Nitric oxide modulation of glutamatergic, baroreflex, and cardiopulmonary transmission in the nucleus of the solitary tract

Ana Carolina Rodrigues Dias,[1] Melissa Vitela,[2] Eduardo Colombari,[1] and Steven W. Mifflin[2]

[1]Department of Physiology, Universidade Federal de São Paulo-Escola Paulista de Medicina, São Paulo, Brazil; and
[2]Department of Pharmacology, University of Texas Health Science Center at San Antonio, San Antonio, Texas

Dias, Ana Carolina Rodrigues, Melissa Vitela, Eduardo Colombari, and Steven W. Mifflin. Nitric oxide modulation of glutamatergic, baroreflex, and cardiopulmonary transmission in the nucleus of the solitary tract. Am J Physiol Heart Circ Physiol 288: H256–H262, 2005; doi:10.1152/ajpheart.01149.2003.—The neuromodulatory effect of NO on glutamatergic transmission has been studied in several brain areas. Our previous single-cell studies suggested that NO facilitates glutamatergic transmission in the nucleus of the solitary tract (NTS). In this study, we examined the effect of the nitric oxide synthase (NOS) inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) on glutamatergic and reflex transmission in the NTS. We measured mean arterial pressure (MAP), heart rate (HR), and renal sympathetic nerve activity (RSNA) from Inactin-anesthetized Sprague-Dawley rats. Bilateral microinjections of L-NAME (10 nmol/100 nl) into the NTS did not cause significant changes in basal MAP, HR, or RSNA. Unilateral microinjection of (RS)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA, 1 pmol/100 nl) into the NTS decreased MAP and RSNA. Fifteen minutes after L-NAME microinjections, AMPA-evoked cardiovascular changes were significantly reduced. N-methyl-D-aspartate (NMDA, 0.5 pmol/100 nl) microinjection into the NTS decreased MAP, HR, and RSNA. NMDA-evoked falls in MAP, HR, and RSNA were significantly reduced 30 min after L-NAME. To examine baroreceptor and cardiopulmonary reflex function, L-NAME was microinjected at multiple sites within the rostro-caudal extent of the NTS. Baroreflex function was tested with phenylephrine (PE, 25 μg iv) before and after L-NAME. Five minutes after L-NAME the decrease in RSNA caused by PE was significantly reduced. To examine cardiopulmonary reflex function, phenylbiguanide (PBG, 8 μg/kg) was injected into the right atrium. PBG-evoked hypotension, bradycardia, and RSNA reduction were significantly attenuated 5 min after L-NAME. Our results indicate that inhibition of NOS within the NTS attenuates baro- and cardiopulmonary reflexes, suggesting that NO plays a physiologically significant neuromodulatory role in cardiovascular regulation.

(RS)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; N-methyl-D-aspartate; baroreceptor and cardiopulmonary reflexes; cardiovascular regulation; renal sympathetic nerve activity

ANATOMIC AND PHYSIOLOGICAL studies suggest interactions between NO and glutamatergic transmission within the nucleus of the solitary tract (NTS) that could modulate cardiovascular regulation (11, 12, 14, 15, 19, 28). L-Glutamate and the synthetic enzyme for production of NO, nitric oxide synthase (NOS), have been colocalized in nerve terminals and neurons within the NTS (8, 13–15, 25, 28). NOS immunoreactivity is also found in other neurons in the medulla oblongata that project to the NTS (3, 25). Activation of glutamatergic receptors in the NTS elicits the production and release of NO and other nitrosyl substances with neurotransmitter/neuromodula-

tor properties (2, 19). Therefore, all of the substrates necessary for NO production are present in the NTS.

Microdialysis or microinjection of NOS inhibitors into the NTS reduces extracellular levels of glutamate (11) and the cardiovascular responses evoked by NTS microinjections of ionotropic excitatory amino acid agonists (12, 19). Iontophoresis of an inhibitor of NOS, N^G-nitro-L-arginine methyl ester (L-NAME), does not alter spontaneous discharge but significantly decreases the number of action potentials evoked by iontophoretic application of (RS)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) in NTS neurons receiving vagal afferent inputs (1). In addition, iontophoresis of a NO donor enhances spontaneous discharge and the number of action potentials elicited by AMPA, suggesting that NO could be facilitating AMPA-mediated neuronal transmission within the NTS (1).

If NO facilitates glutamatergic transmission within the NTS, inhibition of NO production should reduce cardiovascular responses elicited by stimulation of glutamatergic receptors and stimulation of cardiovascular afferent inputs known to utilize glutamatergic transmission (baroreceptor, cardiopulmonary). The specific aim of this study was to examine the participation of NO in the cardiovascular responses elicited by stimulation of N-methyl-D-aspartate (NMDA) and AMPA receptors in the NTS, as well as the functional significance of these interactions in the cardiovascular responses evoked by activation of peripheral baroreceptors and cardiopulmonary receptors.

