOS activity has been demonstrated in central and peripheral sites throughout the autonomic nervous system that controls cardiovascular regulation, including the receptors and effectors of the baroreflex pathway. Localization of neuronal populations that possess nNOS has been achieved by histochemical staining using NADPH-diaphorase and immunohistochemistry (1, 2). This close proximity of the production of NO within central sites involved in cardiovascular regulation has lead to the belief that NO may be involved in the regulation of autonomic outflow. There are a large number of studies examining the central effects of NO on sympathetic outflow by administration of NO agonists or blockers orally, intravenously, intracerebroventricularly, or into specific central sites. The overall consensus is that NO acts as a sympathoinhibitory substance within the central nervous system. The purpose of this review is to summarize recent developments in identifying the role of central NO mechanisms involved in regulating sympathetic outflow in normal and disease states.

NO on Sympathetic Outflow

Oral Administration. Chronic administration of the NO inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) in the drinking water causes a large increase in resting mean arterial pressure and heart rate of rats (3). Ganglionic blockade caused a greater drop of arterial pressure in the L-NAME-treated rats compared with controls. These data suggest that the level of central sympathetic tone in L-NAME-treated rats is much greater than in untreated rats. Similarly,

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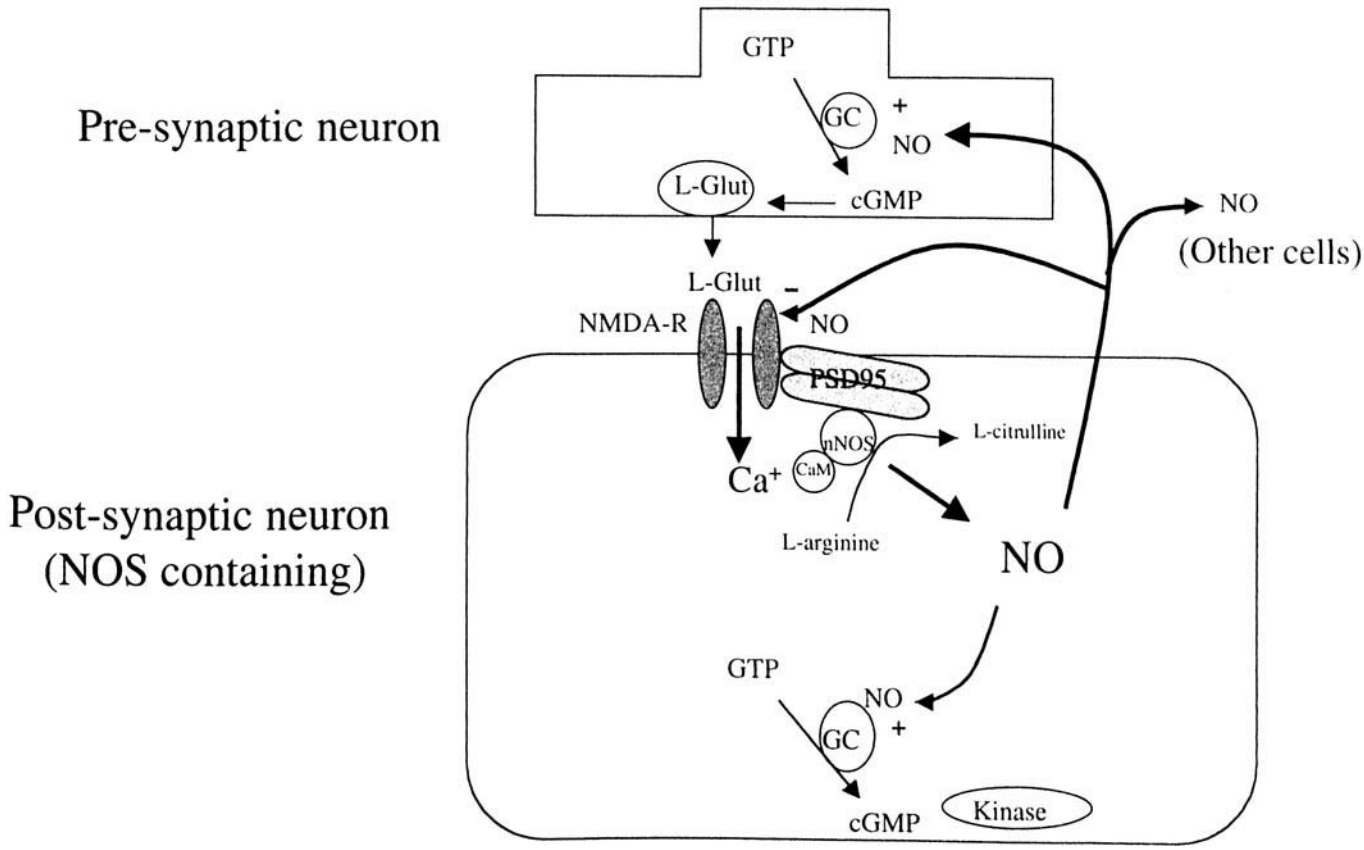

Figure 1. Schematic representations of hypothetical mechanisms for modulation of synaptic glutamatergic neurotranmission by NO. Activation of NMDA-R1 receptor by glutamate released from the presynaptic neuron causes an increase in calcium influx that activates nNOS associated with PSD95 attached to NMDA-R1, through calmodulin. The resulting NO produced in this manner then can diffuse to the presynaptic neuron or neighboring cells (neurons or astrocytes) to activate GC to produce cGMP. cGMP can then affect ion channel function, phosphodiesterase activity, or activate protein kinases to effect various cellular events.

increases in arterial pressure mediated by over activation of the sympathetic nervous system have been observed by other investigators after oral administration of L-NAME in rats (4, 5). Oral administration of organic nitrites caused hemodynamic tolerance to NO donors and significantly increased the hypotensive responses to ganglionic blockade in conscious pigs (6). Furthermore, there was a decrease in the density of neurons that stain for NOS activity in brain stem slices measured with NADPH-diaphorase (6). Taken together, these data suggest that an increase in central sympathetic drive plays an important role in the elevated arterial pressure induced by chronic inhibition of NO synthesis when L-NAME is given orally (3–6).

