# Hemodynamic effects of L-glutamate in NTS of conscious rats: a possible role of vascular nitrosyl factors

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**Colombari, Eduardo, Robin L. Davisson, Richard A. Shaffer, William T. Talman, and Stephen J. Lewis.** Hemodynamic effects of L-glutamate in NTS of conscious rats: a possible role of vascular nitrosyl factors. *Am. J. Physiol.* 274 (*Heart Circ. Physiol.* 43): H1066–H1074, 1998.—This study examined peripheral mechanisms responsible for changes in mean arterial blood pressure, heart rate, and renal, mesenteric, and hindquarter vascular resistances produced by microinjections of L-glutamate (L-Glu) into the nucleus tractus solitarii (NTS) of conscious rats. Microinjection of L-Glu produced an initial pressor response, bradycardia, and vasoconstriction in each vascular bed. Subsequent hindquarter vasodilation was observed. After prazosin was administered, L-Glu produced initial hypotension that was probably due to reduced cardiac output. This hypotension was followed by hindquarter vasodilation. Inhibition of nitric oxide synthesis did not affect the initial hypotension or bradycardia in rats treated with prazosin, but the first microinjection of L-Glu after administration of prazosin and $N^G$-nitro-L-arginine methyl ester (L-NAME) produced significantly greater hindquarter vasodilation than after administration of prazosin alone. Second and third microinjections of L-Glu produced significantly smaller hindquarter vasodilation. We conclude that *1*) hemodynamic effects produced by microinjection of L-Glu into the NTS of conscious rats involves activation of the sympathetic nervous system and *2*) release of preformed nitrosyl factors may mediate vasodilation in the hindquarter vascular bed.

autonomic control; hemodynamics; nitric oxide; excitatory amino acids

MICROINJECTION OF the excitatory amino acid L-glutamate (L-Glu) into the nucleus tractus solitarii (NTS) of conscious rats produces a pressor response and bradycardia (4, 5, 18, 20). The pressor response is virtually abolished by systemic administration of the $\alpha_1$-adrenoceptor antagonist prazosin, whereas the bradycardia is unaffected (4). The bradycardia is abolished by the subsequent systemic administration of the muscarinic receptor antagonist methylatropine (4). These findings suggest that L-Glu mediates its pressor response via activation of sympathetic vasoconstrictor drive to the peripheral vasculature, whereas it mediates the bradycardia by activating the cardiac vagus. In contrast to the pressor responses seen in conscious rats, microinjections of L-Glu into the NTS of anesthetized rats produce dose-related decreases in mean arterial pressure (MAP) and heart rate (HR) (27, 33). The initial decrease in MAP is not associated with changes in mesenteric or renal vascular resistances, although dilation gradually develops in these beds (33). The sequence of changes in hindquarter vascular resistance differs. In the hindquarter bed, vasodilation occurs immediately after microinjection of L-Glu, whereas vasoconstriction develops during the later stage of the depressor response (33). Because these effects of L-Glu are virtually abolished by ganglion blockade (33), they appear to result from changes in autonomic nerve activity.

The activation and/or withdrawal of sympathetic vasoconstrictor drive may be fully responsible for the hemodynamic effects produced by microinjections of L-Glu into the NTS. However, we have provided evidence that postganglionic lumbar sympathetic nerves innervating the hindquarter vasculature contain nitric oxide (NO) synthase (6, 7) and that the direct and reflex-mediated activation of the lumbar chain produces hindquarter vasodilation via release of newly synthesized and preformed pools of nitrosyl factors (6–9, 25). This raises the possibility that microinjections of L-Glu into the NTS may reduce vascular resistance in the hindquarter bed, in part, by an active sympathetic neurogenic vasodilator process utilizing nitrosyl factors.

At present, there is no information about the effects on vascular resistances when L-Glu is injected into the NTS of conscious rats. Such information would contribute to a better understanding of mechanisms through which glutamatergic systems in the NTS regulate arterial pressure. Therefore, the aims of the present study were to *1*) characterize hemodynamic effects produced by microinjection of L-Glu (1 nmol) into the NTS of conscious rats and *2*) evaluate the contribution of sympathetic vasoconstrictor and vasodilator processes and of vascular nitrosyl factors in these hemodynamic effects.

## MATERIALS AND METHODS

*Animals.* All experiments were performed in conscious, freely moving male Sprague-Dawley rats ($n$ = 23) weighing between 300 and 400 g. The animals were housed individually in Perspex cages in a room with a 12:12-h light-dark cycle. Food and water were freely available except during the experiments.

*Surgical procedures.* Seven days before the experiments were performed, the rats were anesthetized by administration of halothane (2%) mixed with oxygen (100%). The rats were placed in a stereotaxic frame (model 1940, David Kopf Instruments), and guide cannulas were implanted into the brain using the stereotaxic coordinates of Paxinos and Watson (24), as described previously (4). Briefly, a 15-mm-long stainless steel cannula (22 gauge) was introduced through a cranial window made caudal to lambda. The cannula was positioned 14 mm caudal to bregma, 0.5 mm lateral to the

midline, and 7.9 mm below the skull surface at the level of bregma. The tip of the guide cannula was placed in the cerebellum 1.0 mm above the dorsal surface of the brain stem. The guide cannula was fixed with methacrylate to both the skull and the screws inserted in the skull. An occluder closed the cannula until the time of experiments. The needle (33 gauge) used for microinjections into the NTS was 1.5 mm longer than the guide cannula and was connected by PE-10 tubing to a 1-μl syringe (Hamilton, Reno, NV).

Two days before the experiments were performed, the rats were anesthetized by an intraperitoneal injection of a mixture of ketamine (120 mg/kg) and acepromazine maleate (12 mg/kg). Femoral arterial and venous catheters (PE-50) were implanted for measurement of pulsatile arterial blood pressure, MAP, and HR and for administration of drugs, respectively. Immediately after catheterization, a midline laparotomy was performed, and miniature pulsed Doppler flow probes were placed around the lower abdominal aorta, superior mesenteric artery, and left renal artery for measurement of hindquarter, mesenteric, and renal blood flow, respectively. The probes were sutured in place, the leads and catheters were tunneled subcutaneously and exteriorized between the scapulae, and the wounds were closed. To protect the probe wires and polyethylene tubing while allowing animals unrestricted movement during recovery and experimental testing, the free ends of the catheters and Doppler leads were led through a stainless steel skin button connected to a spring-swivel assembly that 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 using stainless steel sutures. Details of the Doppler technique, including construction of the probes, reliability of the method for estimation of flow velocity, and quantitative determination of percentage changes in hindquarter, mesenteric, and renal resistances, have been described previously (6, 12, 16).