MATERIALS AND METHODS

Animals. Experiments were performed on male Sprague-Dawley rats (300–400 g; Charles River). Rats were housed in a laboratory animal room, two per cage, with unrestricted access to food and water. All animals were given at least 1 wk to acclimatize before use. All experimental protocols were approved by the Institutional Animal Care and Use Committee and were in accordance with US Public Health Service guidelines.

Preparation. Animals were anesthetized with Inactin (100 mg/kg ip; Sigma) and placed on a thermostatically controlled heating pad. The trachea was cannulated, and the rat was artificially ventilated with room air supplemented with 100% O_2. Catheters were placed in the femoral artery and vein for blood pressure monitoring and injection of drugs. Arterial pressure was measured with a Cobe CDX transducer (Cobe Laboratories, Lakewood, CO). Mean arterial pressure (MAP, mmHg) and heart rate (HR, beats/min) were determined from the pulsatile signal with a Coulbourn blood pressure processor. Gallamine thiethiodide (20 mg/kg initially, supplemented with 5 mg as needed,

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typically every hour) was also given for paralysis. Depth of anesthesia was gauged by the stability of blood pressure and HR and the absence of a pressor response to paw pinch. The rat was placed in a stereotaxic frame, and an occipital craniotomy was performed to expose the dorsal surface of the medulla in the region of obex. Cardiopulmonary receptors were stimulated by right atrial injections of phenylbiguanide (PBG) via a PE-50 tube in the jugular vein.

Renal sympathetic nerve activity. The postganglionic renal nerve bundle was isolated through a flank retroperitoneal incision and mounted, intact, on a bipolar Teflon-coated stainless steel wire electrode (0.0071-in. bare diameter; Cooner Wire, Austin, TX). When the optimal signal was obtained, the nerve was fixed in place with a silicone impression material (polyvinylsiloxane; Super-Dent) and the flank wound was closed. The nerve signal was fed through an alternating current amplifier (P5; Grass Instruments, Quincy, MA) via a high-impedance probe, amplified 20,000–100,000 times, and filtered between 300 and 3,000 Hz. The 0% and 100% reference levels of nerve activity were obtained by recording the noise level after inhibition and stimulation of nerve activity with a 0.15-ml bolus injection of phenylephrine (PE, 100 μg/ml) and sodium nitroprusside (250 μg/ml), respectively. The resting level of renal sympathetic nerve activity (RSNA) was placed within the 0–100% scale, and reductions in RSNA are presented as a percent decrease relative to the resting level of RSNA.

NTS microinjections. Rats were placed in a stereotaxic frame with the head inclined 45° downward for better visualization of the calamus scriptorius. Drugs were injected through glass pipettes by pressurized N_2 pulses with a pressure ejection system. The volume of the injection (100 nl) was determined by viewing the movement of the fluid meniscus inside the pipette by a microscope with a calibrated micrometer. Unilateral AMPA and NMDA microinjections and bilateral L-NAME and N^G-nitro-D-arginine methyl ester (D-NAME) microinjections were made into the NTS with the following coordinates: 0.5 mm rostral to calamus, ±0.5 mm lateral to midline, and 0.5 mm ventral from the surface of the medulla. In the cardiopulmonary and baroreceptor reflex protocols, L-NAME or D-NAME microinjections were made at two sites on each side of the NTS and one site caudal

to the calamus scriptorius (volume of microinjection was 60 nl/site), as shown in Fig. 1. After the experiment, a micropipette containing Chicago sky blue 2% was positioned at the sites of microinjections for postmortem analysis of injection sites.

Histology. Animals were transcardially perfused with saline (0.9%) followed by 10% formalin solution. The brain was removed, and 40-μm sections were cut with a microtome. Sites of microinjection were identified under the microscope after staining by the Nissl method (neutral red). Data obtained from microinjection sites located in the NTS at the level of the calamus scriptorius were used for analysis.

Data analysis. Pulsatile arterial pressure, MAP, HR, and RSNA were recorded with a PC-based data acquisition system (Spike 2; Cambridge Electronic Design, London, UK). RSNA was monitored on a digital oscilloscope (Nicolet Instrument, Madison, WI) and an audio monitor (Grass Instruments). MAP and HR were determined from the arterial pressure pulse (Coulbourn Instruments). Differences in RSNA between treatment and baseline were measured over the same interval period (10–15 s). All data are presented as means ± SE. Results were analyzed by one-way ANOVA with repeated measures followed by Newman-Keuls post hoc test. Significance was accepted for $P < 0.05$.

