Am J Physiol Heart Circ Physiol
280: H2371–H2379, 2001.

NO formation in nucleus tractus solitarii attenuates pressor response evoked by skeletal muscle afferents

JIANHUA LI[1] AND JEFFREY T. POTTS[2]
Harry S. Moss Heart Center and [1]Department of Internal Medicine and [2]Department of Physiology, University of Texas Southwestern Medical Center, Dallas, Texas 75390-9174

Received 23 August 2000; accepted in final form 8 January 2001

Li, Jianhua, and Jeffrey T. Potts. NO formation in nucleus tractus solitarii attenuates pressor response evoked by skeletal muscle afferents. *Am J Physiol Heart Circ Physiol* 280: H2371–H2379, 2001.—We have previously shown that static muscle contraction induces the expression of c-Fos protein in neurons of the nucleus tractus solitarii (NTS) and that some of these cells were codistributed with neuronal NADPH-diaphorase [nitric oxide (NO) synthase]-positive fibers. In the present study, we sought to determine the role of NO in the NTS in mediating the cardiovascular responses elicited by skeletal muscle afferent fibers. Static contraction of the triceps surae muscle was induced by electrical stimulation of the L7 and S1 ventral roots in anesthetized cats. Muscle contraction during microdialysis of artificial extracellular fluid increased mean arterial pressure (MAP) and heart rate (HR) 51 ± 9 mmHg and 18 ± 3 beats/min, respectively. Microdialysis of L-arginine (10 mM) into the NTS to locally increase NO formation attenuated the increases in MAP (30 ± 7 mmHg, $P < 0.05$) and HR (14 ± 2 beats/min, $P > 0.05$) during contraction. Microdialysis of D-arginine (10 mM) did not alter the cardiovascular responses evoked by muscle contraction. Microdialysis of N^G-nitro-L-arginine methyl ester (2 mM) during contraction attenuated the effects of L-arginine on the reflex cardiovascular responses. These findings demonstrate that an increase in NO formation in the NTS attenuates the pressor response to static muscle contraction, indicating that the NO system plays a role in mediating the cardiovascular responses to static muscle contraction in the NTS.

cardiovascular responses; static muscle contraction; blood pressure; heart rate; microdialysis; L-arginine; L-NAME

STUDIES HAVE DEMONSTRATED that nitric oxide (NO) synthase (NOS) is localized in discrete medullary areas (41) involved in cardiovascular regulation (8, 9, 21, 22), including the nucleus tractus solitarii (NTS). These findings indicate a potential role of NO as a neural signal transduction system within these cardiovascular-related medullary regions. Furthermore, it has been reported that NO in the NTS plays a role in the central regulation of cardiovascular function. For example, microinjection of *S*-nitrosocysteine (a NO donor) into the NTS has been reported to elicit a decrease in arterial blood pressure (ABP) in anesthetized rats (23). Microinjection of N^G-monomethyl-L-arginine (L-NMMA; an inhibitor of NOS) into the NTS has been reported to increase ABP and renal sympathetic nerve activity in anesthetized rabbits and rats (13, 40).

The evidence that the muscle afferents terminate in several laminae of the spinal cord as well as ascending to terminate in the NTS has been shown by neuroanatomic tracing studies (7, 20, 29, 30, 33) using injections of horseradish peroxidase into the triceps surae of cats. Furthermore, it has been shown that c-Fos expression in the NTS was induced in treadmill running rats and in anesthetized cats with static contraction of skeletal muscle (17, 24). Moreover, c-Fos-positive neurons were also found in the NTS after barodenervation (26). This suggests that neurons in the NTS are activated by skeletal muscle afferents involved in expression of the pressor response during muscle contraction. The distribution of c-Fos-labeled cells containing NOS has also been evaluated using double-labeling methods. We found that NADPH-diaphorase (NADPH-d)-positive fibers and c-Fos-labeled cells were codistributed in the NTS (25). Therefore, the purpose of the present study was to determine the functional role of NO in the NTS on the cardiovascular responses induced by the activation of skeletal muscle afferents during static contraction. Microdialysis of L-arginine and N^G-nitro-L-arginine methyl ester (L-NAME) into the NTS were used to locally alter the levels of NO.