*Protocol.* After a 2-day period of recovery, the arterial catheters were connected to a Beckman Dynograph coupled pressure transducer (Cobe Lab) and the flow probe leads were connected to a Doppler flowmeter (Dept. of Bioengineering, University of Iowa, Iowa City, IA) for recording MAP, HR, and blood flows. In the first study, a group of rats ($n = 7$) received microinjections of L-Glu into the NTS (1 nmol in 100 nl). This dose of L-Glu is the approximate half-maximal effective dose with respect to changes in MAP and HR in conscious rats (4). Microinjections of L-Glu were given twice before and after systemic administration of the $\alpha_1$-adrenoceptor antagonist prazosin (100 μg/kg iv) and three times after the subsequent systemic administration of the NO synthesis inhibitor $N^G$-nitro-L-arginine methyl ester (L-NAME; (25 μmol/kg iv). The microinjections of L-Glu were given 5–10 min apart. Baseline hemodynamic values were allowed to stabilize for at least 10 min after the injection of either prazosin or L-NAME but before the first microinjection of L-Glu was given. In the second study, the hemodynamic effects produced by the systemic injection of the NO donor sodium nitroprusside (5 μg/kg iv) or the *S*-nitrosothiol, *S*-nitrosocysteine (100 nmol/kg iv) (23), were examined in a group of rats ($n = 8$) before and after administration of prazosin (100 μg/kg iv) and again after subsequent administration of L-NAME (25 μmol/kg iv). In the third study, the hemodynamic effects produced by four successive systemic injections of sodium nitroprusside (5 μg/kg iv) or *S*-nitrosocysteine (100 nmol/kg iv) were examined in rats ($n = 8$) pretreated with the combination of prazosin (100 μg/kg iv) and L-NAME (25 μmol/kg iv).

*Histology.* After the experiments were performed, methylene blue (100 nl of a 2% solution) was microinjected at the same site in the NTS for histological analysis. The animals were killed with an overdose of pentobarbital sodium (100 mg/kg iv) and perfused through the heart with saline followed by 10% buffered Formalin. The brains were stored in buffered Formalin for 2 days, and serial coronal (50 μm) sections were cut and stained by the Nissl method using Giemsa dye (5, 11). Only rats whose microinjection sites were located in the NTS at the level of the calamus scriptorius were used for data analysis. Those animals in which microinjections lay outside the NTS did not manifest hemodynamic effects.

*Statistics.* All data were expressed as means ± SE and were analyzed by repeated-measures analysis of variance (32) followed by Student's modified *t*-test with Bonferroni correction for multiple comparisons between means (31).

## RESULTS

*Hemodynamic effects of prazosin and L-NAME.* The hemodynamic effects produced by systemic injection of the $\alpha_1$-adrenoceptor antagonist prazosin (100 μg/kg iv) and subsequent injection of the NO synthesis inhibitor L-NAME (25 μmol/kg iv) into conscious freely moving rats ($n = 7$) are summarized in Table 1. Prazosin produced a significant, sustained reduction in MAP and hindquarter resistance but did not produce sustained decreases in either renal or mesenteric resistances. The rats also displayed sustained tachycardia. The subsequent injection of L-NAME produced marked and sustained increases in MAP and vascular resistances accompanied by bradycardia.

*Hemodynamic effects produced by microinjection of L-Glu into the NTS.* A typical example of effects elicited by microinjections of L-Glu (1 nmol) into the NTS of a conscious rat before and after the injection of prazosin (100 μg/kg iv) and the subsequent administration of L-NAME (25 μmol/kg iv) are shown in Fig. 1. L-Glu produced a transient pressor response, bradycardia, and decreases in mesenteric, renal, and hindquarter

Table 1. *Hemodynamic effects produced by systemic injection of prazosin and then L-NAME in conscious rats*

| Variable | Before Prazosin | After Prazosin | Change, % | Prazosin + L-NAME | Change, % |
|---|---|---|---|---|---|
| HR, beats/min | 332 ± 15 | 412 ± 26 | +24 ± 4* | 278 ± 17 | −33 ± 7† |
| MAP, mmHg | 108 ± 4 | 79 ± 6 | −26 ± 5* | 131 ± 5 | +66 ± 16† |
| HQR, mmHg/kHz | 42 ± 11 | 22 ± 4 | −48 ± 7* | 72 ± 15 | +227 ± 53† |
| RR, mmHg/kHz | 27 ± 5 | 21 ± 4 | −22 ± 11 | 60 ± 11 | +186 ± 42† |
| MR, mmHg/kHz | 39 ± 7 | 48 ± 10 | +23 ± 10 | 115 ± 19 | +140 ± 27† |

Values are means ± SE of plateau levels. HR, heart rate; MAP, mean arterial blood pressure; HQR, hindquarter resistance; RR, renal resistance; MR, mesenteric resistance. Prazosin (100 μg/kg iv) and $N^G$-nitro-L-arginine methyl ester (L-NAME; 25 μmol/kg iv) were given to 7 conscious rats. %Changes in these values after administration of prazosin or L-NAME are shown, and %changes produced by L-NAME reflect changes from prazosin values. *$P < 0.05$, after prazosin vs. before prazosin; †$P < 0.05$, L-NAME vs. prazosin.