Recently, it was demonstrated that microinjection of an AT1 receptor antagonist (candesartan), but not that of an AT2 receptor antagonist (PD123319), into the nucleus of the solitary tract (NTS) produced greater decreases in arterial pressure, heart rate, and RSNA in L-NAME-treated rats than in control rats. Results of this study suggest that increased sympathetic nerve activity (SNA) contributes to the hypertension caused by chronic NOS inhibition and that activation of the renin-angiotensin system in the NTS is involved at least in part in this increased sympathetic nerve activity *via* angiotensin AT1 receptors (7). A role for the rostroventrolateral medulla (RVLM) has also been implicated in this model of hypertension (8).

Intravenous Administration. Inhibition of NOS by the systemic administration of L-arginine analogues results in vasoconstriction and an increase in mean arterial pressure in both animals and man (9–11). This increase in pressure may partly be due to the elimination of the vasodilation due to peripheral NO (12). However, there is a growing body of evidence that suggests that part of the increase in vasoconstriction and subsequent increase in blood pressure due to systemic administration of NOS blockade are due to an activation of the peripheral sympathetic outflow (3, 13–17). First, ganglionic blockade during intravenous administration of L-NAME significantly decreased arterial pressure in rats (3, 15, 17), suggesting that a sympathetic component may be responsible for the increase in arterial pressure observed during intravenous administration of L-NAME. Second, blockade of the adrenergic beta receptors produces a greater fall in heart rate and blood pressure in the L-NAME-treated rats compared with control rats (3). Third, cooling of RVLM suppressed the increase in arterial pressure produced by administration of L-NAME in barodenervated cats (14). Fourth, sympathectomy significantly reduces the hypertensive response to L-

NAME administration (14). Fifth, direct recording from the renal sympathetic nerve demonstrates a biphasic response to systemic administration of NOS inhibitor N^G-monomethyl-L-arginine (L-NMMA) to anesthetized rats (16). Prior barodenervation abolished the initial sympathoinhibitory response and potentiated the sympathoexcitatory response to L-NMMA (16). This increase in sympathoexcitation was abolished by cervical spinal transection. Conversely, stimulation of endogenous NO synthesis with L-arginine caused a decrease in renal nerve activity, despite a decrease in blood pressure in anesthetized rabbits (13). Intravenous L-NMMA produced an increase in cardiac sympathetic activity in barodenervated rabbits that was reversed by administration of L-arginine. There are a few studies that report a lack of changes in basal RSND in response to N^G-nitro-L-arginine (L-NNA) or selective nNOS inhibition in conscious, intact and sinoaortic-denervated rabbits (18, 19). These discrepancies are not readily explained by differences in species or methodological differences.

Taken together these studies examining the effect of administration of NOS inhibitors in the systemic circulation would suggest a significant central inhibitory role for NO in the control of sympathetic neural outflow.

Intracerebroventricular Administration. Intracerebroventricular administration of L-NAME in anesthetized rats produces an increase in heart rate and arterial blood pressure (20). These increases are blocked by administration of the adrenergic beta blocker, atenolol. In another study, either intracerebral or intracisternal administration of NOS inhibitors produced increases in RSND and blood pressure in rats and rabbits (21–23). Cervical spinal transection abolished these responses to central administration of NOS inhibitors (21). Conversely, administration of L-arginine intracerebroventricularly increases NO synthesis within the CNS and produces a decrease in abdominal sympathetic nerve discharge in rats (24). As shown in Figure 2, intracisternal administration of L-NAME, which inhibits NOS of medullary sites, increased arterial pressure and RSNA, and facilitated rapid adaptation of the arterial baroreflex control of sympathetic nerve activity in anesthetized rabbits (22). In summary, the findings of central administration of modulators of the NO pathways within the cerebral ventricles or intracisternally consistently support the concept of tonic restraint of central sympathetic outflow by NO.

nNOS mRNA and Enzyme Activity. A number of studies have examined the NO system within the CNS by