MATERIALS AND METHODS

General Surgical Preparation

Experiments were performed on 25 anesthetized cats of either sex weighing 3.4–5.5 kg. The animals were anesthetized by inhalation of a halothane-oxygen mixture (2–3%). An endotracheal tube was inserted into the trachea via a tracheotomy to maintain an open airway, and the jugular vein and carotid artery were catheterized for drug administration and measurement of ABP, respectively. Anesthesia was then maintained with a mixture of α-chloralose (80 mg/kg) and urethane (200 mg/kg) injected intravenously. Throughout the experiment, supplemental α-chloralose (15 mg/kg iv) was given if the cats exhibited a corneal reflex or if they withdrew a limb in response to a noxious stimulus.

Address for reprint requests and other correspondence: J. Li, Moss Heart Center and Dept. of Internal Medicine, Univ. of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9174 (E-mail: jianhua.li@UTSouthwestern.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Arterial blood gases and pH were periodically checked (Radiometer, ABL-3; Copenhagen, Denmark) and maintained within normal limits (pH: 7.30–7.40; P_{CO_2}: 32–36 mmHg; P_{O_2}: >80 mmHg) by adjusting the ventilator (model 661, Harvard Apparatus; South Natick, MA) or by injecting a 1 M solution of sodium bicarbonate intravenously. Body temperature was continuously monitored with a rectal probe and maintained between 37.0–38.5°C by a water-perfused heating pad and an external heat lamp.

A laminectomy was performed, exposing the lower lumbar and upper sacral portions of the spinal cord. The dura was opened, and the L7 and S1 spinal ventral roots were carefully separated and cut close to the spinal cord. The peripheral ends of the transected L7 and S1 ventral roots were then placed on platinum bipolar stimulating electrodes, and the exposed spinal cord region was immersed in a pool of warm mineral oil (37°C).

The cat's head was fixed on a stereotaxic apparatus (Kopf Instruments; Tujunga, CA), and a limited craniotomy was performed to expose the dorsal surface of the medulla. The dorsal surface of the medulla ~3–4 mm rostral to the obex was then exposed by carefully reflecting the cerebellum rostrally. Gelfoam was used to minimize any bleeding during this procedure, and warmed (37°C) mineral oil was applied to dorsal surface of the medulla.

The calcaneal bone of hindlimb was cut, allowing the Achilles tendon to be connected to a force transducer for measurement of developed tension during electrically stimulated muscle contraction. The pelvis was stabilized in a spinal unit (Kopf Instruments), and the knee joint secured by attaching the patellar tendon to a steel post.

ABP was measured from the common carotid artery by a pressure transducer (model P23ID, Statham; Oxnard, CA). Mean arterial pressure (MAP) was obtained by integrating the arterial signal with a time constant of 4 s. Heart rate (HR) was derived from the arterial pressure pulse by a Biotach (Gould Instruments; Cleveland, OH). The developed tension during electrically stimulated muscle contraction was measured using the force transducer (FT10, Grass Instruments) clamped to the cut end of the Achilles tendon. All measured variables were continuously recorded on an eight-channel chart recorder (model 2800s, Gould Instruments).

Experimental Protocols

The cats were allowed to stabilize for at least 40 min after surgery. The microdialysis probe (1-mm membrane and 0.5-mm diameter, CMA-10, Bioanalytical System; West Lafayette, IN) was stereotaxically lowered to reach the NTS according to Berman's Atlas (5) ipsilateral to the contracted muscle using stereotaxic carrier (Kopf Instruments) (1.5 mm lateral to midline, 1.0 mm rostral to the obex, and 1.5 mm below the dorsal medullary surface). Two probes were lowered in the NTS for bilateral dialysis of L-NAME in three cats. The probes were continuously perfused at a rate of 5 μl/min with an artificial extracellular fluid (ECF; pH 7.4), which was made fresh for each experiment. The ECF contained 0.2% bovine serum albumin, 0.1% bacitracin, and the following ions (in mM): 6.2 K^+, 134 Cl^-, 2.4 Ca^{2+}, 150 Na^+, 1.3 P−, 13 HCO_3^-, and 1.3 Mg^{2+}.