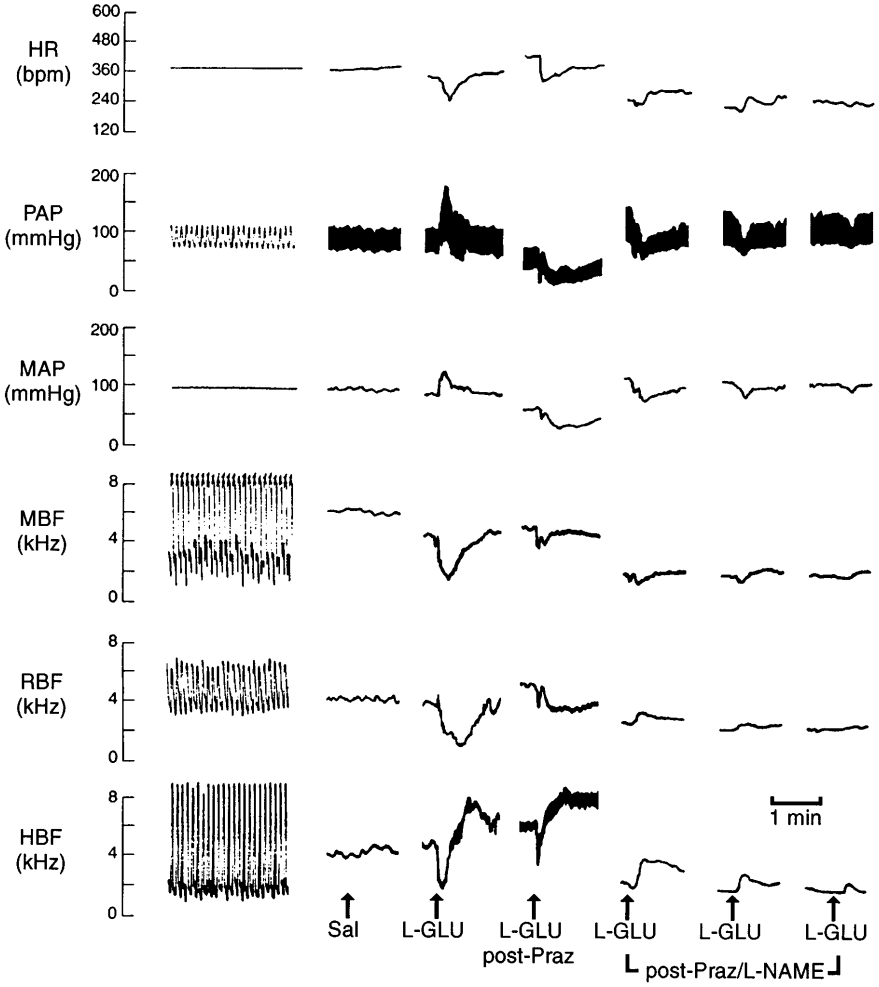


Fig. 1. A typical example of effects of microinjections of L-glutamate (L-Glu; 1 nmol/100 nl) into nucleus tractus solitarii (NTS) of a conscious rat on heart rate (HR), pulsatile (PAP) and mean arterial blood pressure (MAP), and mesenteric (MBF), renal (RBF), and hindquarter blood flows (HBF) after systemic injection of saline (Sal), prazosin (Post-Praz; 100 μg/kg iv), and $N^G$-nitro-L-arginine methyl ester (Post-Praz/L-NAME; 25 μmol/kg iv).

blood flow. The initial decrease in hindquarter blood flow was followed by a pronounced increase in flow. After the administration of prazosin, the microinjection of L-Glu produced an initial reduction in MAP, HR, and blood flows. Although the depressor effect and reduction in renal blood flow were sustained for >1 min, HR and mesenteric blood flow returned to baseline more rapidly. In contrast, there was a pronounced increase in hindquarter blood flow. Ten minutes after administration of L-NAME, the first microinjection of L-Glu still produced a reduction in MAP and minor changes in mesenteric and renal blood flows, but it then caused a pronounced increase in hindquarter blood flow. Each subsequent injection of L-Glu produced progressively smaller reductions in MAP and increases in hindquarter blood flow, whereas the responses of the other hemodynamic variables did not appreciably change.

*Summary of initial hemodynamic effects produced by L-Glu (phase I).* The initial hemodynamic effects produced by the microinjections of L-Glu (1 nmol) into the NTS of conscious rats before and after the systemic administration of prazosin (100 μg/kg iv) and then L-NAME (25 μmol/kg iv) are shown in Fig. 2. Microinjection of L-Glu produced an increase in MAP (+26 ± 5%, $P < 0.05$) that was accompanied by bradycardia (−38 ± 6%, $P < 0.05$) and marked increases in hindquarter resistance (+307 ± 52%, $P < 0.05$), renal resistance (+125 ± 22%, $P < 0.05$), and mesenteric resistance (+123 ± 21%, $P < 0.05$). The vasoconstriction in the hindquarter bed was significantly greater than that in the renal and mesenteric beds ($P < 0.05$ for both comparisons). A second microinjection of L-Glu produced similar responses (see Fig. 2). After the injection of prazosin, microinjection of L-Glu into the NTS produced significant ($P < 0.05$) decreases in MAP (−21 ± 5%) and HR (−51 ± 7%) and a significantly attenuated hindquarter vasoconstriction (94 ± 16%, $P < 0.05$ compared with preprazosin values). Prazosin eliminated the effects of L-Glu on renal vascular resistance (−2 ± 4%, $P > 0.05$ compared with basal resistance) and mesenteric vascular resistance (−17 ± 8%, $P > 0.05$ compared with basal resistance). A second microinjection of L-Glu produced similar responses in these prazosin-treated rats (see Fig. 2). After subsequent systemic administration of L-NAME, microinjections of L-Glu into the NTS produced hemodynamic responses that were not different from those observed before administration of the NO synthesis inhibitor. Each microinjection of L-Glu produced virtually identical decreases in HR before and after the administration of prazosin and after the subsequent administration of L-NAME (see Fig. 2).

