Central nitric oxide modulates hindquarter vasodilation elicited by AMPA receptor stimulation in the NTS of conscious rats

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Rodrigues Dias, Ana Carolina, and Eduardo Colombari. Central nitric oxide modulates hindquarter vasodilation elicited by AMPA receptor stimulation in the NTS of conscious rats. Am J Physiol Regul Integr Comp Physiol 290: R1330–R1336, 2006. First published December 29, 2005; doi:10.1152/ajpregu.00150.2005.—Microinjection of S-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) in the nucleus of the solitary tract (NTS) of conscious rats causes hypertension, bradycardia, and vasoconstriction in the renal, mesenteric, and hindquarter vascular beds. In the hindquarter, the initial vasconstriction is followed by vasodilation with AMPA doses >5 pmol/100 nl. To test the hypothesis that this vasodilation is caused by activation of a nitroxidergic pathway in the NTS, we examined the effect of pretreatment with the nitric oxide synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME, 10 nmol/100 nl, microinjected into the NTS) on changes in mean arterial pressure, heart rate, and regional vascular conductance (VC) induced by microinjection of AMPA (10 pmol/100 nl in the NTS) in conscious rats. AMPA increased hindquarter VC by 18 ± 4%, but after pretreatment with L-NAME, AMPA reduced hindquarter VC by 16 ± 7% and 17 ± 9% (5 and 15 min after pretreatment, P < 0.05 compared with before pretreatment). Pretreatment with L-NAME reduced AMPA-induced bradycardia from 122 ± 40 to 92 ± 32 beats/min but did not alter the hypertension induced by AMPA (43 ± 6 mmHg before pretreatment). Control injections with D-NAME did not affect resting values or the response to AMPA. The present study shows that stimulation of AMPA receptors in the NTS activates both vasodilatory and vasoconstrictor mechanisms and that the vasodilatory mechanism depends on production of nitric oxide in the NTS.

The pressor response and bradycardia elicited by microinjection of L-glutamate into the nucleus of the solitary tract (NTS) of conscious rats are associated with vasoconstriction in the hindquarter, renal, and mesenteric vascular beds. A subsequent vasodilation occurs in the hindquarter bed (4). A similar pattern of an initial general vasoconstriction followed by hindquarter vasodilation can be seen after stimulation of S-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in the NTS (9). The vasodilation produced by microinjection of glutamate in the NTS can be reduced by systemic administration of prazosin and Nω-nitro-L-arginine methyl ester (L-NAME) (4). The origin and pathway involved in this neurogenic vasodilation is still unclear (4), but it was suggested that this vasodilation is caused by peripheral release of preformed nitrosyl factors during sympathetic stimulation (6, 7).

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glutamatergic transmission. Therefore, we sought to investigate whether NO plays a neuromodulatory role in cardiovascular regulation through regional hemodynamic changes elicited by AMPA receptor stimulation within the NTS of non-anesthetized rats.

MATERIALS AND METHODS

Animals. Experiments were performed in conscious, freely moving, male Wistar rats weighing between 280 and 350 g. Animals were maintained on 12:12-h light-dark cycle in temperature-controlled rooms. Food and water were available ad libitum except during the experiments. All experimental protocols were approved by the Institutional Ethics Committee.

Surgical procedures. All surgical procedures, including implantation of guide cannulas, blood flow probes, and femoral arterial catheters, were performed as described previously by Dias et al. (9). Briefly, 5 days before the experiments, rats were anesthetized with ketamine (50 mg/kg ip; Holliday-Scott, Buenos Aires, Argentina) and placed in a stereotaxic frame (model 1940; David Kopf Instruments) with the incisor bar fixed at −3.5 mm. Guide cannulas aimed at the NTS were implanted bilaterally at the following stereotaxic coordinates: 14.5 mm caudal to bregma, 0.5 mm lateral to the midline, and 5.5 mm below the skull surface at the level of bregma. The guide cannula was fixed with methacrylate to the skull and to watch screws inserted in the skull. An oculder closed the cannula until the start of the experiments. The animals were allowed to recover fully from anesthesia and were kept in their home cages for 3–5 days. Forty-eight hours before the experiments were performed, rats were anesthetized with a mixture of halothane (2% in oxygen (100%), and a cannula (PE-10 connected to PE-50; Clay Adams, Parsippany, NJ) was implanted in the femoral artery for measurement of pulsatile arterial blood pressure (PAP), mean arterial pressure (MAP), and heart rate (HR). After catheterization, a midline laparotomy was performed, and miniature pulsed Doppler flow probes (Iowa Doppler Products, Iowa City, IA) were placed around the lower abdominal aorta (1.3 mm of lumen), superior mesenteric artery (1.0 mm of lumen), and left renal artery (0.8 mm of lumen) for measurement of hindquarter, mesenteric, and renal blood flow, respectively. The probes were sutured in place, the leads and the catheter were tunneled subcutaneously and exteriorized between the scapulae, and the wounds were closed. To protect the probe wires and the polyethylene tubing while allowing the animal unrestricted movement, we passed the free ends of the catheters and the Doppler leads through a stainless steel skin button connected to a spring and swivel assembly. The assembly was mounted to a ring stand clamp and suspended above the cage. The skin button was attached to the skin incision in the scapular region with stainless steel sutures. To avoid contamination and minimize postoperative pain, we performed all procedures with sterile tools and solutions. Animals that seemed to show distress or pain after surgeries were euthanized.