After the microdialysis probe was inserted, 1 h was allowed for stabilization of the preparation. During this period, ECF was continuously dialyzed. ABP and HR responses to a static muscle contraction of the right triceps surae muscle were recorded. The muscle contraction was induced by simultaneous electrical stimulation (three times motor threshold; 0.1 ms duration; 40 Hz) of the peripheral ends of the L7 and S1 ventral roots for 1 min to obtain control cardiovascular responses. At least two reproducible cardiovascular responses to static muscle contraction were recorded at 20-min intervals.

Microdialysis of L-arginine. GROUP 1: DOSE-RESPONSE EXPERIMENT FOR L-ARGININE. See Table 1 for protocol summary. This study was performed in four cats. First, the control pressor response to muscle contraction was determined during dialysis of ECF. Increasing concentrations of L-arginine (1–100 mM; Sigma) were then dialyzed. Each concentration was dialyzed for 40 min, followed by muscle contraction as previously described. Finally, ECF was dialyzed after discontinuing L-arginine to determine the recovery of the pressor response. On the basis of the results of the dose-response experiments, 10 mM L-arginine was used in the subsequent experiments.

GROUP 2: TIME-COURSE EXPERIMENT FOR L-ARGININE. See Table 1 for protocol summary. In eight cats, the control cardiovas-

Table 1. *Summary of experimental protocols*

Group 1: dose response of L-Arg (n = 4)							
Time, min	0–60	60–80	80–120	120–160	160–200	200–290	
Dialysis	ECF	ECF	L-Arg (1 mM)	L-Arg (10 mM)	L-Arg (100 mM)	ECF	
Contraction	1	2	3	4	5	6	
Group 2: time course response of 10 mM L-Arg (n = 8)							
Time, min	0–60	60–80	80–95	95–120	120–210		
Dialysis	ECF	ECF	L-Arg (10 mM)	L-Arg (10 mM)	ECF		
Contraction	1	2	3	4	5		
Group 3: dose response of L-NAME (n = 7)							
Time, min	0–60	60–80	80–120	120–160	160–200	200–240	240–330
Dialysis	ECF	ECF	L-NAME (0.2 mM)	L-NAME (2 mM)	L-NAME (20 mM; uni)	L-NAME (20 mM; bi)	ECF
Contraction	1	2	3	4	5	6	7
Group 4: dialysis of D-Arg, L-NAME, and L-Arg (n = 6)							
Time, min	0–60	60–80	80–120	120–160	160–190	190–230	230–290
Dialysis	ECF	ECF	D-Arg (10 mM)	L-Arg (10 mM)	L-NAME (2 mM)	L-Arg (10 mM)	ECF
Contraction	1	2	3	4	5	6	7

Each muscle contraction was performed at the end of each time period. L-Arg and D-Arg, L-arginine and D-arginine, respectively; ECF, extracellular fluid; L-NAME, N^G-nitro-L-arginine methyl ester.

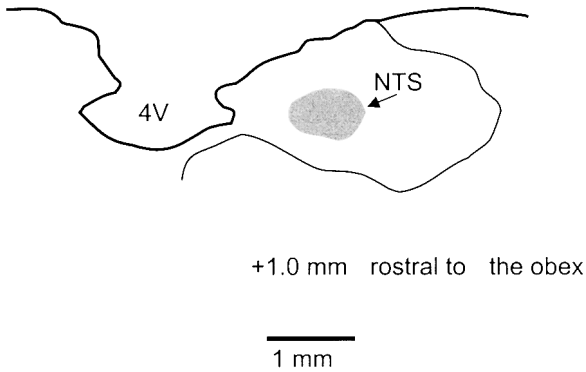

Fig. 1. A representative example of the location of the microdialysis probe in the nucleus tractus solitarii (NTS) from one animal. The probe was placed 1.5 mm lateral, 1.0 mm rostral to the obex, and 1.5 mm below the dorsal medullary surface. Shaded area, region stained by perfusing the probe with 2% Evans blue dye. 4V, fourth ventricle.

cular responses to muscle contraction were determined during ECF dialyzing into the NTS; 10 mM L-arginine was then dialyzed. Fifteen and forty minutes after the dialysis of L-arginine, muscle contraction was repeated as previously described. Ninety minutes after the dialysis of L-arginine, muscle contraction was repeated to check that the cardiovascular responses returned to their control levels.