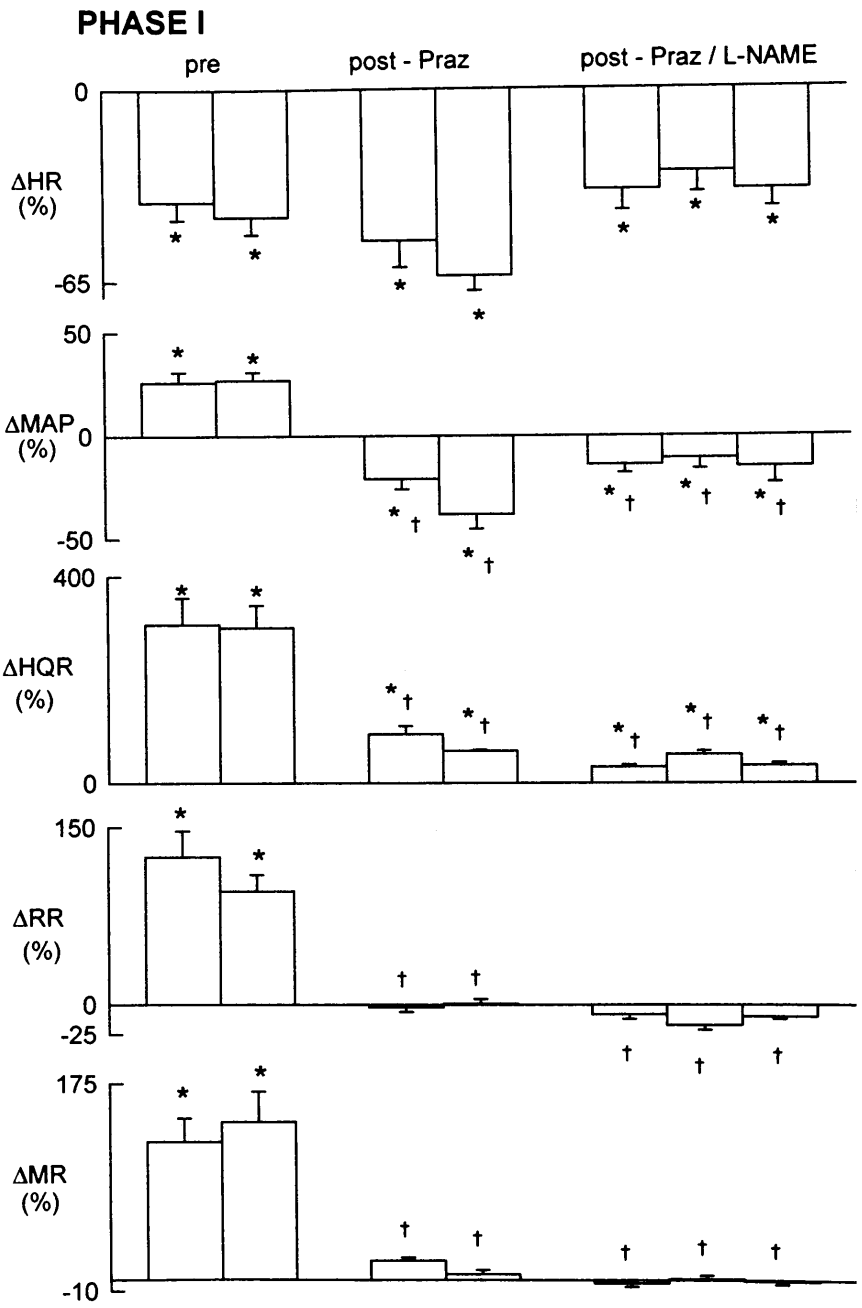


Fig. 2. Initial effects (*phase I*) on HR, MAP, and hindquarter (HQR), renal (RR), and mesenteric vascular resistances (MR) produced by microinjections of L-Glu (1 nmol/100 nl) into NTS of conscious rats ($n = 7$) before (Pre) and Post-Praz and then Post-Praz/L-NAME. L-Glu microinjections were given twice before and after injection of prazosin and three times after subsequent injection of L-NAME. Values are means ± SE of %changes (Δ) from baseline. *$P < 0.05$, significant response; †$P < 0.05$, significant change from Pre values.

*Summary of late-phase hindquarter vasodilation (phase II).* The initial pressor and vasoconstrictor effects produced by microinjection of L-Glu into the NTS were followed by a sustained increase in hindquarter blood flow. The hemodynamic values during the hindquarter vasodilator phase before and after the administration of prazosin and then L-NAME are shown in Fig. 3. This phase consisted of a reduction in hindquarter resistance but no change in other hemodynamic variables. Before prazosin was administered, the two injections of L-Glu into the NTS produced significant ($P < 0.05$) and equivalent reductions in hindquarter resistance of −29 ± 5 and −24 ± 4%, respectively. The two microinjections of L-Glu given after administration of prazosin produced decreases in hindquarter resistance of −27 ± 4 and −24 ± 4%, respectively. These vasodilator responses were equivalent to those observed before the administration of prazosin ($P > 0.05$ for both comparisons). There were no changes in any of the other hemodynamic variables ($P > 0.05$ for all comparisons). After the systemic administration of L-NAME, the first microinjection of L-Glu into the NTS produced a small but significant decrease in MAP (−10 ± 2%) that was accompanied by a pronounced decrease in hindquarter resistance (−43 ± 4%). This vasodilator response was significantly ($P < 0.05$) greater than that observed before administration of the NO synthesis inhibitor. The microinjection of L-Glu also significantly reduced renal resistance (−19 ± 4%, $P < 0.05$). The second and third microinjections of L-Glu produced

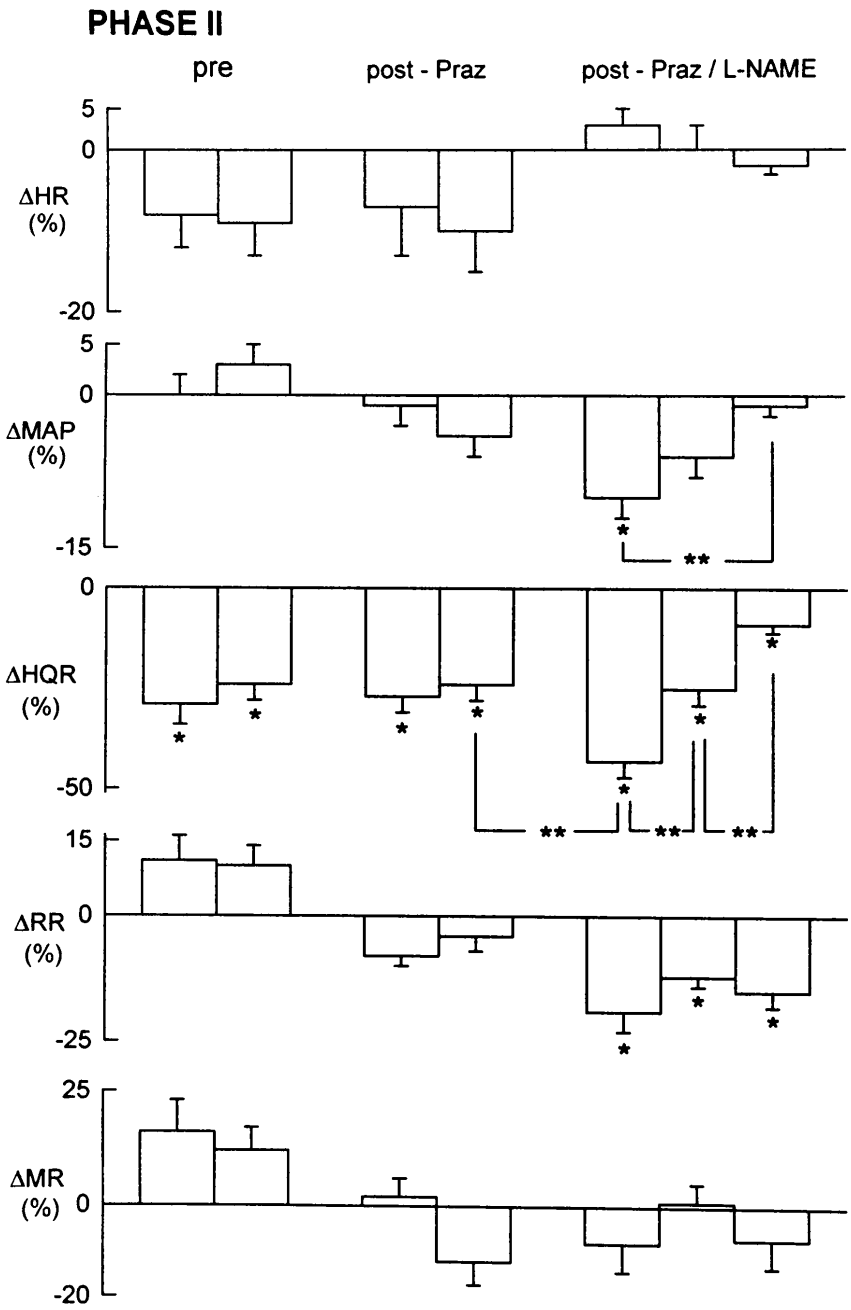


Fig. 3. Changes in HR, MAP, and HQR, RR, and MR during secondary hindlimb vasodilator phase (*phase II*) produced by microinjection of L-Glu (1 nmol/100 nl) into NTS of conscious rats ($n = 7$) Pre and Post-Praz and then Post-Praz/L-NAME. Values are means ± SE of %changes from baseline. *$P <$ 0.05, significant response; **$P < 0.05$, 1st microinjection Post-Praz/L-NAME vs. 2nd and 3rd microinjections Post-Praz/L-NAME or 1st microinjection Post-Praz/L-NAME vs. 2nd microinjection Post-Praz.

progressively smaller decreases in MAP (−6 ± 2 and −1 ± 1%, respectively) and hindquarter resistance (−25 ± 4 and −9 ± 4%, respectively) but similar decreases in renal resistance (−12 ± 2 and −15 ± 3%, respectively).