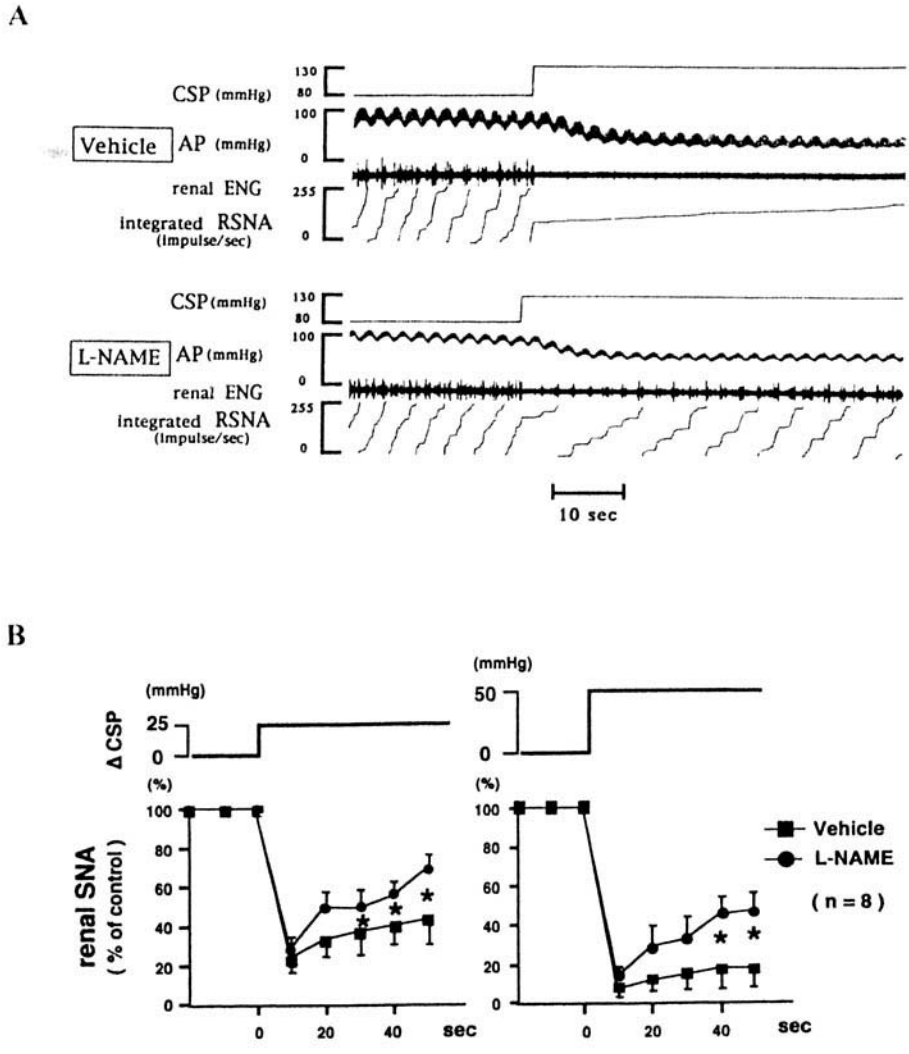

Figure 2. (A) Chart recordings showing the effects of a step-wise increase in the carotid sinus pressure (CSP) of 50 mm Hg on arterial pressure (AP) and RSNA (ENG, raw electroneurogram) after the intracisternal injection of vehicle (top) or L-NMMA (bottom). (B) Effects of a step-wise increase in the CSP of 25 mm Hg (left) and 50 mm Hg (right) on RSNA after the injection of either the intracisternal vehicle or L-NAME. $^*P < 0.05$, L-NAME vs vehicle. (Reproduced with permission from Ref. 22)

examining the genetic message for the production of the enzyme nNOS by the use of reverse transcription-polymerase chain reactions (RT-PCR) technique, the location of active messages by *in situ* hybridization, location, and/or activity by histochemical staining using NADPH-diaphorase or location by immunohistochemistry (25, 26).

The role of NO generation as a mechanism constraining a rise in sympathetic nerve activity is indirectly supported by demonstration of an increase in NO-producing neurons within the brain following experimentally induced stress. (27). Restraint stress activated a high number of NADPH-diaphovase positive neurons (as assessed with c-fos expression) in various autonomic sites in the CNS, including the paraventricular nucleus (PVN)(28). Increases in the mRNA and protein for nNOS in the PVN have been reported in rats exposed to immobilization stress (27). This activation within the PVN appears to be in the parvocellular portion of the PVN, the cells that project to the spinal cord and dictate sympathetic outflow. These observations are consistent with NO regulating sympathetic outflow via the PVN.

nNOS mRNA levels are also altered in response to blood volume and fluid balance challenges. Hypovolemia produced by intraperitoneal injection of polyethylene glycol in rats leads to an increased number of NADPH-diaphovase positive neurons and increased nNOS gene expression in the PVN and supraoptic nucleus (SON) (29). These data would suggest that volume challenge produces an increase in expression as well as activity of nNOS in the PVN and SON. Recovery of arterial pressure after hemorrhage (decrease in blood volume) was augmented in conscious rats treated with intracerebroventricular injections of L-NAME, suggesting that NO contributes to prolonged hypotension resulting from hemorrhage (30). Salt-loading by drinking hypertonic saline or dehydration also produces an increase in nNOS gene expression, immunostaining, and an increased number of NADPH-diaphorase-positive cells in the PVN and SON of rats (26, 31, 32). Consistent with these observations, chronic dehydration observed in hereditary diabetes insipidus (rats that lack vasopressin) also exhibited an increased nNOS gene expression and immunoreactivity in magnocellular neurons in the PVN and SON (33, 34).

Administration within Specific Sites. Studies employing the microinjection of agents that affect the NO pathway into specific central nuclei have allowed localization of possible CNS sites of nitrergic modulation of sympathetic activity. The following discussion will present the work in the order in which the cardiovascular afferent information is processed, with the primary afferents in the NTS, followed by interactions within the medullary level with other medullary sites such as caudalventrolateral medulla (CVLM) and RVLM, followed by interactions with forebrain sites such as the PVN.

Medullary sites. Administration of L-NMMA into the NTS produced an increase in RSND and arterial pressure regardless of whether the baroreceptors were intact or not in anesthetized rabbits as shown in Figure 3 (35). This response was also observed in anesthetized rats (36). Stimulation of endogenous NO activity by administration of L-arginine, a precursor of NO, into the NTS produced a decrease in both RSND and arterial blood pressure (36). Furthermore, perfusion of L-arginine or sodium nitroprusside (SNP; an NO donor) increased neuronal activity in approximately one-half of neurons recorded in the NTS in brainstem slices (37). In this study, the effect of NO on neuronal activity was blocked by methylene blue, suggesting that this response is caused by soluble guanylate cyclase activation. Furthermore, the effect of NO in the NTS on the depressor response may be caused by the facilitatory release of L-glutamate (38). Conversely, we also need to consider the inhibitory role of NO on the actions of L-glutamate on NMDA receptors. It has been suggested that NO induces a blockade of NMDA receptors directly (39, 40)

