Role of Sympathetic Nerve Activity in the Generation of Vascular Nitric Oxide in Urethane-Anesthetized Rats

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The aim of the present study was to examine the involvement of the sympathetic nervous system in the generation or release of vascular nitric oxide. In urethane-anesthetized rats, the administration of the novel nitric oxide synthesis inhibitor L-N-nitro arginine (LNA) (0.02 mmol/kg i.v.) increased mean arterial pressure and renal, mesenteric, and hindquarter vascular resistances. The intravenous administration of L-arginine (60 mg/kg plus 12 mg/kg/min i.v.) produced small reductions in arterial pressure and vascular resistances and abolished the hemodynamic effects of LNA. Pretreatment with the ganglion blocking agent chlorisondamine lowered mean arterial pressure and vascular resistances, abolished the LNA-induced pressor and renal vasoconstrictor response, and attenuated the increases in mesenteric and hindquarter resistances. In contrast, the vasodilator hydralazine lowered mean arterial pressure and vascular resistances to levels equivalent to that of ganglionic blockade; however, the subsequent administration of LNA still produced significant increases in arterial pressure and regional vascular resistances. In ganglion-blocked rats in which pressure and vascular resistances were returned to normal levels by infusion of arginine vasopressin or phenylephrine, the pressor and vasoconstrictor effects of LNA were restored. However, phenylephrine was significantly more efficacious and markedly exaggerated the action of LNA. These results suggest that the sympathetic nervous system plays an important role in modulating the synthesis or release of vascular nitric oxide through the effects of 1) normal sympathetic discharge, 2) humoral activation of α-adrenergic receptors, and 3) vascular tone per se. (Hypertension 1991;17:881–887)

There is now considerable evidence that nitric oxide (NO) is an endogenous vasodilator substance that can be generated by vascular endothelium.1,2 Vascular smooth muscle has been recently demonstrated to also be a potential source of NO.3 In vitro studies have demonstrated that the enzymatic generation of NO from L-arginine can be inhibited in an enantiomeric manner by Nω-monomethyl-L-arginine (LNMMA).4 In vivo the systemic injection of LNMMA produces an L-arginine–reversible hypertension due to an increase in peripheral vascular resistance in all regional beds studied.5 However, Gardiner et al6 have shown that the vascular beds show a differential sensitivity to LNMMA. The results of these in vivo5,6 as well as an extensive number of in vitro studies7 suggest that there is tonic release of NO from the endothelium and perhaps smooth muscle8 and that this compound contributes to the regulation of vascular tone. Despite the compelling evidence that NO tonically regulates smooth muscle tone,9 the precise in vivo mechanisms by which inhibitors of NO production increased vascular resistance have yet to be determined.

On the basis of in vitro studies, the principal mechanism by which NO may influence vascular tone in vivo is via direct relaxation of the vascular smooth muscle.1,2 However, there is considerable evidence that NO may also regulate basal tone via inhibition of neurogenically mediated vasoconstriction. In addition to augmentation of neurogenically induced vasoconstriction by endothelial denudation,8,9 increasing shear stress on endothelium reduces neurogenic vasoconstriction10 and vascular norepinephrine “spill-over” is enhanced without intact endothelium.11 These findings, in addition to those suggesting that inhibition of NO may exert central sympathoexcitatory effects,12 raise the possibility that in addition to the direct increase in vascular tone produced by loss of NO-induced smooth muscle relaxation, hypertension...
resulting from the inhibition of vascular NO synthesis may facilitate the vasoconstrictor function of the sympathetic nerves. In the present study, we sought to determine whether the sympathetic nervous system is involved in the hemodynamic effects produced by L-arginine-reversible inhibitor of NO synthesis.13

Methods

Male Sprague-Dawley rats (n=39) (Biolabs, St. Paul, Minn.) weighing 360±6 g were used in this study. The rats were anesthetized with urethane (1 g/kg i.p.) and surgically implanted with femoral arterial and venous catheters for measurement of pulsatile and mean (MAP) arterial blood pressure, heart rate, and the administration of drugs, respectively. An intratracheal catheter was inserted, and the rats were allowed to breathe a mixture of 95% O2-5% CO2 in room air. The body temperature was maintained at approximately 37°C by a thermostat-controlled heating pad.