*Effects of systemically injected S-nitrosocysteine and sodium nitroprusside.* To determine whether diminished hindquarter vasodilation after L-NAME might have resulted from tachyphylaxis to the effects of NO, we studied effects of NO donors systemically administered after prazosin and L-NAME. The decreases in MAP and hindquarter resistance produced by the systemic injection of *S*-nitrosocysteine (100 nmol/kg iv) or sodium nitroprusside (5 μg/kg iv) to conscious, freely moving rats ($n = 8$) before and after injection of prazosin (100 μg/kg iv) and then L-NAME (25 μmol/kg iv) are summarized in Table 2. Before prazosin was administered, *S*-nitrosocysteine and sodium nitroprusside produced significant ($P < 0.05$) decreases in MAP and hindquarter resistance. The hypotensive and vasodilator effects of *S*-nitrosocysteine and sodium nitroprusside were augmented after administration of prazosin. The hypotensive and vasodilator effects of *S*-nitrosocysteine were further augmented after subsequent administration of L-NAME. The effects of prazosin and L-NAME on baseline hemodynamic values in this protocol were similar to those in the L-Glu experiments (see Table 1).

The percent decreases in hindquarter resistance produced by four consecutive injections of *S*-nitrosocys-

Table 2. *Hemodynamic effects produced by systemic injection of S-nitrosocysteine or sodium nitroprusside in conscious rats before and after administration of prazosin alone or combined with L-NAME*

| Compound | Variable | Before Prazosin | After Prazosin | Prazosin + L-NAME |
|---|---|---|---|---|
| SNC | MAP, mmHg | −17 ± 2 | −29 ± 4* | −46 ± 5† |
| | HQR, mmHg/kHz | −21 ± 3 | −37 ± 4* | −56 ± 7† |
| SNP | MAP, mmHg | −14 ± 2 | −36 ± 4* | −38 ± 4 |
| | HQR, mmHg/kHz | −10 ± 2 | −35 ± 5* | −42 ± 5 |

Values are means ± SE of %changes in parameters. Eight conscious freely moving rats received an injection of *S*-nitrosocysteine (SNC; 100 nmol/kg iv) or sodium nitroprusside (SNP; 5 μg/kg iv) before and after administration of prazosin (100 μg/kg iv) and again after administration of L-NAME (25 μmol/kg iv). *$P < 0.05$, after prazosin vs. before prazosin; †$P < 0.05$, after prazosin + L-NAME vs. after prazosin.

teine (100 nmol/kg iv) or sodium nitroprusside (5 μg/kg iv) in conscious freely moving rats ($n = 8$) pretreated with a combination of prazosin (100 μg/kg iv) and L-NAME (25 μmol/kg iv) are summarized in Table 3. The first injection of *S*-nitrosocysteine or sodium nitroprusside produced substantial hypotensive and vasodilator responses in these rats. Each subsequent injection of *S*-nitrosocysteine or sodium nitroprusside produced very similar responses.

*Microinjections sites within the NTS.* A diagrammatic representation of microinjection sites within the brain of the seven rats used in these studies is shown in Fig. 4. These sites were located within the intermediate NTS. There were no hemodynamic responses when microinjection sites were located outside the NTS (data not shown). The success rate in these experiments was 42%.

## DISCUSSION

The present study confirms that microinjections of L-Glu into the NTS of conscious rats produce a pressor response and bradycardia (4, 5, 18, 20) and that the pressor response is virtually eliminated by pretreatment with the $\alpha_1$-adrenoceptor antagonist prazosin (4). This study demonstrates that the pressor response is associated with marked vasoconstriction of hindquarter arteries and lesser, but still pronounced, increases in vascular resistances in the renal and mesenteric arterial beds. The exaggerated vasoconstriction in the hindquarter in comparison with the other vascular beds may be due to greater activation of sympathetic vasoconstrictor input. However, the exaggerated vasoconstriction in the hindquarter bed would also be consistent with the concomitant withdrawal of sympathetic vasodilator input (6, 8). Microinjection of L-Glu into the NTS produced a significant decrease in MAP and HR in the rats treated with prazosin, but the responses were associated with only minor changes in vascular resistances. These initial decreases in MAP and HR in prazosin-treated rats were blocked by intravenous administration of the muscarinic receptor antagonist methylatropine (4), which suggests that the hypotension was due to a vagally mediated reduction in cardiac output.

Table 3. *Hemodynamic effects produced by four successive systemic injections of S-nitrosocysteine or sodium nitroprusside in conscious rats treated with a combination of prazosin and L-NAME*

| Compound | Injection Number | | | |
|---|---|---|---|---|
| | 1st | 2nd | 3rd | 4th |
| SNC | −47 ± 6 | −52 ± 5 | −46 ± 4 | −51 ± 6 |
| SNP | −36 ± 5 | −39 ± 4 | −40 ± 7 | −37 ± 5 |

Values are means ± SE of %changes in HQR. Eight conscious freely moving rats received four successive injections of SNC (100 nmol/kg iv) or SNP (5 μg/kg iv) before and after combined administration of prazosin (100 μg/kg iv) and L-NAME (25 μmol/kg iv). Note that each injection produced a significant decrease in these hemodynamic parameters at $P < 0.05$. There were no between-injection differences in magnitude of these responses at $P < 0.05$.

