Inhibition of Nitric Oxide Formation in the Nucleus Tractus Solitarius Increases Renal Sympathetic Nerve Activity in Rabbits

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It has been shown that nitric oxide (NO) is synthesized in the central nervous system as well as in vascular endothelial cells. However, the physiological role of NO in cardiovascular regulation by the central nervous system remains unclear. This objective of this study was to examine the possibility that NO plays a role in neural transmission in the nucleus tractus solitarius (NTS) and thus contributes to control of sympathetic nerve activity in rabbits. We examined the effects of \( N^\text{G} \)-monomethyl-L-arginine (L-NMMA), an inhibitor of the formation of NO from L-arginine, microinjected into the NTS on arterial pressure (AP), heart rate (HR), and renal sympathetic nerve activity (RSNA). L-NMMA increased AP and RSNA in rabbits with intact as well as denervated sinoaortic baroreceptors and vagi. L-NMMA increased HR only in rabbits with sinoaortic denervation and vagotomy. Pretreatment with L-arginine microinjected into the NTS, which did not alter baseline AP, HR, and RSNA, prevented the increases in AP and RSNA evoked with subsequent L-NMMA. Pretreatment with L-arginine did not alter the effects of subsequent L-NMMA injections into the NTS. The gain of arterial baroreflex control of RSNA assessed by the slope of the regression line relating changes in AP and those in RSNA caused by intravenous phenylephrine or nitroglycerin did not differ before and after microinjections of L-NMMA. L-NMMA microinjected into the area postrema did not alter AP, HR, or RSNA. These results suggest that in rabbits NO is involved in the mechanism in the NTS that mediates tonic inhibition of RSNA. The effect of inhibition of NO formation in the NTS does not depend on functioning baroreflex mechanisms. (Circulation Research 1993;72:511–516)

**KEY WORDS** • nitric oxide • \( N^\text{G} \)-monomethyl-L-arginine • nucleus tractus solitarius • area postrema • sympathetic nerve activity • baroreflex • rabbits

Previous studies have shown that nitric oxide (NO), which largely accounts for the biological effects of endothelium-derived relaxing factors, \(^1\) is synthesized from L-arginine in the central nervous system \(^2\) as well as in other tissues, including vascular endothelial cells \(^3\), macrophages \(^4\), and neutrophils \(^5\). It is also suggested that in various tissues NO plays a physiological role in local transcellular communication by facilitating cGMP formation in adjacent cells through activation of soluble guanylate cyclase \(^6\).

\( N^\text{G} \)-Monomethyl-L-arginine (L-NMMA), an analogue of L-arginine, is an inhibitor of the formation of NO from L-arginine in various tissues, including the central nervous system \(^1,4,5,7\). Recently, Sakuma et al \(^8\) have shown that L-NMMA administered intravenously in sinoaortic-denervated and vagotomized rats increased renal sympathetic nerve activity (RSNA), which was abolished by spinal section. Togashi et al \(^9\) have shown that an intracisternal injection of L-NMMA increased arterial pressure and RSNA. These results suggest that NO is involved in the regulation of RSNA by the central nervous system, particularly in the brainstem. Furthermore, a recent study by Paola et al \(^10\) has demonstrated in rats that L-NMMA microinjected into the nucleus tractus solitarius (NTS) attenuated the depressor effect evoked with glutamate microinjected into the NTS. Since glutamate acts as a neurotransmitter at the NTS \(^11,12\), the latter findings suggest the possibility that NO is involved in neural transmission in the NTS.

However, Paola et al \(^10\) examined the effect of L-NMMA microinjected into the NTS on the depressor effect of glutamate exogenously administered into the NTS. It is not known whether L-NMMA alters sympathetic nerve activity by inhibiting basal NO formation in the NTS. Moreover, it is not known whether the cardiovascular effects of L-NMMA microinjected into the NTS depends on functioning baroreflex mechanisms or whether L-NMMA alters arterial baroreflex function. The NTS is the site where afferent fibers arising from arterial baroreceptors, chemoreceptors, cardiopulmonary receptors, and other visceral receptors make the first central synapses \(^13\) and thus plays an important role in the integration of autonomic control of the cardiovascular system \(^14\).
The purpose of this study was to further examine the role of NO in neural transmission in the NTS. We examined the effects of L-NMMA microinjected into the NTS on arterial pressure, heart rate, and RSNA in rabbits with intact and denervated sinoaortic baroreceptors and vagi. We also examined the effects of L-NMMA microinjected into the NTS on the gain of arterial baroreflex control of RSNA.

