Nitric Oxide Limits Pressor Responses to Sympathetic Activation in Rat Spinal Cord

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Abstract—N-methyl D-aspartate (NMDA) receptor stimulation is known to activate nitric oxide (NO) synthase, an enzyme present in a high proportion of sympathetic preganglionic neurons. In this study, we have examined the possibility that NO modulates the pressor responses elicited by NMDA receptor stimulation in the spinal cord. In experiments on anesthetized rats, we determined whether intrathecal administration of either 3-morpholinylsydnoneimine chloride (SIN-1), an NO donor, or N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME), an NO synthase inhibitor, affected the response to stimulation of spinal NMDA receptors by NMDA (1 pmol to 1 μmol in 10-μL intrathecal administration). Intrathecal NMDA resulted in dose-dependent increases in blood pressure. SIN-1 (100 nmol) attenuated the pressor responses to NMDA (F\textsubscript{1,70}=12, \(P=0.001\)). Conversely, L-NAME (1 nmol to 1 μmol) augmented the pressor response to NMDA in a dose-dependent manner (F\textsubscript{1,161}=28.3, \(P<0.001\)). The effect of L-NAME to amplify the pressor response to NMDA was reversed by L-arginine but not by D-arginine. These results indicate that endogenous synthesis of NO in the spinal cord limits the pressor response to stimulation of spinal NMDA receptors. (Hypertension. 2000;36:1089-1092.)

Key Words: blood pressure • sympathetic nervous system • nitric oxide synthase • L-NAME

Nitric oxide (NO), synthesized from L-arginine by NO synthase (NOS), mediates endothelium-dependent vasodilation, acts as a neurotransmitter, and is used by macrophages to kill tumor cells and bacteria.\textsuperscript{1} Inhibition of NOS increases arterial pressure by causing peripheral vasoconstriction and by increasing sympathetic nerve activity by central nervous system (CNS) effects.\textsuperscript{2} Inhibition of NOS has been reported to produce diverse actions at CNS sites involved in cardiovascular regulation. NOS inhibitors increase arterial pressure when injected into the rostral ventrolateral medulla\textsuperscript{3} or the hypothalamic paraventricular nucleus\textsuperscript{2} and lower arterial pressure when injected into the caudal ventrolateral medulla.\textsuperscript{5} NOS has been identified in most sympathetic preganglionic neurons (SPN) in the intermediolateral column of the spinal cord,\textsuperscript{6,7} but its function at this site is not yet established. The NO precursor L-arginine has been reported to excite renal sympathetic nerves and the NOS inhibitor N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME) to silence renal sympathetic nerves when injected into the spinal intrathecal space in the rabbit.\textsuperscript{8} If NO activated SPN, then L-NAME might be expected to lower arterial pressure. To date, studies on anesthetized rats have been inconsistent, with some studies reporting pressor responses\textsuperscript{9,10} and others reporting depressor responses\textsuperscript{11} to intrathecal administration of L-NAME.

Because NOS is activated by an increase in intracellular calcium, such as that caused by stimulation of N-methyl D-aspartate (NMDA) receptors, we tested the possibility that NO synthesis modulates the pressor response elicited by NMDA in the spinal cord.

Methods

Fifty-two male Wistar-Kyoto rats (weight 300 to 400 g) were obtained from the Flinders Medical Center Animal House. All experimental procedures conformed to guidelines laid down by the National Health and Medical Research Council of Australia and were approved by the Animal Welfare Committee at Flinders University of South Australia.