RESULTS

Effects of NOS inhibition on responses to NTS microinjections of AMPA. One group of animals (n = 8; basal MAP 104 ± 3 mmHg, basal HR 344 ± 10 beats/min) was unilaterally microinjected with 1 pmol/100 nl of AMPA into the NTS before and 5, 15, 30, and 45 min after the bilateral microinjection of L-NAME (10 nmol). Bilateral microinjection of L-NAME did not change MAP, HR, or RSNA (MAP: basal 104 ± 4, after L-NAME 107 ± 10 mmHg; HR: basal 339 ± 8, after L-NAME 338 ± 5 beats/min; RSNA: basal 45 ± 8%, after L-NAME 32 ± 16%; n = 8). Before L-NAME injections, AMPA microinjection elicited significant reductions in MAP

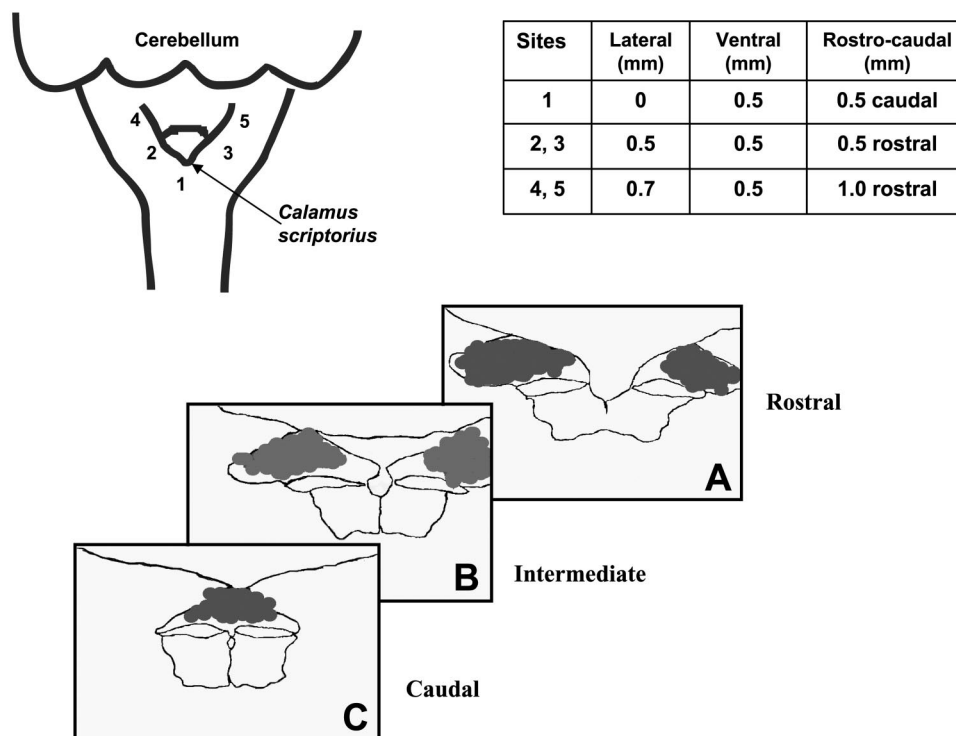| Sites	Lateral (mm)	Ventral (mm)	Rostro-caudal (mm)
1	0	0.5	0.5 caudal
2, 3	0.5	0.5	0.5 rostral
4, 5	0.7	0.5	1.0 rostral

Fig. 1. Schematic representation of the dorsal surface of the medulla indicating the sites (*1–5*) of microinjection into the nucleus of the solitary tract (NTS). *Site 1* was caudal to the calamus scriptorius. *Sites 2* and *3* indicate intermediate NTS and *sites 4* and *5* the rostral portion of NTS. Respective coordinates are presented in the table (*top*). *Bottom*: composite of injection sites (dark gray shading) in coronal sections of brain stem at rostral (*A*), intermediate (*B*), and caudal (*C*) NTS from 24 rats.

(ΔMAP: -33 ± 3 mmHg, $P = 0.002$, $n = 8$), RSNA (ΔRSNA: $-66 \pm 10\%$ from basal, $P = 0.001$, $n = 8$), and HR (ΔHR: -18 ± 7 beats/min, $P = 0.05$, $n = 5$). AMPA-evoked reductions in MAP and RSNA were attenuated 5 and 15 min after NOS inhibition (ΔMAP: 5 min -23 ± 6, 15 min -22 ± 6 mmHg; ΔRSNA: 5 min $-43 \pm 6\%$, 15 min $-46 \pm 9\%$ from basal; $P < 0.05$, $n = 8$; Fig. 2). No changes in the HR response to AMPA microinjections were observed after L-NAME.

To test the specificity of L-NAME on the responses shown above, the same protocol was performed in another group of animals ($n = 7$; basal MAP 103 ± 4 mmHg, basal HR 361 ± 14 beats/min) with the inactive enantiomer of L-NAME (D-NAME, 10 nmol). D-NAME microinjection did not alter basal or AMPA- evoked changes in MAP, HR, or RSNA (Fig. 2).

Effects of NOS inhibition on responses to NTS microinjections of NMDA. Interactions between NMDA and L-NAME within the NTS were examined in a separate group of animals ($n = 8$; basal MAP 101 ± 3 mmHg, basal HR 342 ± 9 beats/min). A representative example of changes in MAP, HR, and RSNA caused by microinjection of NMDA before and after L-NAME is shown in Fig. 3*A*.