Drug administration. The arterial catheter was connected to a Statham P23Db transducer and to a ETT-H200 Bridge amplifier (CB Sciences). Flow probe leads were connected to a Doppler flowmeter (Department of Bioengineering, University of Iowa, Iowa City, IA). PAP (mmHg), MAP (mmHg), HR (beats/min), and hindquarter, renal, and mesenteric regional blood flows (kHz) were recorded in conscious animals after recovery from instrumentation (48 h). Signals were obtained using a digital data acquisition system (PowerLab with Chart version 3.4s; AD Instruments) and monitored on a Macintosh computer (G3; Apple). Drug microinjections into the NTS were made with a needle (33-gauge) that protruded 1.5 mm beyond the tip of the guide cannula. The needle was connected with PE-10 tubing to a 1-µl syringe (Hamilton, Reno, NV). Microinjections of AMPA hydrobromide (AMPA HBr, a glutamatergic ionotropic agonist; RBI) and Nω-nitro-l-arginine methyl ester (l-NAME, an NOS inhibitor; Sigma) or Nω-nitro-d-arginine methyl ester (d-NAME, the inactive enantiomer of l-NAME; Sigma) were made unilaterally after baseline hemodynamic values were established.

Protocols. Hemodynamic effects produced by AMPA injections in the NTS (10 pmol/100 nl) were determined before (control) and 5, 15, and between 30 and 45 min after microinjection of l-NAME or d-NAME (10 nmol/100 nl) into the NTS. l-NAME or d-NAME was injected 20 min after the AMPA control microinjection. The volume of all injections was 100 nl. The dose of AMPA used in this study (10 pmol) consistently induced hindquarter vasodilation in a previous study (9). The dose of l-NAME used in this study is considerably less than that used to block NOS peripherally. We have shown before that microinjection of 10 nmol/100 nl into the NTS does not change resting blood pressure, heart rate, regional blood flow, renal sympathetic nerve activity (10), or resting neuronal discharge of vagus nerve-evoked NTS neurons (8).

Histology. After the experiments, methylene blue (100 nl of a 2% solution) was microinjected in the same NTS site for histological analysis. Animals were anesthetized with pentobarbital sodium (1g/kg iv) and perfused transcardially with saline (0.9%) followed by 10% formalin solution. The brain was removed and stored in buffered formalin for at least 2 days. Coronal sections (40 µm) were cut on a microtome and stained using the Nissl method (16). Only rats whose microinjection sites were located in the intermediate NTS were used for data analysis (Fig. 1).

Data analysis. Relative mesenteric, renal, and hindquarter vascular conductances (MVC, RVC, and HVC, %) were calculated as the ratio of Doppler shift and MAP, and they were expressed as percentages of the baseline as described previously by Dias et al. (9). Responses were divided into phases to facilitate data analysis: maximum hypotension defined phase I, maximum hypertension defined phase II, and maximal hindquarter vasodilation defined phase III. The timing (period) to define each phase on AMPA cardiovascular responses was the same before and after l-NAME or d-NAME treatment. When a phase was not present or did not occur after the treatment, its corresponding timing in the control response was taken for analysis. All data are expressed as means ± SE and were analyzed using SigmaStat statistical software (version 2.03; SPSS). One-way ANOVA with repeated measures was used to compare differences between time points (control, 5 min, 15 min, and 30–45 min), and the Newman-Keuls post hoc test was used to identify values that were significantly different from control values. Significance was set at P = 0.05.
RESULTS