Effect of 3-Morpholinylsydnoneimine Chloride on Pressor Response to Intrathecal NMDA in Anesthetized Rats

Studies were performed under barbiturate anesthesia (60 mg/kg IP sodium pentobarbital and supplementary doses of 30 mg/kg if required). A femoral artery was cannulated for measurement of arterial pressure and a femoral vein was cannulated for administration of drugs (vinyl tube, outer diameter 0.50 mm, Dural Plastics SV 8). Through a small incision in the atlanto-occipital membrane, a stretched polyethylene tube (outer diameter 0.80 mm, Dural Plastics SP 31) was inserted into the spinal subarachnoid space and threaded caudally 8.5 cm so that the tip lay at the thoraco-lumbar junction. Pancuronium bromide (0.4 mg) was administered intravenously, and an additional dose was given if NMDA evoked muscle twitching. The need for supplemental doses of barbiturate was assessed before each dose of pancuronium was administered. The spinal cord was exposed at the C1 level and sectioned. Drugs were administered intrathecally in 10 μL of artificial cerebrospinal fluid (an aqueous solution of 1.4 mmol/L CaCl\textsubscript{2}, 1.0 mmol/L MgCl\textsubscript{2}, 2.6 mmol/L KCl, and 128.6 mmol/L NaCl, phosphate buffered to pH 7.2). Drug solutions...
were made fresh on the day of the experiment. Increasing doses of NMDA (1 pmol to 1 μmol in 10-μL intrathecal administration) were coadministered with 3-morpholinosydnoneimine chloride (SIN-1) (100 nmol/L) or vehicle. Blood pressure was measured for 20 minutes after each dose of NMDA. SIN-1 or vehicle was administered between doses of NMDA+SIN-1 (or NMDA+vehicle), and another 20 minutes was allowed to pass before the next dose of NMDA.

**Effect of L-NAME on Pressor Response to Intrathecal NMDA in Anesthetized Rats**

These experiments were carried out in a fashion similar to those described above except that increasing doses of NMDA were coadministered with 1 of 4 doses of L-NAME (1 nmol, 10 nmol, 100 nmol, or 1 μmol), and L-NAME alone was given between doses of NMDA+L-NAME.

**Effect of L- or D-Arginine Plus L-NAME on Pressor Response to Intrathecal NMDA in Anesthetized Rats**

In these experiments, increasing doses of NMDA were coadministered with one of l-arginine (5 μmol) and L-NAME (100 nmol) or d-arginine (5 μmol) and L-NAME (100 nmol/L). L-NAME was administered together with L- or d-arginine between doses of NMDA.

At the end of each experiment, 10 μL of methylene blue was injected into the intrathecal catheter followed by 10 μL of artificial cerebrospinal fluid, and rats were killed with an overdose of sodium pentobarbital. An autopsy was performed to confirm the location of cerebrospinal fluid, and rats were killed with an overdose of sodium pentobarbital. Arterial pressure was similar in anesthetized, paralyzed, spinally transected rats subsequently given different doses of NMDA.

**Statistics**

Results are shown as mean±SEM. Changes in mean arterial pressure (ie, mean arterial pressure after administration of drugs compared with pretreatment mean arterial pressure) were compared between groups by means of 1- or 2-way ANOVA. Differences between groups were analyzed by the Ryan-Einot-Gabriel-Welsch (REGW) test.

**Results**

**Effects of SIN-1 on Pressor Response to Intrathecal NMDA in Anesthetized Rats**

In anesthetized and paralyzed rats spinally transected to exclude supraspinal effects, pretreatment arterial pressure was 54±3 mm Hg in rats subsequently given NMDA alone and 55±7 mm Hg in rats subsequently given NMDA together with SIN-1 (n=6 per group, P=NS). Intrathecal administration of NMDA (1 pmol to 1 μmol) resulted in dose-dependent increases in blood pressure in both groups of rats (Figure 1, significant NMDA effect, F3,36=19.8, n=6 per group, P<0.001). SIN-1 (100 nmol/L) attenuated the pressor responses to NMDA (significant SIN-1 effect, F1,10=12, n=6 per group, P=0.001).