Bilateral microinjection of L-NAME (10 nmol) did not change MAP (basal 100 ± 4, after L-NAME 95 ± 6 mmHg) and HR (basal 339 ± 10, after L-NAME 321 ± 11 beats/min). RSNA was transiently, but significantly, reduced after L-NAME microinjections (basal $32 \pm 13\%$, after L-NAME $22 \pm 12\%$, $P < 0.031$; $n = 7$) in this set of experiments. RSNA returned to basal levels within a minute after the last microinjection of L-NAME. Before injections of L-NAME, NMDA microinjections (0.5 pmol/100 nl) resulted in significant reductions in MAP, HR, and RSNA (ΔMAP: -35 ± 3 mmHg, $n = 8$; ΔHR: -23 ± 4 beats/min, $n = 7$; ΔRSNA: $-44 \pm 7\%$ from basal, $n = 7$; all $P < 0.001$). Responses to NMDA microinjection were reduced 15 and 30 min after L-NAME (ΔMAP: 15 min -20 ± 4, 30 min -21 ± 6 mmHg, $P < 0.05$, $n = 8$; ΔHR: 15 min -7 ± 3, 30 min -5 ± 3 beats/min, $P < 0.05$, $n = 7$;

Fig. 4). The NMDA-evoked reduction in RSNA was attenuated 30 min after L-NAME (ΔRSNA 30 min: $-17 \pm 7\%$ from basal; $P < 0.05$, $n = 7$). This protocol was performed in another group of rats ($n = 8$; basal MAP 103 ± 3 mmHg, basal HR 337 ± 11 beats/min), using the inactive enantiomer of L-NAME (D-NAME, 10 nmol) to demonstrate the specificity of L-NAME action in NTS. D-NAME microinjection did not alter basal or NMDA-evoked changes in MAP, HR, and RSNA (Fig. 4).

Effects of NTS NOS inhibition on responses to baroreceptor stimulation. Intravenous injection of PE (0.1 ml, 25 μg/kg) increased MAP $\sim$20 mmHg and reduced RSNA $\sim$50%. In our preparation, only slight reductions in HR were observed. PE was injected before multiple NTS microinjections (see Fig. 1) of L-NAME (control), 5–7 min after the last L-NAME injection, and 45 min after the last L-NAME injection (PE recovery). A representative recording of changes in MAP, HR, and RSNA caused by baroreceptor stimulation with PE before (PE control) and after L-NAME is shown in Fig. 3*B*.

Microinjections of L-NAME over much of the rostro-caudal extent of the NTS significantly decreased RSNA (from basal: $39 \pm 6\%$ to $21 \pm 7\%$ after L-NAME; $P = 0.024$, $n = 7$). However, this reduction was transient and RSNA had returned to basal levels before the following PE injection. NTS microinjections of L-NAME did not alter the increase in MAP elicited by PE injection (ΔMAP: PE control 23 ± 2, PE after L-NAME 25 ± 2, PE recovery 24 ± 2 mmHg; $n = 7$). The PE-evoked reduction in RSNA was significantly reduced 5–7 min after L-NAME (ΔRSNA: PE control $-47 \pm 7\%$, PE after L-NAME $-24 \pm 7\%$, PE recovery $-47 \pm 5\%$ from basal; $P = 0.02$, $n = 7$; Fig. 5*A*). The magnitude of the PE-evoked bradycardia was low under control conditions and after L-NAME (ΔHR: PE control -5 ± 1, PE after L-NAME $+1 \pm 3$, PE recovery -5 ± 1 beats/min; $n = 7$). Another group of animals ($n = 7$) had the same protocol performed with the inactive enantiomer D-NAME instead of L-NAME, and no
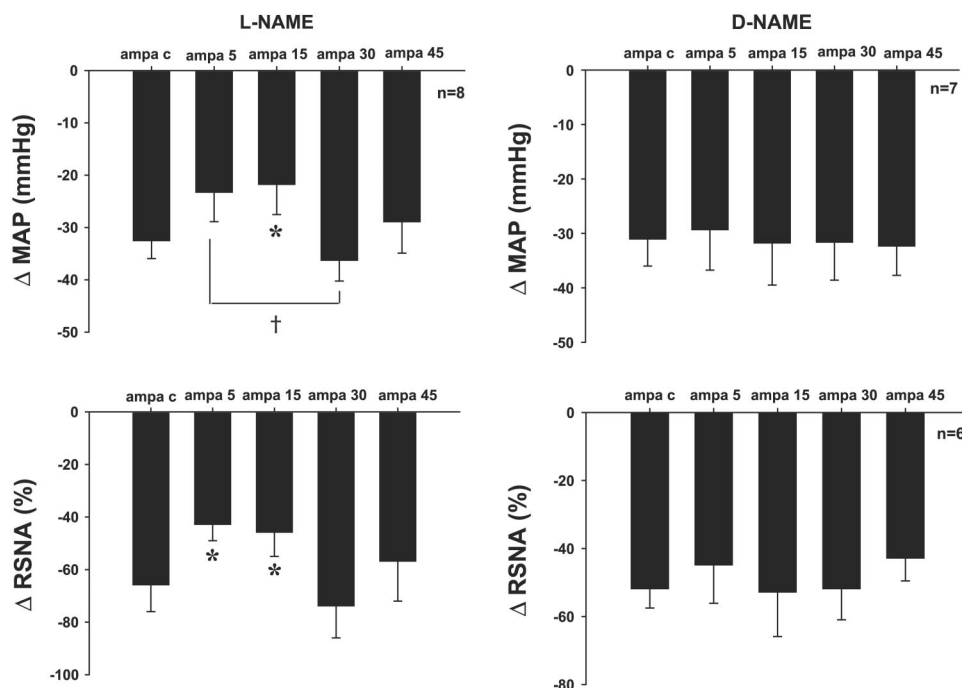