Effects of central NOS inhibition on changes in MAP and HR induced by microinjection of AMPA in the NTS. A group of six rats (baseline MAP: 108 ± 3 mmHg; baseline HR: 340 ± 22 beats/min) received unilateral microinjection with 10 pmol/100 nl AMPA in the intermediate NTS before microinjection of L-NAME and then 5, 15, and 30–45 min after L-NAME (10 nmol/100 nl). Figure 2 (left) shows a typical example of the hemodynamic effects caused by AMPA in a conscious rat in the absence of L-NAME (AMPA control). The response consists of a period of hypotension (phase I, ΔMAP control: −16 ± 11 mmHg) followed by a longer lasting period of hypertension (phase II, ΔMAP control: 35 ± 5 mmHg, P < 0.05). Phase III is defined by the peak of hindquarter vasodilation. By this time, MAP was still elevated (ΔMAP control: 19 ± 4 mmHg, P < 0.05) but was falling back to baseline (Figs. 2 and 3A). The initial hypotension caused by AMPA (phase I) is the result of intense bradycardia (ΔHR control: −122 ± 40 beats/min, P < 0.05; Fig. 3B) that may decrease cardiac output. This response can be abolished with intravenous methylnitrate treatment, as we previously reported (9). The large variation in AMPA-mediated hypotension can be related to the variability in HR fall, considering that experiments were performed on nonanesthetized, freely moving rats.

Unilateral microinjection of L-NAME (10 nmol) into the NTS of conscious rats did not change baseline MAP (MAP before L-NAME: 112 ± 6 mmHg vs. MAP after L-NAME: 114 ± 5 mmHg) or baseline HR (HR before L-NAME: 369 ± 30 beats/min vs. HR after L-NAME: 316 ± 28 beats/min). AMPA-induced changes in MAP were not affected by L-NAME (phase II, ΔMAP before L-NAME: 35 ± 5 mmHg; phase II, ΔMAP 15 min after L-NAME: 43 ± 6 mmHg; Fig. 3A). Microinjection of L-NAME in the NTS did not significantly alter the bradycardia induced by AMPA (phase I, ΔHR before L-NAME: −122 ± 40 beats/min; phase I, ΔHR 5 min after L-NAME: −163 ± 55 beats/min; phase I, ΔHR 15 min after L-NAME: −92 ± 32 beats/min; Fig. 3B).

Effects of central NOS inhibition on regional vascular conductance responses to NTS microinjection of AMPA. The ratio of Doppler shift (blood flow) and MAP was used to calculate...

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**Fig. 2.** Typical tracing showing changes in pulsatile arterial pressure (PAP, mmHg), mean arterial pressure (MAP, mmHg), heart rate (HR, beats/min [bpm]), renal blood flow (RBF, kHz), mesenteric blood flow (MBF, kHz), and hindquarter blood flow (HBF, kHz) elicited by unilateral microinjection of S-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA; 10 pmol/100 nl) before (control), during, and 5, 15, and 45 min after unilateral microinjection of the nitric oxide synthase (NOS) inhibitor Nω-nitro-arginine methyl ester (l-NAME; 10 nmol/100 nl) into the NTS of conscious rats. I, II, and III indicate phase I (peak fall of arterial pressure), phase II (peak rise of arterial pressure), and phase III (peak elevation of HBF), respectively.
relative HVC, RVC, and MVC. In the absence of l-NAME, microinjection of AMPA (10 pmol/100 nl) in the NTS reduced HVC (vasoconstriction) in phase I (phase I, ΔHVC control: −21 ± 10%). Phase II is the period of maximal blood pressure, but with respect to blood flow it is a transitional phase in which vascular conductance is still reduced (phase II, ΔHVC control: −11 ± 8%) but returning to baseline. Vasodilation is observed in the hindquarter bed in phase III (phase III, ΔHVC control: 18 ± 4%, *P < 0.05) (Fig. 3C).