Materials and Methods

General Procedures

Male New Zealand White rabbits (2.2–3.5 kg) were anesthetized with α-chloralose (60 mg/kg i.v.) after induction with thiaylal sodium (25 mg/kg i.v.) and mechanically ventilated (model SN-480-6 ventilator, Shinano, Tokyo) with room air supplemented with oxygen through endotracheal tubing. Supplemental doses of α-chloralose (20 mg/kg i.v.) were given hourly, and the rabbits were immobilized with pancuronium bromide (0.5 mg i.v.). Arterial blood gases were monitored, and ventilation was adjusted to maintain PaO₂ greater than 100 mm Hg, PaCO₂ at 35–45 mm Hg, and pH 7.35–7.45. Body temperature was maintained at 37–40°C by a heating pad and a heating lamp.

A femoral artery was cannulated with a high-fidelity micromanometer (model MPC-500, Millar Instruments, Houston, Tex.) for arterial pressure recording. Heart rate was determined with a cardiotachometer (model 2140, San-ei, Tokyo) triggered by arterial pulsation. A femoral vein was cannulated with PE-90 tubing for drug administration.

After the left flank was opened, the left renal nerve was identified, separated from surrounding connective tissues, cut distally under a dissecting microscope (model OPMI 99, Zeiss, Germany), and covered with mineral oil. RSNA was recorded by bipolar electrodes (silver/silver chloride), preamplified with a high-gain difference amplifier (model MEG-2100, Nihon-Kohden, Tokyo) with a band-pass filter (150–1,000 Hz), fed into a nerve traffic analyzer (model MET-1100, Nihon-Kohden), and converted to spikes by a window discriminator.

Arterial pressure, heart rate, raw RSNA, and the integrated output from the spike counter (integrated RSNA) were simultaneously recorded on an eight-channel optical hard-copy recorder (model 8M14, San-ei) for monitoring experimental conditions and on a magnetic tape recorder using pulse code modulation (model RD-101T, TEAC, Tokyo) for subsequent analysis. Data analysis was performed after digitizing the pretaped data at 100 Hz (12-bit resolution) with a laboratory computer system (model PC-9801, NEC, Tokyo; ADX-98, CANOPUS, Kobe, Japan).

Microinjection of Drugs Into the NTS and Area Postrema

Each rabbit was placed in a stereotaxic frame with the head inclined downward by 45°. An incision was made between the ears, and muscles were dissected to expose the cisterna magna. The atlantooccipital membrane was cut and removed. The dura was incised, and the obex was visualized.

A glass micropipette (50 μm o.d.) was filled with drug dissolved in artificial cerebrospinal fluid containing (mM) NaCl 123, CaCl₂ 0.86, KCl 3.0, MgCl₂ 0.89, NaHCO₃ 25, NaH₂PO₄ 0.5, and Na₂HPO₄ 0.25 and gassed with 5% CO₂–95% O₂ mixture. The pipette was placed in a micromanipulator and positioned in the injection sites. Microinjection was made at two sites at each side of the NTS or at one site of the area postrema (0.2 mm below the dorsal surface) as shown in Figure 1. The sites of injections were defined according to an atlas in the rabbit, with reference to the midline, the dorsal surface of the medulla, and the rostral border of the area postrema (Figure 1). Injections of drugs in the NTS were made in sequence from sites 1 to 4 (Figure 1) so that four injections (one injection per site) were made in each rabbit. The drug dissolved in artificial cerebrospinal fluid (0.2 μl) was injected at each injection site for 10 seconds. The pipette was left in situ for a few minutes after injection and was then removed. Immediately thereafter, the micropipette containing methylene blue was positioned at the site of the drug injection, and methylene blue (0.2 μl) was injected to examine the sites of injection at postmortem examination.

Histological Examination

After completion of the experiments, the brain was perfused with 0.9% saline followed by 10% formalin solution through the heart. The brainstem was removed, and frozen sections (50 μm) were cut serially. The locations of methylene blue staining were identified with a microscope. The animals were excluded from the study if injections were not at the proper sites.