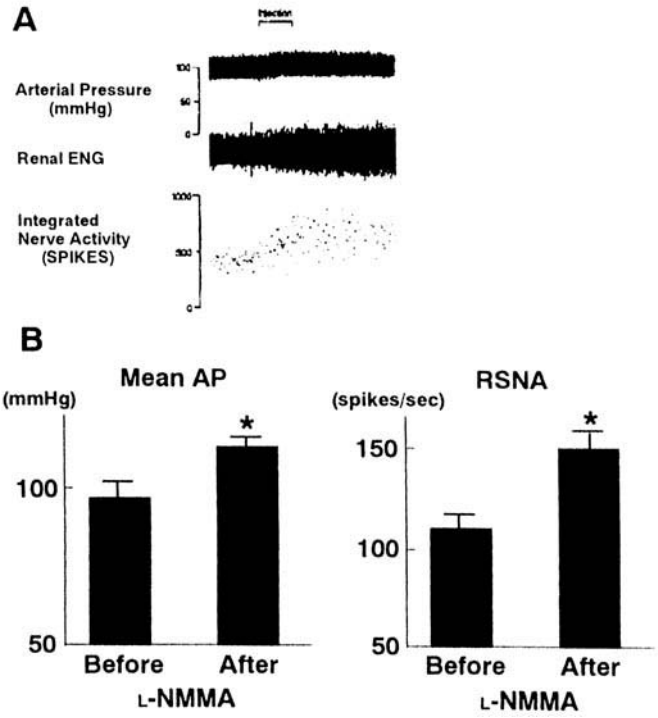

Figure 3. (A) Original recordings of arterial pressure and RSNA with microinjection of L-NMMA into the NTS in rabbits with intact sinoaortic baroreceptors and vagi. ENG, electroneurogram. (B) Effects of L-NMMA microinjected into the NTS on mean arterial pressure (AP) and RSNA in rabbits with intact baroreceptors and vagi. (Reproduced and modified with permission from Ref. 35)

At this site of cardiovascular reflex regulation, the NO system appears to be inhibitory in nature. The RVLM, the eventual sympathoexcitatory site within the medulla, is also modulated by the NO system. Inhibition of the NO system by administration of L-NNA produced an increase in sympathetic activity and arterial blood pressure in barodenervated cats (14) and barointact rats (36). Conversely, administration of either L-arginine or the NO donor SNP produced a decrease in both arterial pressure and RSND in both cats (14) and rats (36). However, there are some studies showing contradictory results (41, 42). These studies demonstrate excitatory effects of NO within the RVLM of freely moving

rats (41, 42). Furthermore, administration of SNP at the CVLM, an inhibitory medullary site, produced an increase in arterial pressure and RSND in anesthetized cats (43). On the other hand, they demonstrated that microinjection of L-NMMA into the CVLM caused the depressor and sympathoinhibitory responses (43). This would suggest an antagonist action of NO at these two medullary sites. It should be noted that there is a study demonstrating that neither L-NNA nor *S*-nitroso-*N*-acetylpenicillamine (SNAP) into the CVLM significantly changed RSNA in anesthetized cats (44). The true nature of the interaction and actions of NO within these sites remains unclear. Thus, overall baroreflex effects of NO in the brainstem are complicated and remain to be elucidated.

Nevertheless, recently, Sakai *et al.* (45) succeeded in examining the role of NO in the NTS using a gene transfer technique. Adenovirus encoding eNOS was transfected into the NTS *in vivo*, which increased production of NO in the NTS and caused a decrease in arterial pressure, heart rate, and urinary norepinephrine excretion in conscious rats (45). These data suggest that overexpression of eNOS within the NTS has an inhibitory effect on arterial pressure in rats.

Overall, the data from injections within specific medullary sites generally support the idea of an endogenous NO mechanism involved in constraining tonic sympathetic drive. Figure 4 shows a schematic diagram outlining the effects of NO on neuronal activity and possible mechanisms in medullary sites.

Hypothalamic sites. There have been relatively few studies that have examined specific sites within the forebrain involved in mediating the effect of NO on sympathetic nervous outflow (46–48). However, the effect of NO in the PVN on sympathetic nerve activity has not been examined until recently. The PVN is known to be a site of integration for autonomic and endocrine-mediated cardiovascular responses (49, 50). PVN neurons project to several areas within the CNS that are known to be important in regulating cardiovascular function. These regions include the NTS and the vagal complex present in the dorsomedial medulla, the RVLM, and the intermediolateral cell column of the thoracolumbar spinal cord, the site of sympathetic preganglionic motor neurons. The presence of NOS-positive neurons in the PVN of the hypothalamus suggests NO may serve as a physiological regulator of the sympathetic nervous system. Recently, perfusion of the PVN with NO-containing cerebrospinal fluid or microinjection of SNP into the PVN has been shown to elicit a significant reduction in arterial blood pressure (46). They concluded that NO in the PVN reduced