A midline laparotomy was performed, and miniature pulsed Doppler flow probes were placed around the left renal and superior mesenteric arteries and the lower abdominal aorta to monitor renal, mesenteric, and hindquarter blood flow velocities, respectively. The probes were sutured in place, the leads were exteriorized, and the wounds were closed. The reliability of the method for the estimation of flow velocity and for quantitative determination of percentage changes in renal, mesenteric, and hindquarter resistances have been described in detail elsewhere.14

Experiment 1

In this study, the effects of pretreatment with either saline (0.9% i.v., n=14) or L-arginine (60 mg/kg plus 12 mg/kg/min in 0.1 M phosphate buffer, pH 7.4 i.v., n=5) on the cardiovascular effects of LNA (0.02 mmol/kg, that is, 4.38 mg/kg i.v.) were examined. Since LNA is effectively insoluble in saline, the aqueous solution was strongly vortexed before removal of the appropriate volume of suspended drug. LNA was administered 15 minutes after reaching steady pressure and flow values after administration of saline or L-arginine.

Experiment 2

In this study, the effects of pretreatment with either the vasodilator substance hydralazine (500 μg/kg i.v., n=5) or the ganglion-blocking agent chlorisondamine (2.5 mg/kg i.v., n=5) on the cardiovascular effects of LNA (0.02 mmol/kg, that is, 4.38 mg/kg i.v.) were examined. Since LNA is effectively insoluble in saline, the aqueous solution was strongly vortexed before removal of the appropriate volume of suspended drug. LNA was administered 15 minutes after reaching stable pressure and flow values after administration of saline or L-arginine.

Experiment 3

In this study, the effects of restoration of arterial blood pressure and vascular resistances to pre-chlorisondamine (2.5 mg/kg i.v.) levels by continuous infusions of either arginine vasopressin (AVP) (250–986 ng/kg/min, 484±118 ng/kg/min, n=5) or phenylephrine HCl (21.5–43.6 μg/kg/min, 26±4 μg/kg/min, n=5) on the cardiovascular effects of LNA (0.02 mmol/kg i.v.) were examined. The LNA was administered 10–15 minutes after reaching stable arterial blood pressure and blood flows.

Drugs

All drugs used in this study were obtained from Sigma Chemical Co., St. Louis, Mo., except for chlorisondamine (CIBA-GEIGY, Summit, N.J.).

Statistics

The data are represented as the mean±SEM. The data were analyzed by repeated-measures analysis of variance with covariance followed by Student's modified t test with the Bonferroni correction for multiple comparisons between means using the modified error mean square terms (for between-group and within-group comparisons) from the covariance analysis.15

Results

Effects of Arginine on L-NO2-Nitro Arginine-Induced Responses

Typical examples of the effects of LNA (0.02 mmol/kg i.v.) on MAP and regional flows in rats pretreated with saline or L-arginine are shown in Figure 1. LNA induced an increase in MAP (without changes in heart rate) and reduced blood flow in the
three vascular beds examined. The maximal responses occurred between 15 and 25 minutes and were sustained at these levels for at least 2 hours. The effects of LNA were markedly attenuated in the rats treated with L-arginine.

A summary of these hemodynamic effects is shown in Figures 2 and 3. The initial values for MAP and renal, mesenteric, and hindquarter resistances for the group that received saline were identical to those of the group pretreated with L-arginine and were not altered by saline. In contrast, L-arginine produced significant reductions in MAP and each of the three vascular resistances. In another study (n=5 for both groups), MAP and vascular resistances of both the saline or L-arginine–treated rats remained stable (p>0.05) for at least 2 hours (i.e., longer than the time needed for the experiments with LNA). LNA produced significant increases in MAP and renal, mesenteric, and hindquarter resistances in the group pretreated with saline. These LNA-induced responses were abolished by pretreatment with L-arginine.

Effects of Ganglionic Blockade

Typical examples of the effects of LNA on MAP and regional blood flows of a rat pretreated with the ganglionic blocker chlorisondamine and of one pretreated with the nonspecific vasodilator hydralazine are shown in Figure 4. These agents produced equivalent reductions in both MAP and regional blood flows but differential effects on the pressor and regional vasoconstrictor effects of LNA. The LNA-induced rise in MAP was blocked by chlorisondamine but not hydralazine, and the LNA-induced decrease in vascular flows was more pronounced in the rats treated with hydralazine. A summary of the effects of these pretreatments on the peak hemodynamic effects of LNA are shown in Figures 5 and 6.