Microdialysis of L-NAME. GROUP 3: DOSE-RESPONSE EXPERIMENT FOR L-NAME DIALYSIS. See Table 1 for protocol summary. In another group of animals (n = 7), L-NAME (Sigma) of increasing concentrations (0.2–20 mM) was dialyzed into the NTS after determining control responses during ECF dialyzing. Each concentration of L-NAME was dialyzed for 40 min. At the end of the 40-min dialyzing period for each concentration, muscle contraction was repeated as previously described. Bilateral dialysis of 20 mM L-NAME into the NTS was performed in three of these cats. ECF was dialyzed after discontinuing L-NAME to determine recovery of the cardiovascular responses to muscle contraction.

Microdialysis of D-arginine and microdialysis of L-NAME followed by L-arginine. GROUP 4: DIALYSIS OF D-ARGININE, L-NAME, AND L-ARGININE. See Table 1 for protocol summary. ECF was dialyzed into the NTS for 40 min to determine control cardiovascular responses to muscle contraction. D-Arginine (10 mM, Sigma) was dialyzed into the NTS before dialyzing L-arginine in five of six cats (see Table 1). Forty minutes after D-arginine dialyzing, muscle contraction was repeated as previously described.

In six cats, L-arginine (10 mM) was then dialyzed into the NTS for 40 min, and muscle contraction was repeated. L-NAME (2 mM) was then dialyzed for 30 min (muscle contractions were also performed after dialyzing L-NAME), and 10 mM L-arginine was dialyzed into the NTS for 40 min. Muscle contraction was performed as previously described at the end of the 40-min L-arginine dialyzing period. Finally, ECF was dialyzed to determine the recovery of the cardiovascular responses to muscle contraction.

Histology

At the end of each experiment, the brain stem was removed and fixed in a solution of 10% phosphate-buffered formalin and then stored at 4°C. After the tissue was adequately fixed, the medulla was blocked and subsequently sliced into 40-μm sections on a cryostat (model 2800 Frigocut-E, Cambridge Instruments). The tracks in the NTS produced by the dialysis probe were determined. In five cats, Evans blue was dialyzed into the NTS for 40 min, and the rostrocaudal extent of dye diffusion was measured using a light microscope. Histological examination showed that the dialysis probes entered 1.5 mm into the dorsal surface of the medulla. The staining spread was 0.8–1.2 mm in the NTS at rostrocaudal extent for a 40-min dialyzing period. The blue dye was not observed outside of the NTS in the examined tissues. A representative example of the location of the microdialysis probe is shown in Fig. 1.

Statistical Analysis

A one-way repeated measure ANOVA was used for statistical comparison of changes in MAP, HR, and muscle tension across time and doses, and a Student-Newman-Keul's post hoc analysis was used to determine differences between