Protocol

Experiment 1. In this experiment, we determined the effects of L-NMMA (16 nmol per site) microinjected into the NTS on arterial pressure, heart rate, RSNA, and the gain of arterial baroreflex control of RSNA in animals with intact sinoaortic baroreceptors and vagi (n=11). Arterial pressure, heart rate, and RSNA were recorded continuously throughout the experiments. L-NMMA was injected at four sites in the NTS in sequence, and the data after completion of four injections were taken as those after L-NMMA. Stable data for a few minutes were used for later analysis.

The gain of arterial baroreflex control of RSNA was assessed with an intravenous infusion of phenylephrine.
(PE, 5–40 μg/kg per minute; n = 6) or nitroglycerin (NG, 5–50 μg/kg per minute; n = 5), which increased or decreased mean arterial pressure, respectively, by approximately 40 mm Hg at a rate of 20 mm Hg per minute (Figure 2). An infusion of PE or NG was begun at least 5 minutes after completion of microinjections of L-NMMA, by which time arterial pressure, heart rate, and RSNA had been stabilized. Either PE or NG was infused in one rabbit. The gain of arterial baroreflex control of RSNA was defined as percent changes in RSNA from control per milliliters of mercury of mean arterial pressure change (%/mm Hg).

The following control studies were done: 1) Artificial cerebrospinal fluid (0.2 μl per site) was injected bilaterally into two sites at each side of the NTS (n = 5). 2) L-NMMA at a dose of 160 nmol (0.2 ml) was injected intravenously (n = 4). 3) L-NMMA (16 nmol per site) was injected into the sites 1 mm lateral as well as 1 mm caudal to the NTS at each side (n = 3). 4) The gain of arterial baroreflex control of RSNA during transient elevation of arterial pressure caused by intravenous infusion of PE was determined before and after microinjections of kainate (1 nmol per site) into two sites at each side of the NTS (n = 4). Microinjections of kainate caused transient decreases in arterial pressure and RSNA by its neuroexcitatory effect followed by marked increases in arterial pressure and RSNA (200–300% of control) by its neurotoxic effect. Thereafter, elevated arterial pressure and RSNA decreased gradually over the period of several hours. We waited for 3–4 hours until RSNA returned to the level comparable to that observed after microinjections of L-NMMA into the NTS, and then the gain of arterial baroreflex control of RSNA was examined with intravenous infusion of PE.

The latter study with kainate was done to confirm that the drugs were injected at the proper sites of the NTS, where afferent fibers from the arterial baroreceptors made synapses. It was expected that kainate microinjected at the proper sites of the NTS abolished arterial baroreflex control of sympathetic nerve activity.16

Experiment 2. In this experiment, we determined the effects of microinjections of L-NMMA (16 nmol per site) into the area postrema on arterial pressure, heart rate, and RSNA in animals with intact sinoaortic baroreceptors and vagi (n = 6). Arterial pressure, heart rate, and RSNA were recorded continuously for 5 minutes after microinjection of L-NMMA.

Experiment 3. In this experiment, we determined the effects of microinjections of L-NMMA (16 nmol per site) into the NTS on arterial pressure, heart rate, and RSNA after pretreatment with L-arginine (16 nmol per site, n = 7) or D-arginine (16 nmol per site, n = 8) in rabbits with intact sinoaortic baroreceptors and vagi. L-NMMA, L-arginine, and D-arginine were injected bilaterally into two sites in each side of the NTS. L-NMMA was injected 5 minutes after the completion of microinjections of L-arginine or D-arginine. Arterial pressure, heart rate, and RSNA were recorded continuously for 5 minutes after completion of L-NMMA injections. In rabbits pretreated with L-arginine, we microinjected kainate (1 nmol per site) into two sites at each side of the NTS (n = 3) at least 10 minutes after L-NMMA injections. This experiment was done to demonstrate that arterial pressure and RSNA could be increased by kainate injection into the NTS.

Experiment 4. In this experiment, we determined the effects of microinjections of L-NMMA (16 nmol per site) into four sites of the NTS on arterial pressure, heart rate, and RSNA in animals with sinoaortic denervation and bilateral vagotomy (n = 6).

Sinoaortic denervation and bilateral vagotomy were performed by bilateral sectioning of the aortic depressor nerves, sympathetic nerves, and vagi and by interrupting all nerves between the internal and external carotid artery, stripping the adjacent adventitia, and painting the region of the carotid sinus with 10% phenol. Completeness of sinoaortic denervation was ensured in each rabbit by the absence of changes in RSNA with an increase in arterial pressure by 20–30 mm Hg caused by an intravenous injection of PE. The experiment was begun at least 2 hours after sinoaortic denervation and bilateral vagotomy, by which time arterial pressure, heart rate, and RSNA had returned to baseline values.