The initial values for pressure and vascular resistances were identical in both groups (p>0.05 for all comparisons). The reductions in MAP and vascular resistances produced by chlorisondamine and by hydralazine were also not different, and these depressor and vasodilator effects were sustained for at least 2 hours.
least 90 minutes (i.e., beyond the time needed to study the actions of LNA). The administration of LNA did not significantly alter MAP or renal resistance in rats pretreated with the ganglionic blocker but did produce a significant increase in mesenteric and hindquarter resistances. Preliminary studies have established that the small increases in resistance were offset by a reduction in cardiac output. In contrast, in the hydralazine-treated rats, LNA increased MAP and all three vascular resistances to values significantly greater than those observed after ganglionic blockade.

**Effects of Vasoconstrictors**

Summaries of the effects of LNA on the MAP and vascular resistances of the ganglion-blocked rats receiving an infusion of either AVP or phenylephrine are shown in Figures 7 and 8. The initial values for pressure and resistance and the effect on these parameters of chlorisondamine were identical (p>0.05 for all comparisons) in each group (with the exception of renal resistance, which was reduced more in the phenylephrine group, -35±5% versus -17±3%). The infusion of AVP and phenylephrine restored the MAP and vascular resistance values to levels not significantly different from those before chlorisondamine (with the exception of mesenteric resistance, which was 34±13% higher).

The administration of LNA produced significant (p<0.05) increases in MAP and vascular resistances (maximal effects within 10–20 minutes) in both the AVP- and phenylephrine-treated rats. However, these responses were much more marked (p<0.05 for all comparisons) in the group treated with phenylephrine.

**Discussion**

The data presented here confirm and extend those of previous studies that have examined the cardiovascular effects of inhibition of the synthesis of NO and have provided evidence that the sympathetic nervous system may play an important role in modulating the generation of this vasodilator substance. The present studies demonstrate that LNA-induced increases in MAP and vascular resistances are 1) abolished by L-arginine, 2) markedly diminished by ganglion blockade but not by an equivalent hypotensive effect of hydralazine, 3) partially or fully restored in ganglion-blocked rats in which MAP and vascular resistances were returned to preganglion blockade levels by the vasoconstrictor agents AVP and phenylephrine, and that 4) phenylephrine is significantly more efficacious in restoring the hemodynamic effects of LNA than is AVP.

To our knowledge, this is the first report on the cardiovascular effects of LNA, which is a more potent inhibitor of NO synthesis in vitro than LNMMMA. The pattern of the hemodynamic responses produced by LNA is certainly similar to that reported for LNMMMA in conscious rats. Moreover, the finding that L-arginine prevents or reverses the
LNA-induced increases in MAP and vascular resistances is also consistent with that found for LNMMMA\textsuperscript{-6,17} and suggests that the LNA-induced hypertension is due to an increase in peripheral vascular resistance that ultimately results from competitive inhibition of the enzymatic formation of vascular NO.

One interpretation of the results with ganglionic blockade is that the hypertensive effects of LNA result from a peripheral or centrally mediated increase in sympathetic neurogenic drive rather than simply by a blockade of the direct NO-induced relaxation of the vascular smooth muscle. Togashi et al\textsuperscript{12} found that LNMMMA (10–125 µg/kg i.v.) produced hypertension that was associated with an initial decrease followed by an increase in both preganglionic adrenal and postganglionic renal sympathetic nerve activity. These authors also reported that the decrease in renal sympathetic nerve activity was abolished by a combination of vagotomy and sinoaurtic denervation and moreover that the intracisternal injection of LNMMMA (1 mmol) produced a decrease in renal sympathetic nerve activity. In preliminary studies, we found that neither the initial nor sustained LNA-induced increases in MAP in urethane-anesthetized rats were associated with a change in renal sympathetic nerve activity, even though the activity increased slightly 20–40 minutes after administration of LNA.