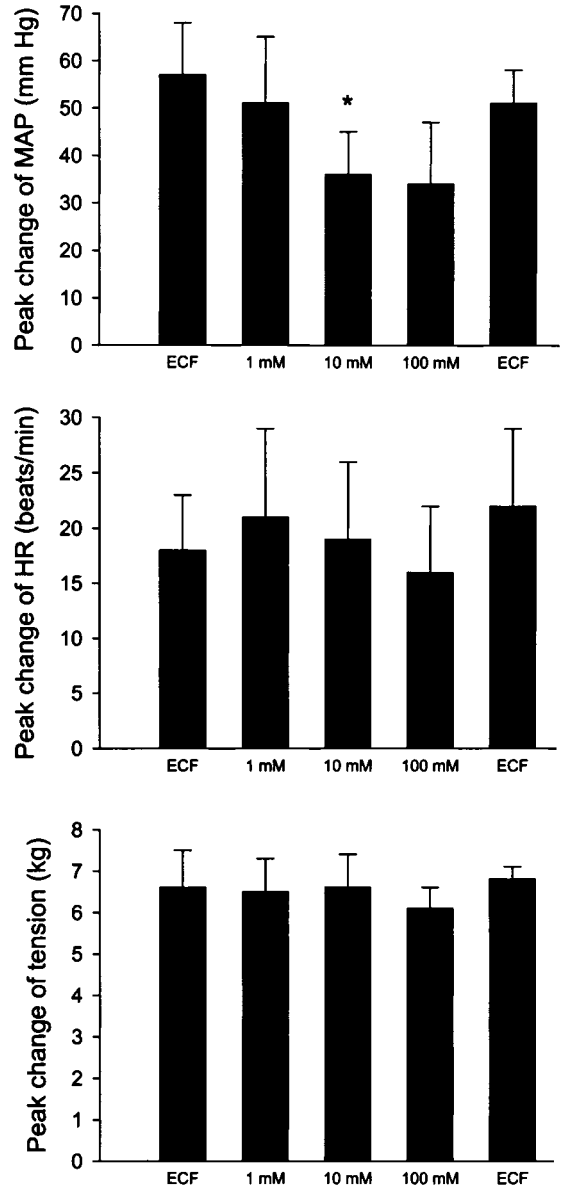

Fig. 2. Effects of microdialysis of 1–100 mM L-arginine into the NTS on the peak changes of mean arterial pressure (MAP) and heart rate (HR) during static muscle contraction. Each concentration was dialyzed for 40 min. Values are means ± SE; n = 4 animals. *$P < 0.05$, significant difference vs. extracellular fluid (ECF) control (before dialysis of 1 mM L-arginine) and ECF recovery.

Table 2. *Effect of 10 mM L-Arg dialyzed in the NTS on baseline MAP and HR and their reflex responses evoked by static muscle contraction*

	Dialysis of ECF (Control)	Dialysis of L-Arg		Dialysis of ECF (Recovery)
		15 min	40 min	
		MAP, mmHg		
Control	102 ± 6	96 ± 6	92 ± 4	98 ± 4
Peak	153 ± 10*	131 ± 12*	122 ± 8*	150 ± 12*
		HR, beats/min		
Control	197 ± 8	186 ± 6	180 ± 6	186 ± 4
Peak	215 ± 8*	200 ± 5*	194 ± 5*	207 ± 4*
		Tension, kg		
Control	0.8	0.8	0.8	0.8
Peak	8.1 ± 0.6*	7.7 ± 0.6*	8.6 ± 0.6*	8.5 ± 0.6*

Values are means ± SE; $n = 8$ animals. Peak tension is the difference between the absolute maximum and resting tension. NTS, nucleus tractus solitarii; MAP, mean arterial pressure; HR, heart rate. *Significant increase above control of baseline ($P < 0.05$).

groups. All values are expressed as means ± SE. For all analysis, differences were considered significant if $P < 0.05$.

RESULTS

Microdialysis of L-Arginine

Dose-response experiments for L-arginine (n = 4). The artificial ECF was dialyzed continuously into the NTS to obtain the control MAP response to muscle contraction before 1 mM L-arginine. A concentration of 1–100 mM L-arginine was then microdialyzed. Basal MAP before each of the induced muscle contractions was 95 ± 6 mmHg (during ECF for control), 94 ± 4 mmHg (during 1 mM L-arginine), 90 ± 5 mmHg (during 10 mM L-arginine), 91 ± 7 mmHg (during 100 mM L-arginine), and 91 ± 5 mmHg (during ECF for recovery), respectively ($P > 0.05$), whereas the basal HR was 190 ± 8, 186 ± 6, 186 ± 8, 188 ± 10, 184 ± 10 beats/min, respectively ($P > 0.05$). The cardiovascular responses induced by muscle contractions during the dialysis are shown in Fig. 2. Therefore, 10 mM L-arginine was chosen for subsequent experiments.