Statistical Analysis

Paired t tests were used to examine the effect of each intervention in a group in experiments 1–4. Unpaired t tests were used to compare arterial pressure and RSNA between the experiments with L-NMMA and kainate in experiment 1. Analyses of variance (ANOVAs) were used to compare the effect of L-NMMA on the arterial baroreflex gains (experiment 1) and to examine if the responses to L-NMMA were different among the groups. Pair comparisons after an ANOVA were made by Duncan's test.18 A value of p < 0.05 was considered to be statistically significant. All data are expressed as mean ± SEM.

Results

Experiment 1

Figure 3 shows representative recordings of arterial pressure, raw RSNA (renal electroneurogram), and integrated RSNA with microinjections of L-NMMA into the NTS. Arterial pressure and RSNA increased soon after the injections, and the effects of L-NMMA lasted for at least 15 minutes after the completion of the injections. Table 1 summarizes the effects of L-NMMA
on arterial pressure, heart rate, and RSNA. L-NMMA microinjected into the NTS (16 nmol per site) increased arterial pressure \((p<0.01)\) and RSNA \((p<0.01)\) with no significant changes in heart rate in animals with intact sinoaortic baroreceptors and vagi. The gain of arterial baroreflex control of RSNA or heart rate assessed by intravenous PE or NG did not differ before and after microinjections of L-NMMA (Figures 4 and 5). Microinjections of artificial cerebrospinal fluid into the NTS, intravenous infusions of L-NMMA (160 nmol), and microinjections of L-NMMA 1 mm lateral and 1 mm caudal to the NTS at each side (16 nmol per site) produced no significant changes in arterial pressure, heart rate, and RSNA (data not shown). Microinjections of kainate markedly increased arterial pressure and RSNA, which gradually returned toward the base-

\(\text{FIGURE 4.} \quad \text{Graph showing percent changes in renal sympathetic nerve activity (%SNA) during increases and decreases in arterial pressure (AP) before (closed circles) and after (open circles) N^\text{O}-\text{monomethyl-L-arginine (NMMA) was injected into the nucleus tractus solitarius.}\}

line values over several hours. Arterial baroreflex control of RSNA was examined 3–4 hours after microinjection of kainate, at which time arterial pressure \((112\pm28 \text{ mm Hg})\) did not significantly differ from that before microinjections of kainate \((109\pm3 \text{ mm Hg})\), whereas RSNA \((161\pm9 \text{ spikes per second})\) was still higher than the value before microinjections of kainate \((109\pm17 \text{ spikes per second})\) \((p<0.05)\). RSNA at 3–4 hours after microinjections of kainate was not significantly different from the value after microinjections of L-NMMA into the NTS \((p>0.1)\). Arterial baroreflex control of RSNA assessed by intravenous infusion of PE was nearly abolished at 3–4 hours after microinjections of kainate (Figure 6).

**Experiment 2**

Microinjection of L-NMMA into the area postrema (16 nmol) produced no significant changes in arterial pressure \((107\pm4 \to 104\pm4 \text{ mm Hg})\), heart rate \((247\pm11 \to 247\pm11 \text{ beats per minute})\), and RSNA \((113\pm8 \to 111\pm7 \text{ spikes per second})\).

**Experiment 3**

After pretreatment with L-arginine microinjected into the NTS (16 nmol per site), which by itself had no

\(\text{FIGURE 3.} \quad \text{Representative recordings of arterial pressure and renal sympathetic nerve activity with microinjections of N^\text{O}-\text{monomethyl-L-arginine (L-NMMA) into the nucleus tractus solitarius in rabbits with intact sinoaortic baroreceptors and vagi. ENG, electroneurogram. Sequential microinjections of L-NMMA into two sites at each side of the nucleus tractus solitarius (from site 1 to 4 in Figure 1) progressively increased arterial pressure and renal sympathetic nerve activity. A horizontal bar indicates the period during which four injections of L-NMMA into the nucleus tractus solitarius were made.}\}

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**Table 1. Effects of N^\text{O}-\text{Monomethyl-L-arginine Microinjected Into the Nucleus Tractus Solitarius on Arterial Pressure, Heart Rate, and Renal Sympathetic Nerve Activity in Rabbits With and Without Intact Baroreceptors and Vagi**