Fig. 2. Change in mean arterial pressure (ΔMAP; *top*) and renal sympathetic nerve activity (ΔRSNA; *bottom*) elicited by microinjection of (*RS*)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA, 1 pmol/100 nl) into the NTS before (ampa c) and after [5, 15, 30 and 45 min (ampa 5, 15, 30, 45)] N^G-nitro-L-arginine methyl ester (L-NAME; *left*, $n = 8$) and N^G-nitro-D-arginine methyl ester (D-NAME; *right*, $n = 7$) microinjections. *$P < 0.05$, different from control; †$P < 0.05$, ampa 5 = ampa 15 and different from ampa 30.
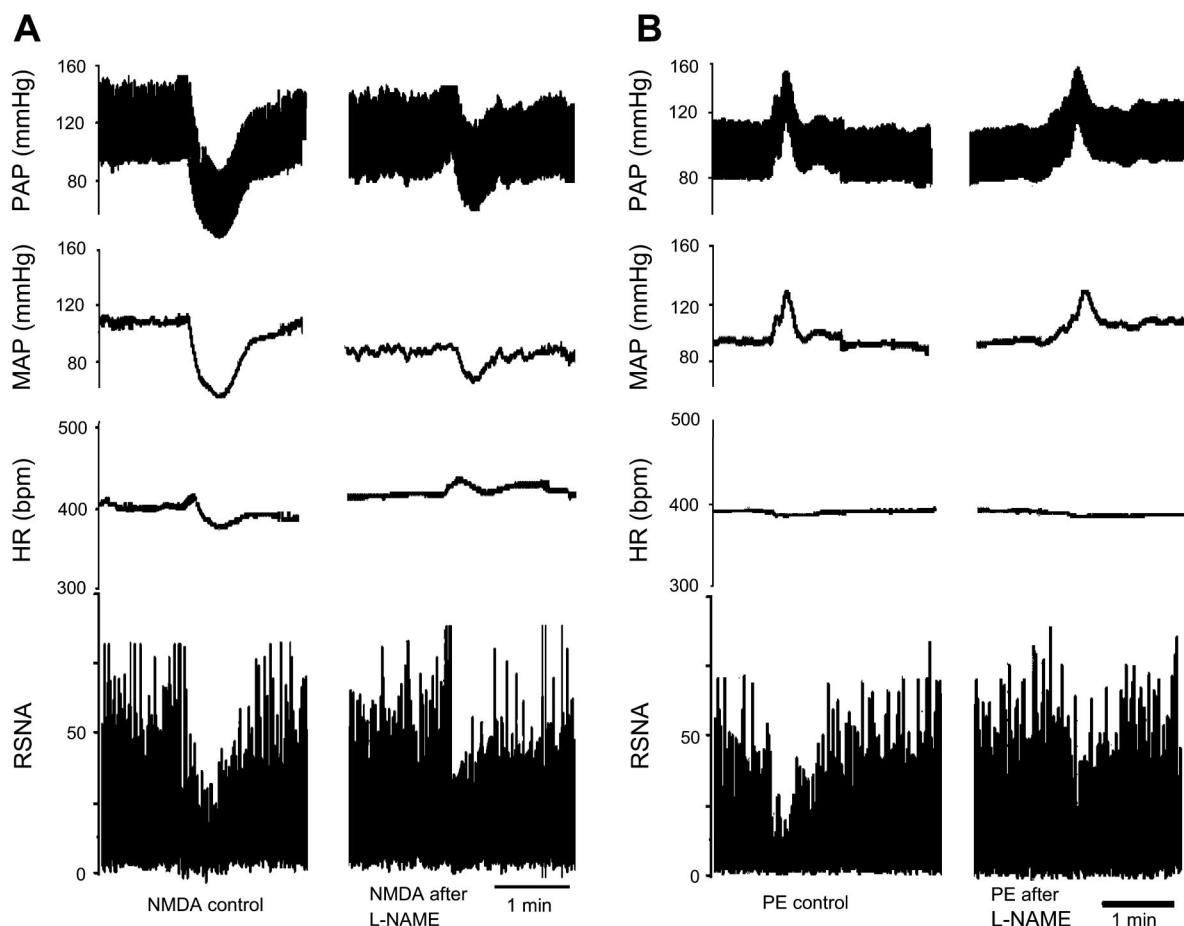
Fig. 3. Representative recordings of pulsatile arterial pressure (PAP), MAP, heart rate [HR, beats/min (bpm)], and RSNA after microinjection of *N*-methyl-D-aspartate (NMDA) before (NMDA control) and after L-NAME into the NTS (*A*). *B*: responses to baroreceptor stimulation during phenylephrine (PE)-induced increases in blood pressure before (PE control) and after L-NAME microinjections.