**Effect of L-NAME on Pressor Response to Intrathecal NMDA in Anesthetized Rats**

Arterial pressure was similar in anesthetized, paralyzed, spinally transected rats subsequently given different doses of L-NAME (1 nmol; 61±5 mm Hg, n=9; 10 nmol, 65±2 mm Hg, n=7; 100 nmol, 64±2 mm Hg, n=6; 1 μmol, 67±4 mm Hg, n=6, F3,32=0.5, P=NS). Intrathecal administration of NMDA (1 pmol/L to 1 μmol/L) resulted in dose-dependent increases in blood pressure in rats receiving L-NAME (Figure 2, significant NMDA effect, F3,161=63.4, P<0.001). L-NAME amplified the pressor response to NMDA in a dose-responsive manner (Figure 2, F3,161=28.3, P<0.001). Post hoc testing with the REGW test revealed significant differences between groups. The pressor responses to intrathecal NMDA were similar in rats receiving 1 nmol and 10 nmol L-NAME. The NMDA-induced pressor responses in rats receiving 100 nmol L-NAME were significantly larger than the responses in rats receiving lower doses of L-NAME. Rats receiving 1 μmol L-NAME showed significantly larger NMDA-induced pressor responses than those in any of the other groups.
Effect of NOS Inhibition in Presence of L- or D-Arginine in Anesthetized Rats

Pretreatment arterial pressure was 67±4 mm Hg in rats allocated to receive L-arginine (5 μmol) coadministered with L-NAME (100 nmol) and 63±2 mm Hg in rats allocated to receive D-arginine (5 μmol/L) coadministered with L-NAME (100 nmol). Increasing doses of NMBA produced dose-related pressor responses in both L-arginine–treated and D-arginine–treated rats (Figure 3, significant NMDA effect, F_{1,70}=8.8, P<0.005, significant NMDA by l-arginine interaction, F_{6,70}=3.3, P<0.01).

Discussion

Many neurons in the CNS contain NOS, a calmodulin-dependent enzyme, and generate NO in response to increased intracellular calcium. NO in turn binds to soluble guanylate cyclase, eliciting large increases in the second messenger, cyclic GMP. In the CNS, activation of NMDA receptors is likely to be an important mechanism leading to NOS activation. Indeed, it was the observation that the excitatory neurotransmitter glutamate elicited large increases in cyclic GMP that led to the suggestion that NO might function as an intercellular messenger in the CNS.

In this study, we have explored possible interactions between NMDA receptor activation and NO in the spinal mechanisms controlling blood pressure. The major finding of this study is that intrathecal administration of the NO donor SIN-1 attenuated the pressor responses induced by intrathecal NMDA, whereas the intrathecal administration of the NOS inhibitor L-NAME amplified these responses. The effect of L-NAME was a result of inhibition of NOS, since it was attenuated by l-arginine but not by d-arginine. These data demonstrate that endogenously generated NO limits the pressor response elicited by activation of spinal NMDA receptors.

NOS is found in more than half of all sympathetic preganglionic neurons, but the functional significance of NO in controlling cardiovascular and other autonomic functions is uncertain. Hakim et al reported that the NO precursor L-arginine, excited renal sympathetic nerves and that the NOS inhibitor L-NAME silenced renal sympathetic nerves when injected into the spinal intrathecal space in the rabbit. No significant change in arterial pressure was found in this study, a finding that was attributed to the small injection volumes that may have only affected a few SPN. If NO activated SPN projecting to cardiovascular targets, then NOS inhibitors would be expected to lower arterial pressure and NO donors would be expected to raise arterial pressure. Experiments in which NO donors or NOS inhibitors have been administered intrathecally to anesthetized rats have yielded inconsistent results. Lee et al reported that administration of a NOS inhibitor lowered arterial pressure. In contrast, Garcia et al and Koga et al reported that intrathecal administration of L-NAME elicited pressor responses. These inconsistencies in response suggest that the effects of NO are complex and might be critically dependent on the experimental conditions. This situation could arise if NO effects are mediated by modulation of the actions of other neurotransmitters rather than by direct neuronal activation or inhibition. Inhibition by an NMDA antagonist of the pressor response to L-NAME and inhibition by L-NAME of the pressor response and tachycardia elicited by intrathecal administration of carbachol are observations consistent with a modulatory rather than a direct role for NO in blood pressure control. In the present study, the dose-response relation of NMDA administered intrathecally was determined in the presence of a NO donor, SIN-1, or a range of doses of the NOS inhibitor L-NAME. The NO donor SIN-1 attenuated the pressor response to NMDA, an effect that was most prominent at relatively low doses of NMDA. Because activation of NMDA receptors is a potent stimulus for NO synthesis, an inhibitory effect of exogenous NO may be less apparent when endogenous NO synthesis is increased by NMDA receptor activation. Blockade of NO synthesis with L-NAME produced a marked shift in the dose-response relation to NMDA. The lowest dose of NMDA (1 μmol/L) elicited a 29-mm Hg pressor response when coadministered with L-NAME (1 μmol), whereas a 1000-fold-higher NMDA dose (1 nmol) only increased arterial pressure by 25 mm Hg when administered without L-NAME. The effect of L-arginine is probably a result of inhibition of NOS because L-arginine reverses the amplification of the pressor response to NMDA induced by L-NAME.