<table>
<thead>
<tr>
<th>Intact</th>
<th>No arginine pretreatment ((n=11))</th>
<th>Pretreated with (\text{L-arginine (n=7)})</th>
<th>Pretreated with (\text{D-arginine (n=8)})</th>
<th>SAD+Vx ((n=6))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Mean AP (mm Hg)</td>
<td>99±3</td>
<td>106±4*†</td>
<td>101±4</td>
<td>101±4</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>242±10</td>
<td>233±8</td>
<td>250±16</td>
<td>248±14</td>
</tr>
<tr>
<td>RSNA (spikes/sec)</td>
<td>121±10</td>
<td>150±13*†</td>
<td>108±7</td>
<td>102±6</td>
</tr>
</tbody>
</table>

SAD, sinoaortic denervation; Vx, vagotomy; AP, arterial pressure; bpm, beats per minute; RSNA, renal sympathetic nerve activity. Values are mean±SEM before and after N^\text{O}-monomethyl-L-arginine microinjection.

*\(p<0.01\) vs. control value (paired \(t\) test); †\(p<0.01\) and ‡\(p<0.05\) vs. changes in the L-arginine–treated group (Duncan’s test); §\(p<0.02\) vs. control value (paired \(t\) test).
significant effects on arterial pressure (from 96±5 to 100±4 mm Hg), heart rate (from 256±18 to 253±18 beats per minute), and RSNA (from 109±5 to 118±6 spikes per second), microinjections of L-NMMA into the NTS (16 nmol per site) produced no significant changes in arterial pressure, heart rate, and RSNA (Table 1). In those rabbits with L-arginine treatment, microinjections of kainate increased arterial pressure (from 102±2 to 141±16 mm Hg) and RSNA (from 120±9 to 228±24 spikes per second). In contrast, after the pretreatment with d-arginine, which also had no significant effects on arterial pressure (from 101±5 to 101±6 mm Hg), heart rate (from 255±14 to 249±13 beats per minute), and RSNA (from 129±9 to 125±6 spikes per second), L-NMMA microinjected into the NTS increased arterial pressure (p<0.01) and RSNA (p<0.01) (Table 1).

Experiment 4

In rabbits with sinoaortic denervation and bilateral vagotomy, L-NMMA microinjected into the NTS (16 nmol per site) increased arterial pressure (p<0.02), heart rate (p<0.02), and RSNA (p<0.01), respectively (Table 1). The effects of L-NMMA appeared soon after the injection and lasted for at least 20 minutes.

Discussion

The major finding of this study is that L-NMMA microinjected into the NTS increased arterial pressure and RSNA in rabbits with intact as well as denervated sinoaortic baroreceptors and vagi. Furthermore, pretreatment with l-arginine prevented but pretreatment with d-arginine did not influence the effects of subsequent L-NMMA on arterial pressure and RSNA. L-Arginine or d-arginine microinjected into the NTS by itself did not alter arterial pressure and RSNA. Since it has been shown in the central nervous system as well as in other tissues that L-NMMA inhibits formation of NO from l-arginine and that l-arginine attenuates or blocks the inhibitory effect of L-NMMA on NO formation,2-4,5,7,19 these results strongly suggest that NO is involved in neural transmission at the NTS that mediates tonic inhibition of arterial pressure and RSNA. It appears unlikely that the effects of L-NMMA on arterial pressure and RSNA resulted from nonspecific mechanisms such as mechanical deformation of the NTS, since microinjections of artificial cerebrospinal fluid in the same quantity into the NTS did not alter arterial pressure and RSNA. It also is unlikely that L-NMMA exerted a toxic effect on the NTS neurons, since the effects of L-NMMA on arterial pressure and RSNA were blocked by the pretreatment with l-arginine microinjected into the NTS.