significant changes were observed in basal levels of MAP, HR, or RSNA or in PE-evoked alterations in these variables.

Effects of NTS NOS inhibition on responses to cardiopulmonary receptor stimulation. The site of action of the intra-atrial PBG injection was localized to the cardiopulmonary region by sequential denervations. In six rats intra-atrial injection of 0.1 ml PBG (8 μg/kg) decreased MAP by 22 ± 2 mmHg and HR by 27 ± 4 beats/min. After bilateral subdiaphragmatic vagotomy, PBG decreased MAP by 26 ± 3 mmHg and HR by 26 ± 4 beats/min. After bilateral cervical vagotomy PBG evoked an increase in MAP of 9 ± 9 mmHg and a decrease in HR of 4 ± 10 beats/min.

Microinjections of L-NAME over much of the rostro-caudal extent of the NTS elicited a significant decrease in RSNA (from basal: 49 ± 6% to 27 ± 6% after L-NAME; $P = 0.004$, $n = 5$). However, this reduction was transient and RSNA returned to basal levels before the following PBG injection. Intra-atrial injection of PBG evoked hypotension, bradycardia, and a reduction in RSNA. PBG-evoked responses were significantly reduced 5–7 min after L-NAME (ΔMAP: PBG control −36 ± 5, PBG after L-NAME −19 ± 5, PBG recovery −34 ± 4 mmHg, $P < 0.001$, $n = 5$; ΔHR: PBG control −24 ± 7, PBG after L-NAME −5 ± 2, PBG recovery, −17 ± 7 beats/min, $P = 0.020$, $n = 5$; ΔRSNA: PBG control −35 ± 5%, PBG after L-NAME −15 ± 2%, PBG recovery −28 ± 4%, $P =$

0.017, $n = 5$; Fig. 5*B*). Another group of animals ($n = 6$) had the same protocol performed with the inactive enantiomer D-NAME instead of L-NAME, and no significant changes were observed in basal levels or PBG-evoked changes in MAP, HR, and RSNA.

DISCUSSION

Our previous single-cell study (1) demonstrated NO facilitation of excitatory amino acid-evoked excitation of NTS neurons and suggested a functional role for NO within the NTS in cardiovascular regulation. The present study supports the idea that NO plays a functionally important role within the NTS as a modulator of responses to excitatory amino acids as well as responses to baroreceptor and cardiopulmonary receptor activation.

In our preparation, inhibition of NOS within the NTS did not alter basal MAP or HR, suggesting little, if any, tonic NO release. Variable change or no change in basal levels of MAP or HR after NTS microinjection of NO donors or NOS inhibitors has been reported by different labs. Inhibition of NOS (5, 18) or antisense-induced reduction in neuronal NOS (nNOS) (17) were reported to increase MAP. Other studies reported that inhibition of NOS (2, 22, 24) and specific inhibition of nNOS (12, 22) do not change basal MAP or HR. Using gene
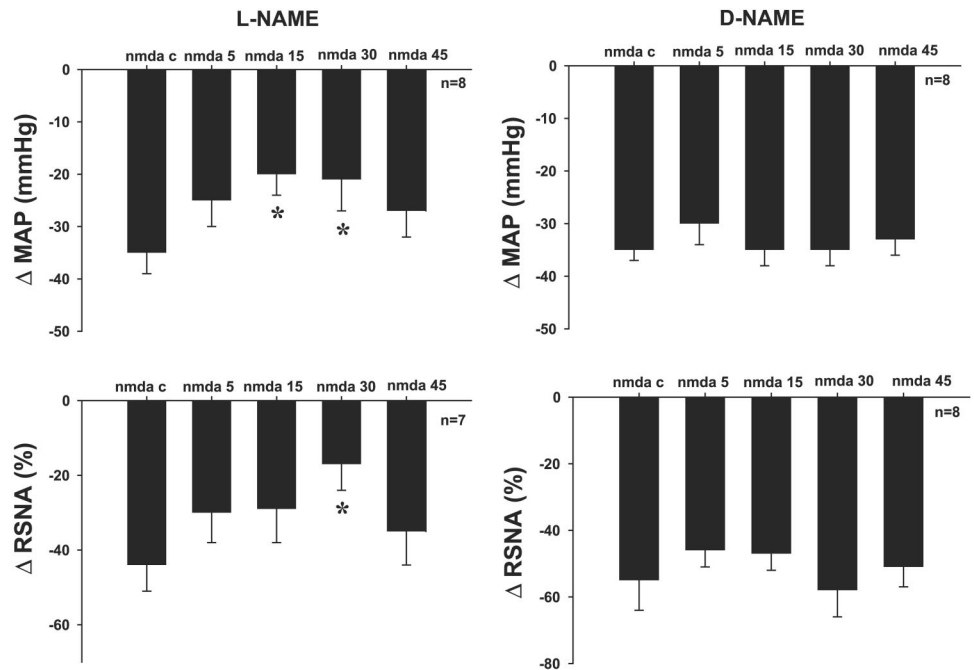
L-NAME