Time-course experiments for L-arginine. The resting MAP and HR were not significantly altered before and after microdialysis of 10 mM L-arginine into the NTS (Table 2). The changes in MAP and HR in response to static muscle contraction before 10 mM L-arginine, 15 and 40 min after L-arginine, and during recovery are shown in Fig. 3. After dialysis of 10 mM L-arginine, the pressor response during static muscle contraction was attenuated at 15 min (peak change of MAP: 35 ± 7 mmHg). Compared with the control value of 51 ± 9 mmHg, a significant difference was found 40 min after L-arginine dialyzing (30 ± 7 mmHg). Ninety minutes after 10 mM L-arginine dialysis was discontinued, the pressor response to static muscle contraction returned to the control level (52 ± 11 vs. 51 ± 9 mmHg, $P > 0.05$). No significant difference in the HR response was observed before microdialysis of 10 mM L-arginine (18 ± 3 beats/min), 15 min (14 ± 2 beats/min) and 40 min (14 ± 2 beats/min) after dialyzing, and during the 90- to 120-min recovery period (21 ± 2 beats/min, $P > 0.05$). Thus microdialysis of 10 mM L-arginine into the NTS for 40 min significantly attenuated the peak change of MAP during static muscle contraction. This effect was recovered after 90–120 min.

Microdialysis of L-NAME

In dose-response experiments with L-NAME (0.2–20 mM, $n = 7$), resting MAP and HR were not signifi-

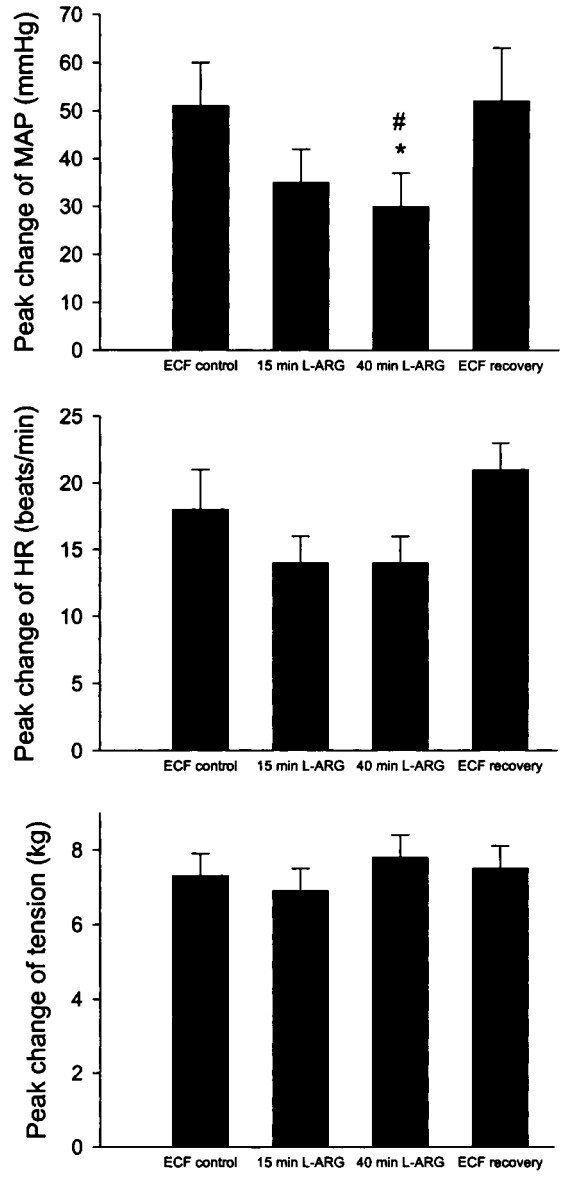

Fig. 3. Peak changes in MAP and HR in response to static contraction of the triceps surae muscle during dialysis of ECF before 10 mM L-arginine (L-Arg; control), 15 and 40 min after microdialysis of 10 mM L-Arg in the NTS, and during recovery (during dialysis of ECF). MAP and HR responses of control to muscle contraction were obtained during ECF dialyzing into the NTS before dialysis of 10 mM L-Arg. MAP and HR responses of recovery to muscle contraction were obtained during the 90-min ECF dialyzing period after discontinuing dialysis of 10 mM L-arginine. Values are means ± SE; $n = 8$ animals. *$P < 0.05$, significant difference vs. before microdialysis of 10 mM L-Arg (control); #$P < 0.05$, significant difference vs. recovery.