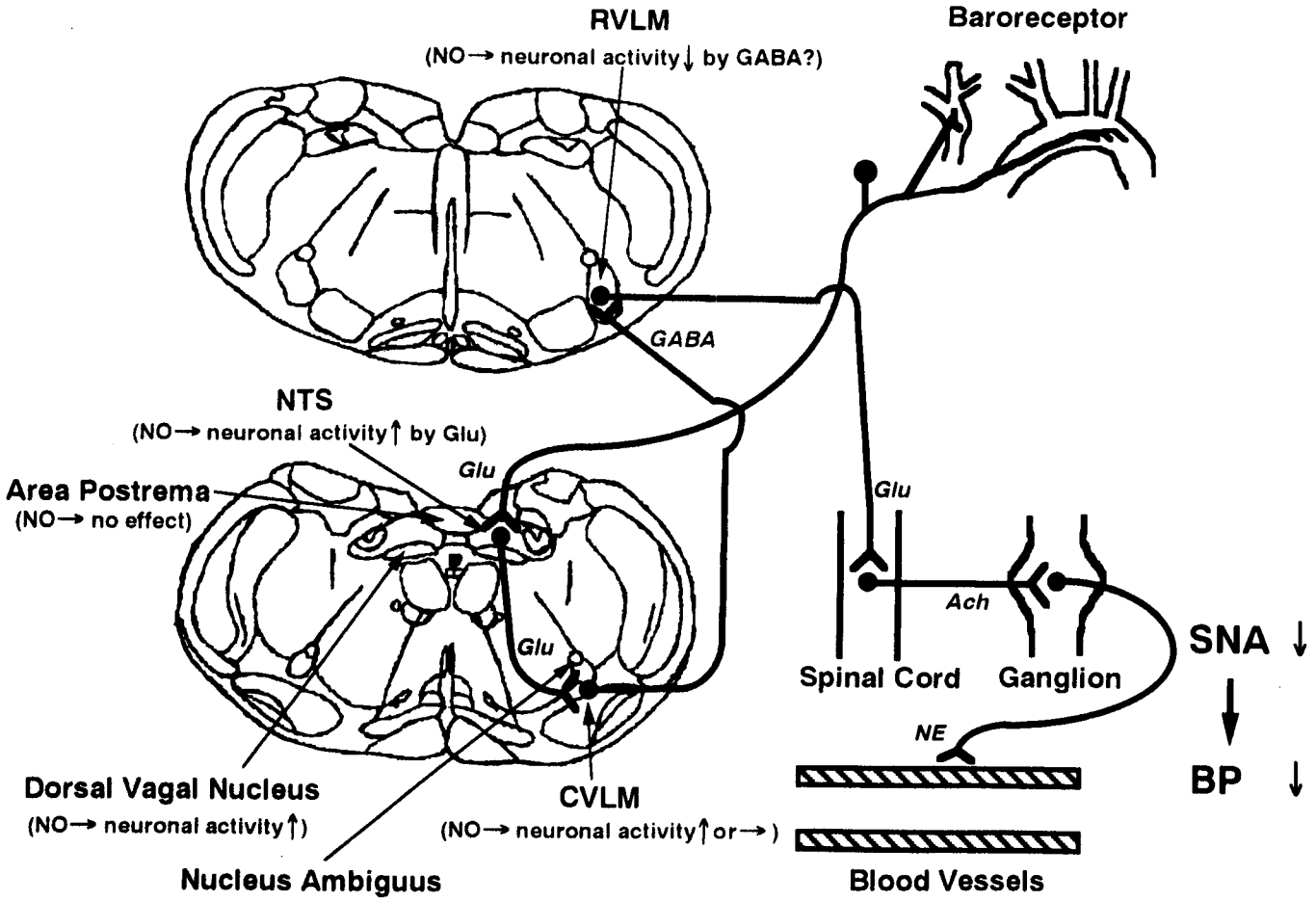

Figure 4. A scheme illustrating the effects of NO on the neuronal activity in the medulla and consequent overall SNA. This scheme also includes the pathway from input from arterial baroreceptors to consequent effect on blood vessels.

arterial blood pressure *via* changes in sympathetic outflow, although sympathetic nerve discharge was not measured. This was the first functional evidence showing that NO within the PVN may play a role in regulating cardiovascular parameters. Furthermore, NOS is densely localized in the PVN of the hypothalamus (1, 25, 51, 52), which led us to speculate that endogenous NO mechanisms in the PVN may be responsible for regulating RSND and thus arterial blood pressure.

To test the above hypothesis, we sought to examine if endogenous NO within the PVN contributes to the regulation of renal sympathetic outflow and if exogenous NO in the PVN produces changes in RSND. We used two inhibitors of NOS and vasoactive compounds to test the specificity of the responses of renal nerve discharge, arterial blood pressure, and heart rate.

We observed that microinjection of an inhibitor of NOS, L-NMMA, increased RSND, arterial blood pressure, and heart rate (48). These data indicate that the endogenous NO system within the PVN is involved in mediating sympathetic outflow. We considered that the increase of blood pressure was, at least, partially mediated by an increase of sympathetic outflow because microinjection of L-NMMA also led to a concurrent increase in efferent renal sympathetic outflow (Fig. 5A). This effect of L-NMMA on renal sympathetic outflow and blood pressure was not due to nonspecific effects of L-NMMA, because the microinjection of the biologically inactive isomer of L-NMMA, D-NMMA, did not cause any significant change in RSND, arterial blood pressure, or heart rate. Furthermore, administration of alkyl esters of L-arginine, such as L-NAME, another inhibitor of NOS, into the PVN also produced an increase in RSND, blood pressure, and heart rate (48). Specificity of NOS inhibitors is further substantiated with the observation that administration of L-arginine reversed the increases in RSND, blood pressure, and heart rate produced by L-NAME. In addition, subsequent administration of L-NAME failed to produce the increase in RSND, blood pressure, and heart rate observed prior to administration of L-arginine. These results indicate that endogenous NO mechanisms within the PVN contribute to regulation of changes in RSND. The vasoconstrictive effect of L-NMMA seems unlikely to be responsible for this increase in sympathetic nerve activity because microinjection of phenylephrine did not elicit an increase in RSND or heart rate. We interpret this lack of effect by phenylephrine to indicate that vasoconstrictor actions of L-NMMA or L-NAME locally within the PVN do not contribute to the responses in renal nerve discharge and heart rate (48).