D-NAME

Fig. 4. ΔMAP (*top*) and ΔRSNA (*bottom*) elicited by microinjection of NMDA (0.5 pmol/100 nl) into the NTS before (nmda c) and after [5, 15, 30, and 45 min (nmda 5, 15, 30, 45)] L-NAME (*left*, $n = 8$) and D-NAME (*right*, $n = 8$) microinjections. *Different from control, $P < 0.05$.

transfer, Waki et al. (29) reduced expression of endothelial NOS (eNOS) within the NTS and reported no change in basal MAP but a reduction in HR. The variable results from these studies could be due to the species used, the strain of rat used, the presence or absence of anesthesia, as well as the precise means used to increase or decrease NO. For example, the use of L-NAME should inhibit both nNOS and eNOS. However, no consistent pattern is apparent that would explain the results from different labs.

We observed a transient decrease in the resting level of RSNA after bilateral and multiple L-NAME microinjections in the NTS. Within a minute after the last microinjection no

significant changes of MAP or HR were observed. It is not likely that the transient reduction in RSNA was an artifact, because it was not seen during NTS injections of the inactive enantiomer D-NAME. The lack of sustained changes in RSNA could reflect a specific effect of L-NAME within the NTS region covered by the microinjection, or it could be a reflection of small changes in multiple sympathetic outflows that are insufficient to result in a change in MAP. The lack of sustained change in basal MAP and RSNA after L-NAME injections could also be due to remaining baroreflex buffering, because injections of L-NAME at multiple sites within NTS only reduced PE-evoked inhibition of RSNA by ~50%. The lack of
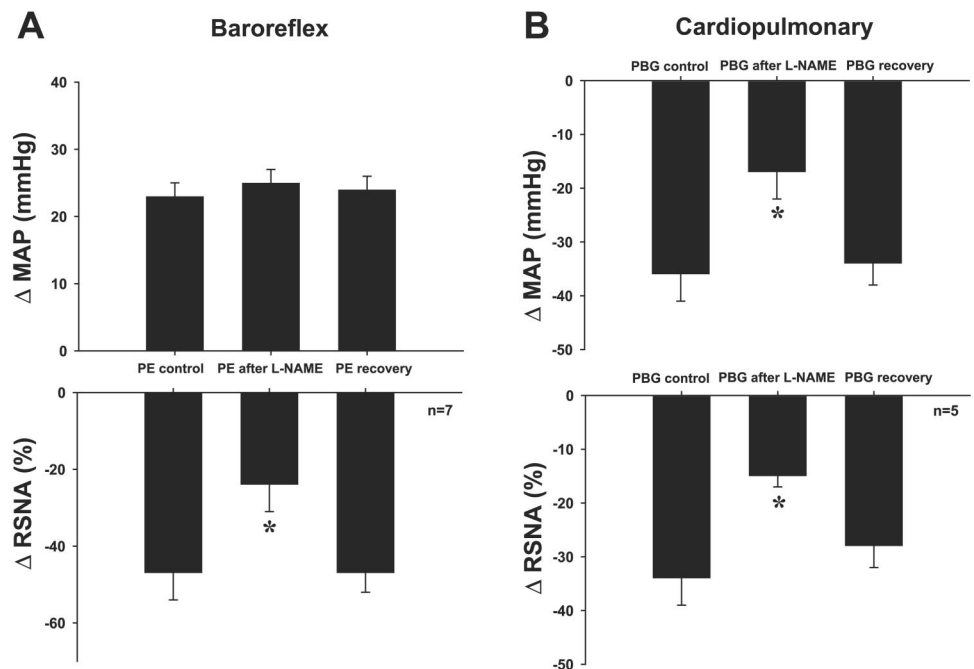
A   Baroreflex

B   Cardiopulmonary

Fig. 5. ΔMAP and ΔRSNA elicited by infusion of PE (baroreflex, $n = 7$; *A*) and phenylbiguanide (PBG, cardiopulmonary, $n = 5$; *B*) before (control) and after L-NAME microinjections into the NTS. *Different from control, $P < 0.05$.

sustained change in MAP and RSNA after L-NAME injections is consistent with our single-unit iontophoretic study (1), which found no change in basal discharge frequency of NTS neurons after iontophoretic application of L-NAME. Keeping in mind the caveats discussed above, in our preparation NO within the NTS does not appear to significantly contribute to tonic maintenance of MAP or RSNA.