After microinjection of l-NAME into the NTS, the maximum vasoconstriction induced by AMPA first obtained in phase I shifted to phase II (phase II, ΔHVC control: −11 ± 8%; phase II, ΔHVC 5 min after l-NAME: −61 ± 7%; phase II, ΔHVC 15 min after l-NAME: −30 ± 7%; and phase II, ΔHVC 45 min after l-NAME: −39 ± 9%, *P < 0.05, n = 6). Vasodilation observed in phase III was abolished in AMPA microinjections performed 5 and 15 min after l-NAME (phase III, ΔHVC control: 18 ± 4%; phase III, ΔHVC 5 min after l-NAME: −16 ± 7%; and phase III, ΔHVC 15 min after l-NAME: −17 ± 9%, *P < 0.05, n = 6). The response to AMPA began to recover by 45 min after l-NAME (phase III, ΔHVC 45 min after l-NAME: 3 ± 6%). Changes in HVC caused by AMPA at 5, 15, and 45 min after l-NAME are shown in Fig. 3C.

AMPA (10 pmol/100 nl) microinjected into the NTS caused significant vasoconstriction in the renal and mesenteric beds during phase II (phase II, ΔRVC control: −35 ± 8%; phase II, ΔMVC control: −36 ± 6%, *P < 0.05, n = 5). Vasoconstriction appeared attenuated 15 min after l-NAME (phase II, ΔRVC 15 min after l-NAME: −26 ± 10%; phase II, ΔMVC 15 min after l-NAME: −17 ± 5%, n = 5), but this difference was not statistically significant (Fig. 3D and E).

As a control for nonspecific effects of l-NAME, we injected the same amount of d-NAME (10 nmol) in a second group of animals (n = 5, baseline MAP: 109 ± 7 mmHg; baseline HR: 322 ± 17 beats/min). Microinjection of d-NAME by itself did not cause any significant change in baseline values (MAP before d-NAME: 108 ± 6 mmHg vs. MAP after d-NAME: 109 ± 7 mmHg; HR before d-NAME: 340 ± 16 beats/min vs. HR after d-NAME: 352 ± 16 beats/min). d-NAME also did not affect the changes in MAP, HR, and regional vascular conductance elicited by AMPA microinjection. Thus the hindquarter vasodilation observed in phase III with AMPA microinjection (phase III, ΔHVC control: 17 ± 6%) remained after pretreatment with d-NAME (phase III, ΔHVC 5 min after d-NAME: 23 ± 10%; phase III, ΔHVC 15 min after d-NAME: 19 ± 12%; and phase III, ΔHVC 45 min after d-NAME: 30 ± 13%, n = 5).

DISCUSSION

This is the first study showing the physiological role of the interaction between the non-NMDA receptor (AMPA) activation and NO produced endogenously in the NTS of conscious rats. The present data show that inhibition of NO production within the NTS changes the hemodynamic responses elicited by AMPA receptor stimulation. Activation of the AMPA receptor in the NTS of conscious rats induces an initial reduction in hindquarter blood flow that is followed by a large increase (9). Increased blood flow is due to hindquarter vasodilation. The origin and pathway of this vasodilation was not clear, but studies of Davisson et al. (6, 7) suggested a neurogenic hindquarter vasodilation mediated by release of preformed stores of nitrosyl factors after sympathetic...
stimulation. Colombari et al. (4) showed a hindquarter vasodilation elicited by activation of glutamatergic receptors in the NTS that was abolished after systemic L-NAME administration. The systemic administration of L-NAME probably blocked NOS in endothelial cells and nerve terminals in the vasculature and anywhere else NOS was present.

In the present study, we observed that hindquarter blood flow changes elicited by AMPA stimulation were modified after L-NAME microinjection within the NTS. Our group (10) recently reported that inhibition of NOS in the NTS of anesthetized rats attenuates baroreflex and cardiopulmonary reflex inhibition of renal nerve discharge, which suggests that NO can be functionally important in modulation of cardiovascular reflexes through the CNS. The pool of preformed NO present within the NTS neurons and that formed and/or released by stimulation of glutamatergic receptors would, in some way, activate a system to cause vasodilation and an increase in hindquarter blood flow. The response observed at the hindquarter bed combines AMPA stimulation response (initial vasoconstriction) and vasodilatatory system activation: 1) quick and accentuated fall in blood flow that returns to basal levels when blood pressure increases, and 2) vasodilation of the hindquarter bed that becomes “visible” when vasoconstriction is finished. In the same way, when the NO-mediated pathway is inhibited (NOS inhibition), the hindquarter vasoconstrictor effect of AMPA lasts longer and is enhanced in magnitude, as noticed, because blood pressure level is increased.