In further support of this hypothesis we observed that the microinjection of SNP, an NO donor, elicited a reduction of RSND, arterial blood pressure, and heart rate. The reduction of blood pressure was, at least, partially mediated by a reduction of sympathetic outflow because microinjection of SNP also led to a concurrent reduction in efferent

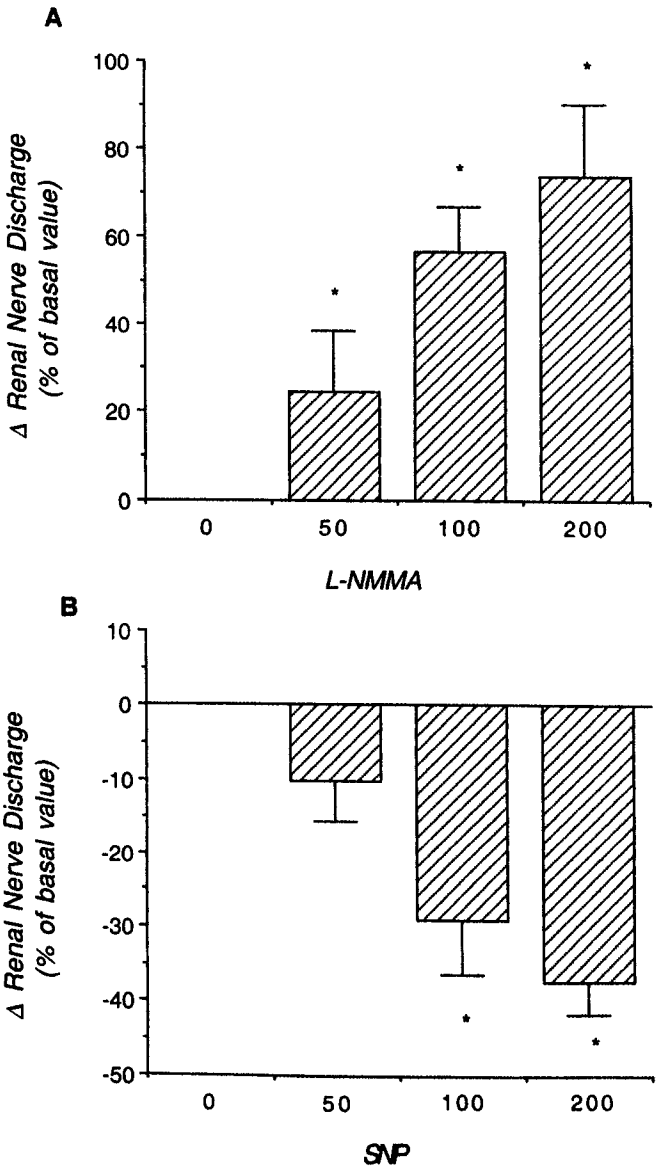

Figure 5. Response of change in RSND, to the microinjection of L-NMMA (A) and SNP (B) into PVN of rats. Graphs represent mean values for each group ± SEM. An asterisk indicates $P < 0.05$ compared with baseline. (Reproduced with permission from Ref. 48)

renal sympathetic outflow (Fig. 5B). This effect of SNP on renal sympathetic outflow and blood pressure was not due to its local vasoactive effect within the PVN because the microinjection of another vasodilator, hydralazine, had no effect on RSND, blood pressure, or heart rate. According to the study by Horn *et al.* (46), this effect of SNP on arterial blood pressure and sympathetic nerve discharge were most likely mediated by NO that was released by SNP.

Furthermore, the effect of L-NMMA, L-NAME, and SNP were site specific for the PVN; those injections, which were more than 0.5 mm away from the PVN, had no significant effect on the renal sympathetic nerve discharge, arterial blood pressure, or heart rate. Adjacent sites to the PVN within the hypothalamus are not responsible for the NO-mediated changes in RSND, arterial blood pressure, or

heart rate. Our data suggests that the endogenous NO system within the PVN modulates sympathetic outflow by an inhibitory mechanism (48).

In addition to its modulatory role in the sympathetic nervous system, NO in the brain may play an important role in fluid balance homeostasis (53). Chronic salt loading has been shown to increase NADPH-diaphorase staining, a histochemical marker for NOS activity, in the posterior pituitary (32). Similarly, there is increased NOS gene expression and NOS-immunoreactive cells in the PVN and SON in response to salt loading. Water deprivation also induced a significant increase in NOS gene expression in the PVN and SON (54). It has been shown that inhibition of NOS enhances the release of oxytocin and vasopressin during osmotic challenge, such as dehydration or salt loading, suggesting an inhibitory role of NO on these systems (31, 55). These data indicate that NO is inhibitory to the release of oxytocin and vasopressin from the PVN. The results of this study are consistent with an inhibitory effect of NO on neurons in the PVN that dictate sympathetic outflow, particularly RSND. It is of interest to note that renal nerve activity is known to contribute to changes in renal excretion of salt and water (56). The increase in arterial blood pressure after NOS blockade may be because of an increase in vasopressin or an increase in sympathetic nerve activity. We believe that the increase in arterial pressure was not entirely due to the release of vasopressin since the increase in arterial pressure was concurrent with an increase in efferent renal nerve activity.

At present, the precise cellular mechanism through which NO acts within PVN to inhibit sympathetic outflow is unknown. Perfusion of the PVN with NO in cerebrospinal fluid has been shown to increase the concentrations of some amino acids in the perfusates, including γ-aminobutyric acid (GABA) (46). The endogenous GABA system within the PVN has been reported to exert a tonic inhibitory effect on the sympathetic nervous system (57). Thus, it was proposed that the effect of NO within the PVN may be mediated by the release of GABA (46). Both NO and GABA are known to provide inhibitory inputs to the PVN of the hypothalamus and are involved in the control of sympathetic outflow. We recently examined the interaction of NO and GABA in the regulation of RSND in rats. Microinjection of SNP into the PVN elicited significant decreases in RSND, arterial blood pressure, and heart rate that were eliminated by a blockade of the GABA system (Fig. 6A). Conversely, microinjection of L-NAME elicited significant increases in the RSND arterial blood pressure. These sympathoexcitatory responses were masked by prior blockade of the GABA system with bicuculline, a $GABA_A$ receptor antagonist (Fig. 6B). The sympathoexcitatory effect of L-NAME was also eliminated by activation of the GABA system with muscimol, a GABA receptor agonist (Fig. 6B). These data indicate that the inhibitory effect of endogenous NO within