Our results are consistent with previous studies that demonstrated facilitatory interactions between excitatory amino acids and NO within the NTS. Inhibition of NOS within the NTS decreases cardiovascular responses evoked by microinjection of glutamate and NMDA into the NTS (2, 19). Within the NTS, selective inhibition of nNOS reduced cardiovascular responses evoked by microinjection of AMPA (12) or NMDA (12, 28). Within the NTS, increases in NO have been shown to induce glutamate release (11) whereas microinjections of AMPA and NMDA have been shown to increase NO levels (11, 19).

The present study confirms the physiological significance of interactions between NO and glutamatergic transmission by measuring the effects of NOS inhibition on cardiovascular responses elicited by activation of NMDA and AMPA receptors in the NTS. We also characterized the effects of NOS inhibition on baroreceptor and cardiopulmonary receptor reflex activation. Previous studies demonstrated that these afferents utilize glutamatergic receptors within the NTS (4, 21, 26, 31) and that NO may play a role in the reflex responses to activation of these receptors (6, 10). NMDA- and AMPA-elicited responses were significantly reduced after L-NAME microinjection into the NTS. Cardiopulmonary receptor evoked reductions in MAP, HR, and RSNA were attenuated by NOS inhibition. Baroreceptor-evoked reductions in RSNA were also attenuated. The small HR responses during baroreceptor stimulation are problematic and may be related to the muscle paralytic agent used (gallamine), which has muscarinic antagonist effects. However, cardiopulmonary receptor activation evoked reasonable reductions in HR, so it may be a question of the relative intensity of the stimuli.

Several studies have examined the effects of NO within the NTS on baroreflex and cardiopulmonary reflex function. Lewis et al. (10) demonstrated that the processing of cardiopulmonary afferent inputs within the NTS involves activation of soluble guanylate cyclase. Paton's group (22, 29) showed that endothelial NOS attenuates baroreflex control of HR whereas L-NAME inhibition of NOS or selective neuronal NOS inhibition had no effect on baroreflex control of HR (22). It should be noted that Waki et al. (29) used spontaneous changes in systolic pressure and pulse interval as indexes of baroreflex gain, and the specificity of these measures has been recently questioned (16). Other groups have reported that alterations in the NTS NO system have no significant effect on baroreflex function (5, 7, 30), and others have shown that inhibition of NOS (24), and more specifically nNOS, attenuates baroreflex regulation of HR (20, 27) but not RSNA (20). The reason(s) for the differences between studies is not obvious; however, species, anesthetic, drug(s) used to interfere with NO, as well as microinjection parameters (single vs. multiple injections), stimulus used to evoke baroreflex responses, as well as output being measured (HR vs. sympathetic nerve discharge) are all variables that could contribute to the differences between studies.

In addition, the relative intensity of the stimulus used to evoke reflex responses might also contribute to differences between studies of NO and baroreflex function. For example, we found no change in the meager (5 beats/min) baroreflex-evoked bradycardia following NTS injections of L-NAME, whereas PBG-evoked bradycardia (27 beats/min) was significantly attenuated. "Single-point" stimuli do not permit construction of reflex curves; therefore, the present study could not determine whether NO facilitates reflex function over the entire operating range or over a restricted portion. A previous single-unit recording study suggested that NO facilitation of excitatory amino acid-evoked discharge was most marked at high levels of discharge frequency (1); therefore, in our preparation NO may only facilitate reflex function in response to large stimuli and/or high neuronal discharge frequency. We cannot exclude possible interactions within the NTS between the NO system and other neurotransmitters and/or inhibitory amino acids known to modulate baroreceptor and cardiopulmonary receptor reflexes (9, 23).

Our previous finding (1) that NO enhances excitatory amino acid-evoked discharge in NTS neurons receiving vagal afferent inputs provides a basis for the attenuation of baroreflex and cardiopulmonary reflex inhibition of renal nerve discharge after inhibition of NOS within the NTS reported in the present study. We propose that L-NAME reduces NO facilitation of excitatory amino acid-evoked discharge during activation of baroreceptor or cardiopulmonary afferent fibers. This reduces the activation of NTS neurons receiving these peripheral afferent inputs and leads to reduced reflex responses.

The source of the NO mediating the observed effects could be within vagal afferent terminals (13, 25) and/or within NTS neurons (8, 14) possessing AMPA and/or NMDA receptors (15). NO released by the postsynaptic neuron could act on the same and/or adjacent cells, including the presynaptic neuron, where it could modulate the release or production of other neurotransmitters. The present data reinforce the idea that interactions between glutamatergic receptors and NO within the NTS can be functionally important in the modulation of cardiovascular reflexes and raise the question of the potential role of these interactions in pathophysiological states such as hypertension.

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