The untreated rats displayed a pronounced decrease in hindquarter resistance once the initial pressor and vasoconstrictor effects produced by the microinjection of L-Glu had subsided. It is unlikely that the decrease in vascular resistance was due to withdrawal of sympathetic drive and resultant loss of $\alpha_1$-adrenoceptor-mediated vasoconstriction, because this hindquarter vasodilation was unaffected by prazosin. After both prazosin and L-NAME were administered, the first microinjection of L-Glu produced exaggerated hindquarter vasodilation. Such persistent neurogenic hindquarter vasodilatation occurs with the first stimulus regardless of the timing of the stimulus after blockade (9) even though breakdown of NO can be expected within seconds of its synthesis (21). Therefore, hindquarter vasodilation in response to L-Glu is not simply due to an increase in NO synthase activity and subsequent release of newly synthesized NO (14). However, the second and third microinjections of L-Glu produced progressively and substantially smaller responses, suggesting depletion of vasodilating factors after blockade of NO synthesis. We conjecture that the vasodilator may be a nitrosyl factor that is released from sympathetic nerves and is more stable than NO itself. We have demonstrated that postganglionic lumbar sympathetic nerves innervating the hindquarter vasculature contain NO synthase (7) and stain for NADPH diaphorase (6), a marker for neuronal NO synthase (13). In addition, we have provided evidence that direct and reflex-mediated activation of postganglionic lumbar sympathetic nerves produces hindquarter vasodilation that may involve release of newly synthesized and preformed pools of nitrosyl factors from these nerves (6–9, 25). Taken together, these findings raise the possibility that the hindquarter vasodilation produced by the microinjection of L-Glu into the NTS of conscious rats may be mediated by the release of preformed pools of nitrosyl factors from the NO synthase-positive lumbar sympathetic nerves innervating this bed. The progressive diminution of the hindquarter vasodilator responses in L-NAME-treated rats would be consistent with release and gradual depletion of these preformed pools of nitrosyl factors that cannot be regenerated in the absence of NO synthesis. In a previous study (9), we

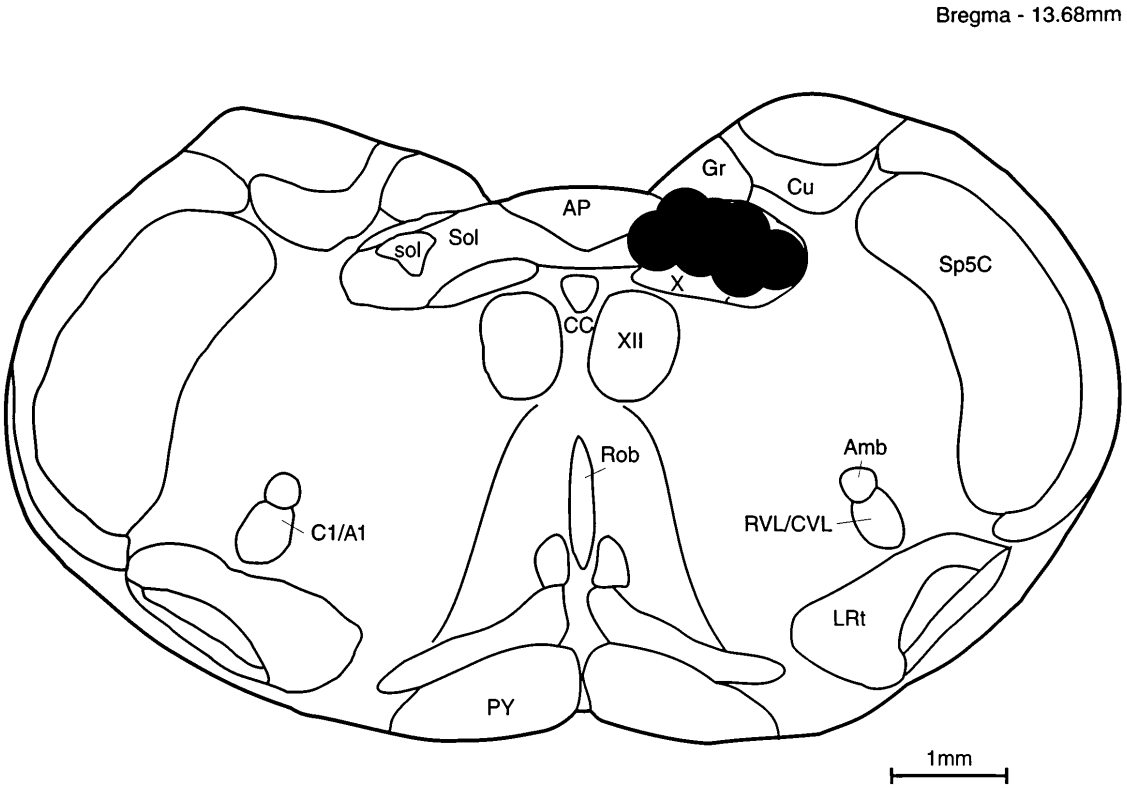


Fig. 4. Diagrammatic representation of microinjection sites within NTS of rats ($n$ = 7) used in these studies. Abbreviations are from atlas of Paxinos and Watson (24): Amb, nucleus ambiguus; AP, area postrema; CC, central canal; Cu, cuneate fasciculus; RVL, rostral ventrolateral medulla; CVL, caudal ventrolateral medulla; C1/A1, epinephrine and norepinephrine cells within RVL/CVL; Gr, gracile nucleus; Sol, nucleus solitary tract; sol, solitary tract; Rob, raphe obscurus nucleus; PY, pyramidal tract; LRt, lateral reticular nucleus; Sp5C, spinal trigeminal nucleus, caudal; X, dorsal motor nucleus of vagus; XII, hypoglossal nucleus.

found that the first episode of air-jet stress produced an equivalent and pronounced hindquarter vasodilation whether it was given 10 or 30 min after the administration of L-NAME. Therefore, it is unlikely that the progressive diminution of the hindquarter vasodilation observed in the present study is due to the increasingly greater inhibition of NO synthesis in the lumbar sympathetic nerve terminals or vascular endothelium between the time of the first and third microinjections of L-Glu (given 10 and 30 min after L-NAME).

There also is considerable evidence that preformed pools of nitrosyl factors exist in vascular smooth muscle (3, 15, 19, 30). Ignarro (14) has postulated that such preformed pools of nitrosyl factors exist within vascular endothelial cells as well. Although the precise identity of these nitrosyl factors has not been established, possible candidates include *S*-nitrosothiols such as the putative endothelium-derived relaxing factor *S*-nitrosocysteine (23), dinitrosyl-iron(II) complexes (29) or iron nitrosyls (26). Determination of whether pools of these nitrosyl factors actually exist in the NO synthase-positive sympathetic terminals must await the development of immunohistochemical or histochemical methods for visualizing these nitrosyl factors.

Although the above findings are consistent with the release of nitrosyl factors from preformed pools in lumbar sympathetic nerve terminals, there are several other possible explanations for these findings. For example, decreases in hindquarter resistance could be due to activation of a cholinergic vasodilator system that causes release of endothelium-derived nitrosyl factors. We doubt that cholinergic mechanisms are responsible in that hindquarter vasodilation produced by direct activation of the lumbar sympathetic chain is unaffected by the muscarinic receptor antagonist methylatropine (O. S. Possas and S. J. Lewis, unpublished observations). In those studies the decrease in hindquarter vascular resistance produced by electrical stimulation of the lumbar chain (3 V, 20 Hz for 10 s) in pentobarbital-anesthetized rats ($n$ = 5) before and after administration of methylatropine (1 mg/kg iv) was $-48 \pm 6$ and $-43 \pm 7\%$ ($P > 0.05$), respectively.