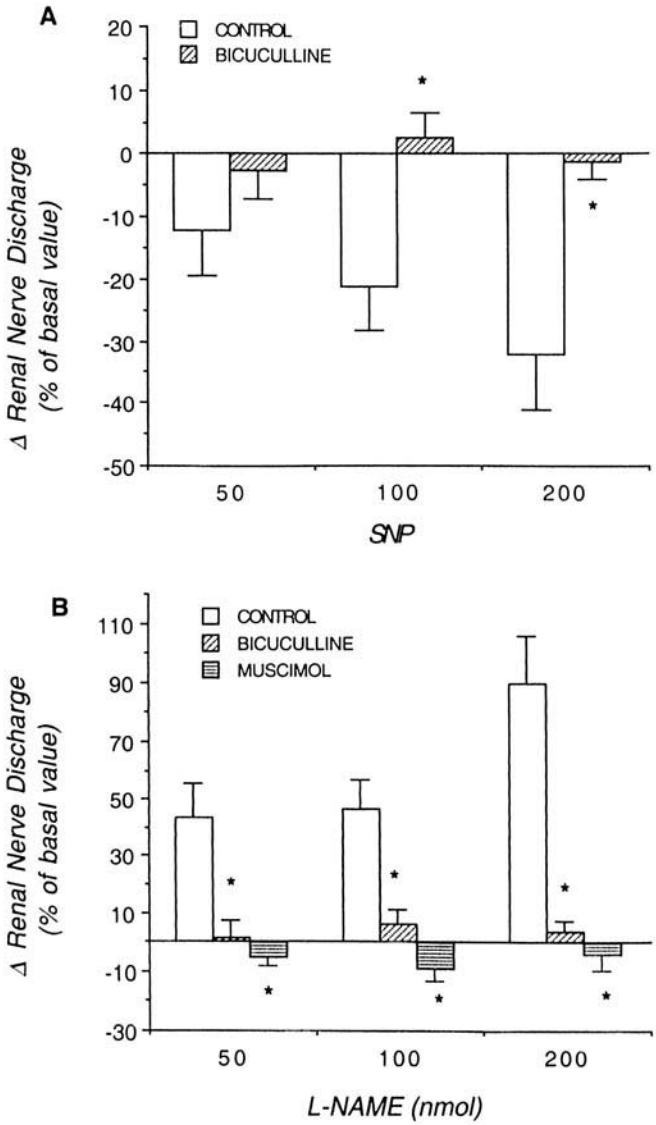

Figure 6. (A) Change of efferent RSND to microinjection of SNP into the PVN both in the absence (open bar) and presence (cross-hatched bar) of blockade of endogenous GABA system with bicuculline in the PVN. (B) Change of efferent RSND, to microinjection of L-NAME into the PVN in the absence (open bar) of muscimol or bicuculline and presence of muscimol (cross-hatched bar) or bicuculline (hatched bar) into the PVN. Graphs represent mean value for each group ± SEM. An asterisk indicates $P < 0.05$ compared with control. (Reproduced with permission from Ref. 47)

the PVN on the RSND is mediated by a GABA mechanism (47).

These studies demonstrate that NO within the PVN regulates sympathetic outflow *via* an inhibitory mechanism possibly involving a GABA system. We propose that NO production by neuronal NOS causes GABA release that in turn produces a reduction in RSNA (Fig. 7). Furthermore, we propose that decreased NO input and/or abnormalities in post-NO mechanisms within the PVN may contribute to the increased sympathetic nerve activity commonly observed during disease states such as heart failure and hypertension.

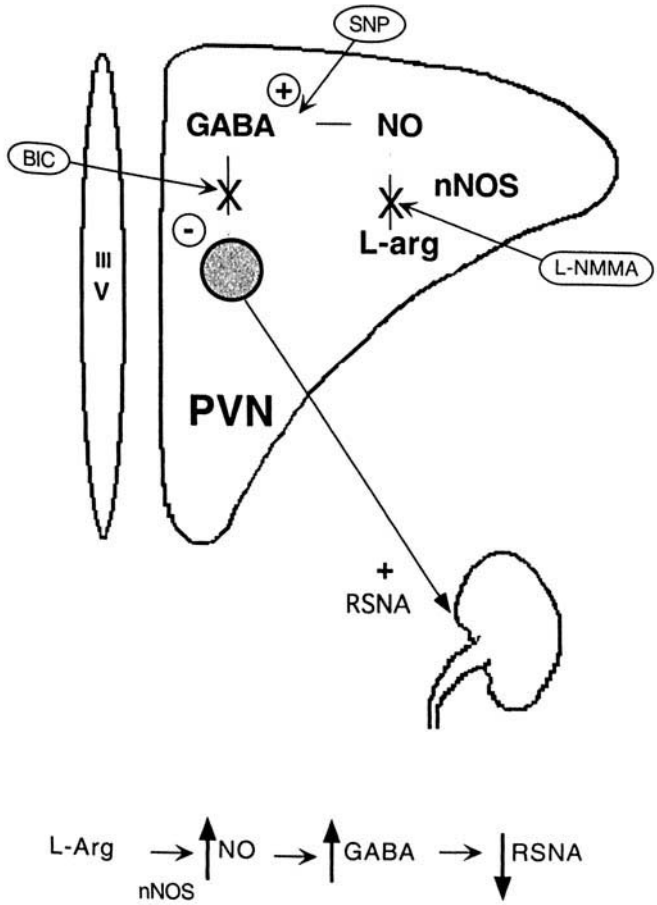

Figure 7. Schematic representations of our working model of interaction between NO and GABA systems to influence renal sympathetic nerve discharge. NO is formed from L-arg with the action of NOS. NO then stimulates (+) the release of GABA, which in turn inhibits (–) PVN neurons responsible for producing activation of RSND.