L-NAME is a potent in vitro and in vivo NOS inhibitor that competes with L-arginine for the substrate-binding site at the enzyme (39). Blockade of NOS activity by injection of L-NAME in the NTS seems to be dose related (18), because in high concentration, L-NAME is more selective to endothelial NOS and local vasoconstriction can cause cell damage. The dose used in our studies did not change baseline blood pressure, heart rate, regional blood flow, or renal nerve activity (10). NO present and synthesized in NTS neurons has inter- and intracellular effects. We do not know whether the results observed in the present study during NOS inhibition can be attributed to postsynaptic modulation, because all of the drugs were microinjected. Our group’s previous single-unit study (8) showed that iontophoresis of L-NAME does not alter spontaneous discharge but significantly decreases the number of action potentials evoked by iontophoretic application of AMPA in NTS neurons receiving vagal afferents inputs, suggesting that NO facilitates glutamate transmission through AMPA receptors within the NTS neurons.

In addition to our previous studies by our group (8, 10) and other findings in the literature, the present study suggests that NO formed and released within the NTS may modulate the response elicited by glutamatergic transmission, including the effects observed on the hindquarter limb. NO was first characterized in the CNS as an intercellular messenger responsible for the increase in cGMP levels after activation of glutamatergic receptors (14). It is known that central NO is produced by NOS, which is activated by Ca2+ through calmodulin. NO activates soluble guanylyl cyclase (sGC), stimulating the production of cGMP. The anatomic relationship among sGC immunoreactivity, nNOS, glutamate, and glutamate receptors suggests that NO and glutamate interactions in the NTS involve sGC (31). The evidence that central NO is involved in peripheral functions has been growing. NO can influence brain development, memory, synaptic plasticity, and modulation of neuroendocrine responses (12). Anatomic and other studies in the last decade support the idea that NO modulates NTS function once nNOS is found in cell bodies, terminals, and fibers of the NTS (2, 21, 26, 28, 30).

We propose a NO-mediated vasodilatatory system (nitroxi- dergic pathway) within the NTS responsible for the modulation of the sympathetic outflow (vasoconstrictor response) when glutamatergic afferents are activated. The integrated response observed with AMPA microinjection into the NTS would be a result of the activation of both systems: vasoconstrictor and vasodilatatory. If one system is blocked, what we see is only

Fig. 4. Schematic representation showing our hypothesis of the mechanism activated by NO formed and/or released within the NTS in modulation of glutamatergic transmission during stimulation of the cardiovascular inputs. SP, substance P; l-glu, l-glutamate.
the cardiovascular effect of one system (vasoconstrictor) without the modulatory influence of the other (vasodilatory).

The exact pathway of central formed NO in modulating glutamatergic receptor-mediated responses in NTS is not fully understood. Increases in extracellular NO levels have been shown to induce glutamate release (24), and microinjections of AMPA and NMDA have been shown to increase NO release (24, 33). It was recently shown that the distribution of NTS neurons that project to the pressor area of rostral ventrolateral medulla (RVLm) is similar to that containing nNOS neurons, suggesting that NO within the NTS may influence cardiovascular function through the NTS-RVLm pathway (20).

We think there are two distinct mechanisms leading to production/release of NO. The first one (see “1” in Fig. 4) involves intracellular production and release of NO by AMPA receptor stimulation within NTS neurons. The pool of NO that is formed facilitates glutamatergic transmission and activates the pathway (see “2” in Fig. 4) that will cause vasodilatorly response by release of NO at the sympathetic nerve terminals in the hindquarter vascular bed. The exact interaction of NTS and other medullary sites resulting in the activation and/or deactivation of this pathway is still unclear.

Figure 4 shows how NO may be involved in the changes we found in hindquarter blood flow. Activation of cardiovascular afferents such as baroreceptors, chemoreceptors, and cardiopulmonary receptors increases the release of glutamate in the NTS. Glutamate acts on AMPA receptors at the postsynaptic membrane and triggers the production of NO (1). Because NO is a diffusible compound, it may act in the same and/or adjacent cells and stimulate the release of glutamate through presynaptic receptors, and it also may change the release of other neurotransmitters such as GABA, substance P, and ACh. The NO mobilized by AMPA receptor activation within the NTS would be responsible for hindquarter vasodilation (activation of the vasodilatory nitrooxidergic pathway) to restore blood pressure levels.

Overall, the present data show that centrally formed NO can affect glutamatergic transmission within the NTS to modulate cardiovascular responses. The specific function or mechanism of this interaction remains to be elucidated, but it could also involve the action of NO in controlling release of other neurotransmitters.

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GRANTS

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