The mechanism by which NO might inhibit pressor responses induced by NMDA is unclear. Soluble guanylate cyclase is a major target for NO. However, this enzyme is not activated in neurons that produce NO because the increases in Ca²⁺ necessary to activate NOS inhibit soluble

![Figure 3](http://hyper.ahajournals.org/)

**Figure 3.** Effect of NOS inhibition in presence of L- or D-arginine. Change in mean arterial pressure (Δ MAP) in anesthetized and paralyzed rats spinal transected to exclude supraspinal effects given escalating doses of NMDA (1 μmol/L) together with 5 μmol L-arginine+100 nmol, L-NAME (•) or 5 μmol, d-arginine+100 nmol, L-NAME (○). L-arginine+L-NAME attenuated pressor response to NMDA compared with d-arginine+L-NAME (significant l-arginine effect, F_{1,70}=8.8, P<0.005, significant NMDA by l-arginine interaction, F_{6,70}=3.3, P<0.01).
guanylate cyclase. NO is freely diffusible, and significant NO concentrations are likely to be observed up to 200 μmol/L from its site of production. Hence, NO generated within a neuron could act on terminals impinging on that neuron as well as nearby neurons. NO has been shown to influence neurotransmitter release, and it is probably by this mechanism that NO can enhance either excitatory or inhibitory synaptic potentials in SPN. Under conditions in which inhibitory synaptic inputs predominate, enhancement of inhibitory synaptic potentials by NO might decrease sympathetic outflow.

A direct effect of NO on NMDA receptor function is, perhaps, a more likely explanation for our findings. NMDA is capable of activating SPN directly. Consequently, the pressor response to intrathecal NMDA is more likely to be a result of activation of NMDA receptors on SPN rather than an indirect effect from activation of unidentified spinal interneurons. NO inhibits NMDA receptors in other neurons. The NO donor SIN-1 attenuates NMDA-induced increases in intracellular calcium and inhibits NMDA-induced currents in striatal neurons. This is a cyclic GMP–independent effect of NO on the redox modulatory site of the NMDA receptor that appears to be mediated by nitrosylation of a specific cysteine residue on the NR2A subunit of the NMDA receptor. Our results are consistent with a NO-mediated inhibition of NMDA receptors. In our experimental paradigm, this inhibition attenuates the sympathetic activation and pressor response elicited by NMDA receptor activation in the spinal cord. Thus, the effect of NO on NMDA-mediated responses in SPN that we have observed may affect the baroreflex and other cardiovascular responses that are dependent on the activity of RVLM neurons.

In summary, we have demonstrated that NO attenuates the pressor responses to NMDA receptor–mediated neuronal excitation and is likely to modulate descending excitatory signals mediating cardiovascular responses to diverse stimuli.

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References

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