Although the present studies suggest that the initial pressor action of LNA is not due to increased sympathetic activity per se, basal tonic discharge may directly contribute to the maintenance of the hypertensive effect of LNA. We propose that the hypertension following the systemic administration of inhibitors of NO synthesis, an effect that is virtually abolished by ganglionic blockade, involves augmentation of neurogenically derived vasoconstrictor tone by 1) maintenance or increase of sympathetic input to the vasculature, 2) facilitation of the release of norepinephrine due to the removal of NO-induced presynaptic inhibition,\textsuperscript{11} and 3) enhanced vasoconstrictor effects of norepinephrine due to the loss of the direct vasorelaxant effects of NO.\textsuperscript{8,10} Vargas et al\textsuperscript{17} have recently reported that the hypertensive effects of LNMMMA (3–30 mg/kg i.v.) are also markedly diminished in ganglion-blocked urethane-anesthetized rats. Moreover, in preliminary studies we have found that a 0.1 mmol/kg dose of LNA produces an immediate hypertension in ganglion-blocked rats; this hypertension is short-lived (i.e., less than 10 minutes). This finding strongly suggests that the
generation of NO may depend on the integrity of the sympathetic nervous system.

Another possible explanation for the diminished effects of LNA in ganglion-blocked rats is that the accompanying hypotension and vasodilation reduce the basal synthesis or release of NO and thus prevent the effects of LNA on synthesis of NO. This possibility is unlikely since LNA produces sustained increases in MAP and vascular resistances in rats treated with the non-endothelium-dependent vasodilator hydralazine, which lowered MAP and vascular resistances to levels equal to those produced by chlorisondamine.

There is no direct evidence that sympathetic nerves innervate the endothelium of large arteries or that norepinephrine released from the nerve terminals can diffuse through the vessel wall in high enough concentrations to activate endothelial α-adrenergic receptors. However, based on the results of extraluminal application of norepinephrine, neuronally released norepinephrine is much more likely to reach the endothelial cells of small arteries. Moreover, with the recent observation that vascular smooth muscle also generates NO, it is possible that the sympathetic input may directly (i.e., independent of the effects on muscle contraction) modulate the synthesis or release of NO via stimulation of α-adrenergic receptors on the endothelium as well as vascular smooth muscle.

The finding that LNA induces a rise in pressure and vascular resistances in ganglion-blocked rats in which systemic and regional hemodynamics were restored to control levels by infusion of the α-adrenergic receptor antagonist phenylephrine suggests that the generation of NO occurs at "normal" levels of pressure and vascular tone and that this generation of NO can occur independent of sympathetic input. These results are consistent with in vivo and in vitro evidence that there is basal release of NO from vascular tissue.

An important finding of the present study was that LNA produced much greater increases in MAP and vascular resistances in ganglion-blocked rats in which the hemodynamics were restored to control levels by an infusion of the α-adrenergic receptor agonist phenylephrine compared with those receiving AVP. These results suggest that the activation of α-adrenergic receptors leads to a marked enhancement of the synthesis or release of NO as opposed to that occurring simply by restoration of basal vasomotor tone as was accomplished with AVP. Although there is general agreement that stimulation of endothelial α-adrenergic receptors induces the generation of NO in vitro, there is conflicting evidence as to whether α-adrenergic receptor agonists induce the release of NO from vascular tissues. For example, many studies have demonstrated that the removal of endothelium augments contractions produced by α-adrenergic receptor agonists, including phenylephrine.

In summary, the present study provides evidence that the sympathetic nervous system plays an important role in the tonic vasodilator actions of vascular NO in urethane-anesthetized rats. The potential mechanisms include 1) sympathetic discharge enhances the generation of NO by a direct interaction with adrenergic receptors, 2) neurogenically derived vasoconstriction enhances the generation of NO, 3) circulating adrenergic receptor agonists also augment generation of NO by direct receptor interaction and production of vasomotor tone, and 4) NO may modulate neurogenically mediated vasoconstriction by presynaptic inhibition of norepinephrine release as well as reducing the responsiveness of the smooth muscle to the neurotransmitter. A fifth possibility, namely that the sympathetic nerves may release NO, is suggested by recent findings that NO synthetase exists in autonomic neurons supplying blood vessels. On the basis of the extensive number of in vitro studies, it appears that a dysfunction in the synthesis or release of vascular NO may be involved in the
etiology of various forms of genetic and experimentally induced hypertensive models. The present findings suggest this represents a fundamental abnormality in the physiological relation between sympathetic discharge and a prominent local vasodilator system.

References

KEY WORDS • nitric oxide • sympathetic nervous system • rat studies
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