NO on Sympathetic Outflow: Disease States

Hypertension and NO. The importance of neuronal NO in many sites that participate in central autonomic regulation leads to the prediction that disease states associated with altered autonomic outflow may also exhibit altered central NO mechanisms/system. In hypertension, gene expression of nNOS is reported to be elevated in the hypothalamus and ventro lateral medulla (VLM), but not changed in cortex, cerebellum, and brainstem of spontaneously hypertensive rats (SHR) (58, 59). While other studies have suggested a decreased NOS activity based on reduced content of nitrite and nitrate in hypothalami of SHR compared with Wistar-Kyoto rats. (60). A recent study has suggested that endogenous NO in the NTS contributes to the impaired reflex control of heart rate, and a lower NOS activity in the dorsal brain stem (61). Central inhibition of NOS with L-NAME produced smaller pressor responses in stroke-prone SHRs, suggesting that endogenous NO was not able to decrease sympathetic outflow in these rats (62). Mineralocorticoid infusion induced hypertension demonstrated decreased nNOS mRNA levels in the hypothalamus and VLM. Hypertension that accompanies chronic renal failure demonstrates increased levels of nNOS mRNA in the posterior hypothalamus, PVN, locus coeruleus, NTS, and VLM (63). We have recently shown that the number of NADPH-diaphorase-positive cells are decreased in a model of renal wrap hypertension (64). These data would suggest that central NO restrains sympathetic outflow during these forms of hypertension. In contrast, changes in nNOS mRNA levels in PVN and caudal VLM have been found to be altered according to the phase of development and/or maintenance of two-kidney, one-clip renal hypertension. As time- (developmental) related studies have not been carried out using these models of hypertension, the question of the cause and effect of changes in the NO system and hypertension remain unresolved.

Heart Failure and NO. The heart failure condition is known to produce attenuated vasodilation in response to agonists known to operate *via* an NO mechanism (65–68). Concomitantly, levels of endogenous eNOS protein and mRNA for eNOS in peripheral tissue are reduced in the heart failure state (69). However, there are no studies examining the NO system within the CNS in heart failure except for a few recent studies from our laboratory (70–72). We have recently examined gene expression of nNOS in discrete brain regions in a group of rats with heart failure and sham-operated control rats (70). Experiments were performed 4 to 5 weeks after left coronary artery ligation in rats with greater than 30% infarct of the left ventricular myocardium. Sham rats had no observable damage to the myocardium. Total RNA was purified from microdissected tissue blocks containing hypothalamus, dorsal pons, dorsal medulla, RVLM, and CVLM. Changes in nNOS mRNA were semiquantified in each region by use of RT-PCR in which known concentrations of deletion mutants were coamplified as internal standards. Compared with controls, significant decreases in nNOS mRNA were found in the hypothalamus, dorsal pons (43%), and dorsal medulla (34%) of rats with heart failure. There were no statistically significant differences in RVLM and CVLM between the control and heart failure groups. Concomitant with these changes in central sites, the plasma concentration of norepinephrine was significantly higher in rats with heart failure. Changes in nNOS gene expression in the hypothalamus, dorsal medulla, and dorsal pons support the hypothesis that decreased NO mechanism may contribute to the increased sympathetic outflow in rats with heart failure (70).

Although the majority of the NOS within this section of the hypothalamus was within the PVN and SON, we cannot be certain that the downregulation is primarily within the PVN. We subsequently examined NADPH-diaphorase-positive neurons as a marker of nNOS activity within central sites (2, 73) to determine the specific areas that demonstrate altered nNOS activity during heart failure (71). These studies show that the number of nNOS-positive neurons in the PVN is significantly decreased in rats with heart failure either 6 or 16 weeks after coronary artery ligation. In contrast to the changes in the PVN, there were no significant

changes in the SON. Furthermore, there was an increase in the number of NOS-positive cells in the subfornical organ (SFO) and the organum vasculosum of the lamina terminalis (OVLT) of rats with heart failure. There appears to be specific decrease in nNOS within the PVN. This evidence indicates that neurons in the autonomic portion of the PVN (49) has decreased nNOS activity in rats with heart failure, consistent with the hypothesis that decreased inhibition by the NO system within the PVN results in an increased sympathetic outflow in rats with heart failure (71). Recently, we have observed that blockade of the endogenous NO mechanism by the microinjection of L-NMMA into the PVN increases the efferent RSND, mean arterial pressure, and heart rate in both sham-operated control rats and rats with heart failure. However, these responses to L-NMMA were significantly reduced in rats with heart failure as compared with the sham-operated control group (74). Conversely, microinjection of NO donor, SNP, into the PVN resulted in significant decreases in efferent renal sympathetic nerve discharge and mean arterial pressure in the sham-operated control group, but not in the rats with heart failure. These data suggest that the reduced renal sympathoinhibition mediated by endogenous NO within the PVN may contribute to the elevated sympathetic nerve activity during heart failure (74).

Summary and Conclusions

There is strong histochemical evidence for the presence of NOS throughout the autonomic nervous system. The presence of NOS in the central areas of the brain involved in regulating sympathetic outflow lead us to believe that these central sites may be involved in regulating overall sympathetic outflow. The finding from various studies after administration of NOS blockade, orally, intravenously, intracerebroventricullary, or within specific sites in the CNS are all in general agreement with the view that the central NO system is inhibitory to overall sympathetic outflow. Studies focused on the NTS and VLM are the exception and suggest both inhibitory and excitatory roles of the NO system. It is possible that the role of NO in regulating sympathetic output may be different in different autonomic centers. Nevertheless, these studies indicate that central NO systems are intimately involved in regulating sympathetic outflow. Furthermore, recent studies conducted in disease states known to have altered sympathetic outflow suggest that an abnormality in the central NO system may be partially responsible for the altered sympathetic outflow in these disease states. The true nature of the role of NO systems in dictating sympathetic outflow in these disease states remains to be fully explored and examined.

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