Vasodilation in response to L-Glu could also result from activation of β-adrenoceptors (1). However, our own studies demonstrate that the vasodilator effects of the β-adrenoceptor agonists isoproterenol and epinephrine (1) are not diminished by L-NAME (6, 28) and are, therefore, unlikely to be mediated by release of nitrosyl factors.

It could also be possible that peptides derived from nerve terminals or vascular endothelium (see Ref. 2) could be involved in the hindquarter vasodilation seen in this study. However, studies from our laboratory do not support this mechanism. We have found that hindquarter vasodilation produced by systemic injection of several peptides, including pituitary adenylate

cyclase-activating polypeptide (28), vasoactive intestinal polypeptide, and calcitonin gene-related polypeptide (E. J. Whalen and S. J. Lewis, unpublished observations), are unaffected by the administration of L-NAME and are therefore not likely to be related to release of nitrosyl factors.

Finally, it is possible that the hindquarter vasodilation is mediated by a neurogenically derived agent (peptide or other). Because the actions of this putative agent progressively diminish on repeated administration in L-NAME-treated rats, the agent might mobilize nitrosyl factors from preformed pools in vascular endothelium or lumbar sympathetic nerve terminals. Alternatively, the vasodilator actions of this agent could be independent of nitrosyl factors that nonetheless could, through their own action on vascular smooth muscle, modulate responses to the unknown agent. There is currently no evidence to support this possibility.

It is possible that the loss of hindquarter vasodilation may be due to the diminution of the vasorelaxant potencies of NO or nitrosyl factors. However, the present study demonstrates that four successive systemic injections of the NO donor sodium nitroprusside or the *S*-nitrosothiol, *S*-nitrosocysteine, produced similar responses in L-NAME-treated rats. The progressive loss of hindquarter vasodilation in response to repeated microinjections of L-Glu into the NTS may simply be due to the development of tachyphylaxis to glutamate in the NTS or the actions of L-NAME in this nucleus, because it has been demonstrated that L-Glu evokes the release of an endothelium-derived relaxing factor-like substance in the NTS (10). However, it may be unlikely that tachyphylaxis to glutamate is responsible for our observations, because multiple microinjections of L-Glu into the NTS of conscious rats produce similar pressor and bradycardic responses (20). Moreover, in the present study, each of the seven microinjections of L-Glu given in the NTS (including those given after the administration of L-NAME) produced virtually identical decreases in HR. In addition, the microinjection of L-Glu into the NTS of the rats treated with prazosin and L-NAME produced a significant vasodilation in the renal bed. Although we have not determined the mechanisms responsible for this vasodilation, each of the microinjections of L-Glu produced virtually identical falls in renal resistance. Consequently, it appears that tachyphylaxis does not readily develop to the hemodynamic responses produced by the multiple microinjection of L-Glu into the NTS of conscious L-NAME-treated rats.

Ma et al. (17) reported that the systemic injection of L-NAME (10 mg/kg iv) directly influenced the activity of neurons within the NTS. Therefore, it is possible that the use-dependent loss of hindquarter vasodilation in rats treated with L-NAME may be due to the actions of the NO synthase inhibitor in the NTS. If this were true, then the inhibition of NO synthase in the NTS would have selectively affected the regulation of autonomic outflow to the hindquarter. Because the vasodilator responses produced by systemic injection of *S*-nitrosocysteine were augmented in prazosin/L-NAME-treated rats, it is unlikely that the progressive loss of the L-Glu-mediated hindquarter vasodilation is due to the diminished vasodilator capacity of endogenous nitrosyl factors. The exaggerated effects of *S*-nitrosocysteine and sodium nitroprusside in prazosin-treated rats are probably due to the loss of baroreflex-mediated activation of $\alpha_1$-adrenoceptors that would normally buffer the vasodilation. The further exaggeration of the effects of *S*-nitrosocysteine in the rats treated with L-NAME and prazosin is possibly due to the upregulation of the signal transduction mechanisms by which this *S*-nitrosothiol relaxes vascular smooth muscle (see Ref. 22).

In conclusion, the present study has characterized the hemodynamic responses produced by microinjections of L-Glu into the NTS of conscious rats. These microinjections of L-Glu produced an initial pressor response and vasoconstriction in peripheral vascular beds via the activation of sympathetic neurogenic $\alpha_1$-adrenoceptor-mediated processes. The microinjection of L-Glu also produced a pronounced vasodilation in the hindquarter bed that may be mediated by the release of preformed pools of nitrosyl factors within the vasculature. As mentioned, the hindquarter vasodilation may be due to activation of the lumbar sympathetic vasodilator system, which releases nitrosyl factors. In further support of this possibility, we have obtained preliminary evidence that the maximal hindquarter vasodilation produced by the direct activation of the lumbar sympathetic chain (3.0 V, 20 Hz, 5-ms duration for 10 s) in pentobarbital-anesthetized rats is unaffected by the muscarinic receptor antagonist methylatropine (1 mg/kg iv, $n = 5$) or the β-adrenoceptor antagonist propranolol (1 mg/kg iv, $n = 5$). At present, we are not certain whether the neurogenic vasodilator system is a "final common pathway" for vasodilation in the hindquarter bed or, rather, is activated only by specific stimuli.

This work was supported by National Heart, Lung, and Blood Institute Grants HL-32205 and HL-143888, a Merit Review and Career Award through the Department of Veterans Affairs, Conselho Nacional de Pesquisa (CNPq-520059/96–2), and Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP-96/6075-5).

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Received 13 November 1997; accepted in final form 18 December 1997.

## REFERENCES

1. **Berecek, K. H., and M. J. Brody.** Evidence for a neurotransmitter role for epinephrine derived from the adrenal medulla. *Am. J. Physiol*. 242 (*Heart Circ. Physiol*. 11): H593–H601, 1982.
2. **Bevan, J. A., and J. E. Brayden.** Nonadrenergic neural vasodilator mechanisms. *Circ. Res*. 60: 309–326, 1987.
3. **Chaudry, H., M. Lynch, K. Schomacker, R. Birngruber, K. Gregory, and I. Kochevar.** Relaxation of vascular smooth muscle induced by low-power laser irradiation. *Photochem. Photobiol*. 58: 661–669, 1993.
4. **Colombari, E., L. G. H. Bonagamba, and B. H. Machado.** Mechanisms of pressor and bradycardic responses to L-glutamate microinjected into the NTS of conscious rats. *Am. J. Physiol*. 266 (*Regulatory Integrative Comp. Physiol*. 35): R730–R738, 1994.
5. **Colombari, E., J. V. Menani, and W. T. Talman.** Commissural NTS contributes to pressor responses to glutamate injected into

the medial NTS of awake rats. *Am. J. Physiol.* 270 (*Regulatory Integrative Comp. Physiol.* 39): R1220–R1225, 1996.