Table 3. *Effect of L-NAME dialyzed into the NTS on baseline MAP and HR and their reflex responses evoked by static muscle contraction*

	Dialysis of ECF (Control)	Dialysis of L-NAME				Dialysis of ECF (Recovery)
		0.2 mM (Uni)	2 mM (Uni)	20 mM (Uni)	20 mM (Bi)	
			MAP, mmHg			
Control	91 ± 4	92 ± 2	88 ± 6	89 ± 5	84 ± 4	86 ± 6
Peak	143 ± 8*	145 ± 11*	143 ± 13*	150 ± 18*	163 ± 15*†	150 ± 15*
			HR, beats/min			
Control	176 ± 6	178 ± 6	178 ± 5	183 ± 6	187 ± 4	187 ± 6
Peak	194 ± 10*	194 ± 10*	196 ± 7*	200 ± 8*	210 ± 8*	207 ± 10*
			Tension, kg			
Control	0.8	0.8	0.8	0.8	0.8	0.8
Peak	3.8 ± 0.4*	4.1 ± 0.6*	4.4 ± 0.6*	3.9 ± 0.5*	3.5 ± 0.4*	3.5 ± 0.8*

Values are means ± SE; $n = 7$ animals, of which 3 animals received bilateral (bi) dialysis of 20 mM L-NAME. Peak tension is the difference between the absolute maximum and resting tension. *Significant increase above control of baseline ($P < 0.05$); †significant increase compared with ECF dialyzing before dialysis of L-NAME.

cantly altered (Table 3). Control MAP and HR responses to muscle contraction were 52 ± 9 mmHg and 18 ± 4 beats/min, respectively. There were no significant differences in the changes in MAP and HR to static muscle contraction after 40 min of unilateral dialysis of L-NAME at a concentration of 0.2 mM (53 ± 9 mmHg and 16 ± 3 beats/min), 2 mM (55 ± 7 mmHg and 18 ± 3 beats/min), and 20 mM (61 ± 9 mmHg and 17 ± 3 beats/min). However, bilateral dialysis of 20 mM L-NAME into the NTS ($n = 3$) significantly increased the MAP response evoked by muscle contraction (79 ± 19 vs. 57 ± 15 mmHg). There was no significant difference in the change in HR to muscle contraction before (20 ± 5 beats/min) and after (23 ± 6 beats/min) bilateral dialysis of 20 mM L-NAME. Ninety minutes after dialysis of 20 mM L-NAME was discontinued, MAP and HR responses to contraction were 64 ± 22 mmHg and 20 ± 6 beats/min, respectively. The results are shown in Fig. 4.

Microdialysis of D-Arginine and Microdialysis of L-NAME Followed by L-Arginine

In five of six cats, 10 mM D-arginine, the inactive stereoisomer, was microdialyzed into the NTS for 40 min. The peak increases in MAP and HR evoked by contraction were 57 ± 14 mmHg and 18 ± 5 beats/min, respectively. D-Arginine had no significant effect on the pressor and HR responses to static muscle contraction compared with the control group (during dialysis of ECF) before D-arginine dialyzing (52 ± 11 mmHg and 17 ± 4 beats/min, respectively) (see Fig. 5).

In six cats, the contraction during dialysis of ECF increased MAP and HR by 54 ± 11 mmHg and 20 ± 4 beats/min at peak, respectively, whereas the peak increases in MAP and HR in response to static contraction 30 min after L-NAME were 56 ± 6 mmHg and 19 ± 5 beats/min, respectively. There were no significant differences in the increase in MAP and HR to static muscle contraction between dialysis of ECF and L-NAME. However, attenuation of the pressor response by 10 mM L-arginine was prevented after dialysis of L-NAME (peak change of MAP: 55 ± 10 mmHg) compared with when 10 mM L-arginine was microdialyzed in the NTS (39 ± 10 mmHg) (Fig. 5). Figure 5 also shows that there were no significant differences in the changes of MAP and HR between control (54 ± 11 mmHg and 20 ± 4 beats/min) and L-NAME followed by L-arginine (55 ± 10 mmHg and 15 ± 3 beats/min). In these experiments, the resting MAP and HR were not altered significantly after dialysis of L-arginine, L-NAME, and L-NAME followed by L-arginine (Table 4).