Recently, Sakuma et al6 have shown that intravenous L-NMMA increased arterial pressure and RSNA in rats with intact as well as denervated sinoaortic baroreceptors and vagi, suggesting that NO plays a role in the central regulation of sympathetic nerve activity. However, it remains unclear where L-NMMA acts to increase arterial pressure and RSNA in the central nervous system. Our results indicate that the NTS was a site at which L-NMMA exerted its action. The sites of injections confined in the NTS were confirmed histologically in each rabbit. Microinjections of L-NMMA into the adjacent regions of the NTS did not alter arterial pressure or RSNA. Microinjection of L-NMMA in the area postrema also did not change arterial pressure, heart rate, or RSNA. The latter study was done since Togashi et al6 have shown in rats that intracisternal injection of L-NMMA increased arterial pressure and RSNA. It appears that the excitatory effects of L-NMMA injected intracisternally do not result from changes in neuronal activity of the area postrema. The mechanism by which intracisternal L-NMMA increased arterial pressure and RSNA6 is not known. However, it is possible that L-NMMA injected intracisternally might have directly affected neurons in the NTS or other neurons located at or underneath the surface of the brainstem. In this respect, it is interesting to note the finding in a recent study that L-NMMA microinjected into the rostral ventrolateral medulla in anesthetized cats increased arterial pressure and RSNA.20

FIGURE 5. Graph showing changes in heart rate (HR, in beats per minute [bpm]) during increases and decreases in arterial pressure (AP) before (closed circles) and after (open circles) Nω-monomethyl-L-arginine (NMMA) was injected into the nucleus tractus solitarius.

FIGURE 6. Graph showing percent changes in renal sympathetic nerve activity (%SNA) during increases in arterial pressure (AP) before (closed circles) and after (open circles) kainate (KA) was injected into the nucleus tractus solitarius. *p<0.01 by two-way analysis of variance.
The NTS is the site where afferent fibers arising from the arterial and cardiopulmonary baroreceptors make the first central synapses.\textsuperscript{13,14} Therefore, we examined whether L-NMMA microinjected into the NTS exerted its effects by inhibiting central transmission of baroreflexes. Our results clearly indicate that the effects of L-NMMA were independent of baroreflex mechanisms, since they were observed in rabbits with sinoaortic denervation and bilateral vagotomy. Sakuma et al\textsuperscript{15} also have shown that the effects of intravenous L-NMMA were observed in rats without functioning baroreflexes. Furthermore, the gains of arterial baroreflex control of RSNA in response to increases or decreases in arterial pressure induced by intravenous PE or NG, respectively, did not differ before and after microinjections of L-NMMA. The latter results suggest that NO may not be involved in central transmission of baroreflex mechanisms into the NTS. In respect to the failure of L-NMMA to alter the arterial baroreflex gains, we considered the possibility that some NTS neurons involved in arterial baroreflex mechanisms had been unaffected by L-NMMA microinjected in the two sites at each side of the NTS. However, this possibility was unlikely since kainate microinjected into the similar sites of the NTS markedly attenuated the gain of arterial baroreflex control of RSNA. One may raise the question that the effects of L-NMMA on the NTS neurons were short lasting and thus were no longer present when arterial baroreflex control of RSNA was examined. However, this possibility was unlikely since it was observed that the increase in arterial pressure and RSNA lasted for at least 15 minutes in rabbits in which the arterial baroreflex was not examined (Figure 3). The examination of arterial baroreflex control of RSNA was completed within 10 minutes after L-NMMA injections. Thus, our results indicate that inhibition of NO formation by L-NMMA in the NTS resulted in the increase in arterial pressure and RSNA without affecting the gain of arterial baroreflex. This effect of L-NMMA did not depend on functioning arterial and cardiopulmonary baroreflexes. Obviously, we do not know from our results the mechanisms underlying the effects of L-NMMA in the NTS. However, it is tempting to speculate that L-NMMA inhibited the neural effect of glutamate mediated by the subtype of N-methyl-D-aspartate (NMDA) receptors. This consideration is based on the previous findings in the cerebellum that NO formation was coupled with NMDA receptor activation.\textsuperscript{19,22} Furthermore, Paola et al\textsuperscript{10} have shown that the depressor effect of glutamate microinjected into the NTS was attenuated by pretreatment with L-NMMA. Andresen and Yang\textsuperscript{22} have recently suggested that in the NTS non-NMDA receptors mediate primary synaptically transmitted afferent input from the arterial baroreceptors. The latter finding may account for our result that L-NMMA microinjected into the NTS increased arterial pressure and RSNA without affecting the arterial baroreflex. Further studies are needed to clarify the mechanisms by which L-NMMA microinjected in the NTS alters arterial pressure and RSNA.

In summary, the results of this study strongly suggest that NO is involved in the mechanism at the NTS that mediates tonic inhibition of RSNA. The neural effect evoked with inhibition of NO formation in the NTS does not depend on a functioning baroreflex mechanism.

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