6. **Davisson, R. L., A. K. Johnson, and S. J. Lewis.** Nitrosyl factors mediate active neurogenic hindquarter vasodilation in the conscious rat. *Hypertension* 23: 962–966, 1994.
7. **Davisson, R. L., O. S. Possas, R. A. Shaffer, S. P. Murphy, and S. J. Lewis.** Neurogenically derived nitrosyl factors mediate sympathetic vasodilation in the hindlimb vasculature of the rat. *Am. J. Physiol.* 272 (*Heart Circ. Physiol.* 41): H2369–H2376, 1997.
8. **Davisson, R. L., R. A. Shaffer, A. K. Johnson, and S. J. Lewis.** Stimulation of lumbar sympathetic nerves may produce hindlimb vasodilation via the release of pre-formed stores of nitrosyl factors. *Neuroscience* 72: 881–887, 1996.
9. **Davisson, R. L., R. A. Shaffer, A. K. Johnson, and S. J. Lewis.** Use-dependent loss of active sympathetic neurogenic vasodilation after nitric oxide synthase inhibition in conscious rats. Evidence for the presence of preformed stores of nitric oxide-containing factors. *Hypertension* 28: 347–353, 1996.
10. **Di Paola, E. D., M. J. Vidal, and G. Nisticò.** L-Glutamate evokes the release of an endothelium-derived relaxing factor-like substance from the rat nucleus tractus solitarius. *J. Cardiovasc. Pharmacol.* 17, *Suppl.* 3: S269–S272, 1991.
11. **Gomori, G.** Histochemical staining methods. In: *Methods in Medical Research*, edited by M. B. Visscher. Chicago, IL: Year Book, 1951.
12. **Haywood, J. R., R. A. Shaffer, C. Fastenow, G. D. Fink, and M. J. Brody.** Regional blood flow measurement with pulsed Doppler flowmeter in conscious rat. *Am. J. Physiol.* 241 (*Heart Circ. Physiol.* 10): H273–H278, 1981.
13. **Hope, B. T., G. J. Michael, K. M. Knigge, and S. R. Vincent.** Neuronal NADPH diaphorase is a nitric oxide synthase. *Proc. Natl. Acad. Sci. USA* 88: 2811–2814, 1991.
14. **Ignarro, L. J.** Nitric oxide: a novel signal transduction mechanism for transcellular communication. *Hypertension* 16: 477–483, 1990.
15. **Kubaszewski, E., A. Peters, S. McClain, D. Bohr, and T. Malinski.** Light activated release of nitric oxide from vascular smooth muscle of normotensive and hypertensive rats. *Biochem. Biophys. Res. Commun.* 200: 213–218, 1994.
16. **Lacolley, P. J., S. J. Lewis, and M. J. Brody.** Role of sympathetic nerve activity in the generation of vascular nitric oxide in urethane-anesthetized rats. *Hypertension* 17: 881–887, 1991.
17. **Ma, S., F. M. Abboud, and R. B. Felder.** Effects of L-arginine-derived nitric oxide synthesis on neuronal activity in nucleus tractus solitarius. *Am. J. Physiol.* 268 (*Regulatory Integrative Comp. Physiol.* 37): R487–R491, 1995.
18. **Machado, B. H., and L. G. H. Bonagamba.** Microinjection of L-glutamate into the nucleus tractus solitarii increases arterial pressure in conscious rats. *Brain Res.* 576: 131–138, 1992.
19. **Matsunga, K., and R. F. Furchgott.** Responses of rabbit aorta to nitric oxide and superoxide generated by ultraviolet irradiation of solutions containing inorganic nitrite. *J. Pharmacol. Exp. Ther.* 259: 1140–1146, 1991.
20. **Mauad, H., E. Colombari, L. G. H. Bonagamba, and B. H. Machado.** Glycine blocks the pressor response to L-glutamate microinjected into the nucleus tractus solitarii of conscious rats. *Braz. J. Med. Biol. Res.* 28: 699–704, 1995.
21. **Moncada, S., R. M. J. Palmer, and E. A. Higgs.** Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.* 43: 109–142, 1991.
22. **Moncada, S., D. D. Rees, R. Schulz, and R. M. J. Palmer.** Development and mechanism of a specific supersensitivity to nitrovasodilators after inhibition of vascular nitric oxide synthesis in vivo. *Proc. Natl. Acad. Sci. USA* 88: 2166–2170, 1991.
23. **Myers, P. R., R. L. Minor, Jr., R. Guerra, Jr., J. N. Bates, and D. G. Harrison.** Vasorelaxant properties of the endothelium-derived relaxing factor more closely resemble *S*-nitrosocysteine than nitric oxide. *Nature* 345: 161–163, 1990.
24. **Paxinos, G., and C. Watson.** *The Rat Brain in Stereotaxic Coordinates*. Sydney, Australia: Academic, 1986.
25. **Possas, O. S., and S. J. Lewis.** NO-containing factors mediate hindlimb vasodilation produced by superior laryngeal nerve stimulation. *Am. J. Physiol.* 273 (*Heart Circ. Physiol.* 42): H234–H243, 1997.
26. **Stamler, J. S., D. J. Singel, and J. Loscalzo.** Biochemistry of nitric oxide and its redox-activated forms. *Science* 258: 1898–1902, 1992.
27. **Talman, W. T., M. H. Perrone, and D. J. Reis.** Evidence for L-glutamate as the neurotransmitter of baroreceptor afferent nerve fibers. *Science* 209: 813–815, 1980.
28. **Travis, M. D., E. J. Whalen, and S. J. Lewis.** Heterologous desensitization of β-adrenoceptor signal transduction in vivo. *Eur. J. Pharmacol.* 328: R1–R3, 1997.
29. **Vedernikov, Y. P., P. I. Mordvintcev, I. V. Malenkova, and A. F. Vanin.** Similarity between the vasorelaxing activity of dinitrosyl iron cysteine complexes and endothelium-derived relaxing factor. *Eur. J. Pharmacol.* 211: 311–317, 1992.
30. **Venturini, C. M., R. M. J. Palmer, and S. Moncada.** Vascular smooth muscle contains a depletable store of a vasodilator which is light activated and restored by donors of nitric oxide. *J. Pharmacol. Exp. Ther.* 266: 1497–1500, 1993.
31. **Wallenstein, S., C. L. Zucker, and J. L. Fleiss.** Some statistical methods useful in circulation research. *Circ. Res.* 47: 1–9, 1980.
32. **Winer, B. J.** *Statistical Principles of Experimental Design*. New York: McGraw-Hill, 1971, p. 752–809.
33. **Yin, M., C. C. Lee, H. Ohta, and W. T. Talman.** Hemodynamic effects elicited by stimulation of the nucleus tractus solitarii. *Hypertension* 23, *Suppl.* 1: I73–I77, 1994.