DISCUSSION

In the present study, microdialysis of L-arginine into the NTS significantly attenuated the pressor response to static muscle contraction, whereas dialysis of L-NAME significantly reduced the effects of L-arginine. Dialyzing the same concentration of D-arginine into the NTS did not alter the pressor response to muscle contraction. These results show that increasing NO synthesis in the NTS attenuates the pressor response evoked by static muscle contraction. Furthermore, bilateral dialysis of 20 mM L-NAME into the NTS potentiated the pressor response to static muscle contraction. Thus these data suggest that the NO system in the NTS modulates the cardiovascular responses to activation of skeletal muscle afferents during static muscle contraction.

The NTS has been considered a terminating site for afferent fibers from arterial baroreceptors, chemoreceptors, cardiopulmonary receptors, and other visceral receptors involved in the integration of cardiovascular responses (2, 8, 21, 22). Previous studies using a horseradish peroxidase tracing technique have demonstrated that afferent fibers from skeletal muscle have direct monosynaptic connections to the NTS, as well as forming second-order neuronal projections from laminae I and laminae V to the NTS (20, 29, 33). Static contraction of the triceps surae muscle produced a

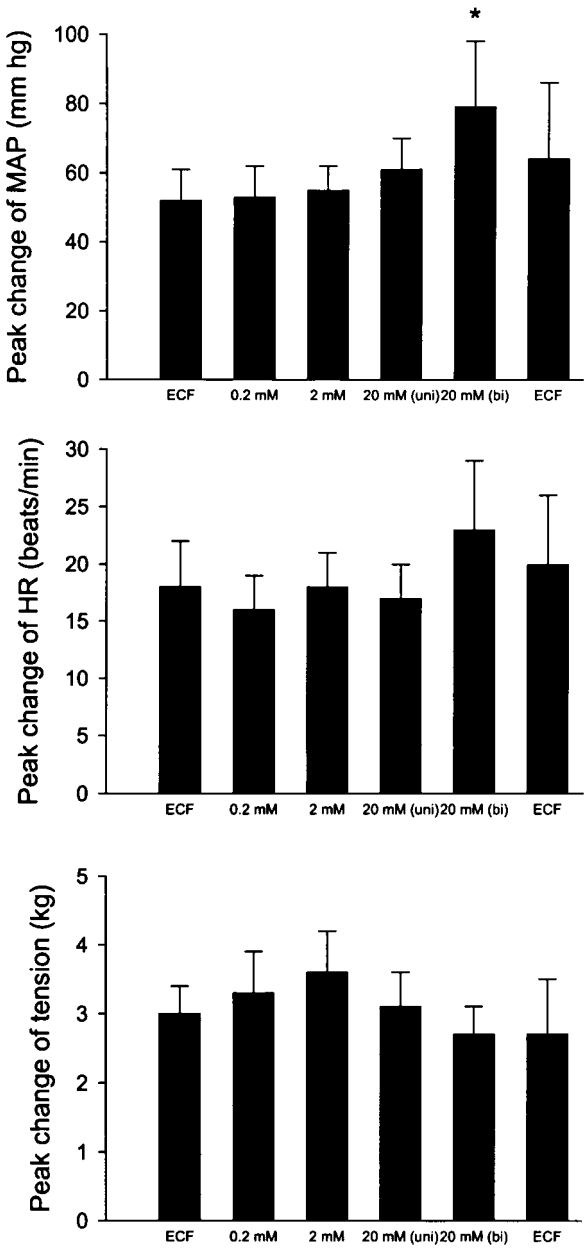

Fig. 4. Peak changes in MAP and HR in response to static muscle contraction after microdialysis of ECF (control), 0.2, 2, and 20 mM (unilateral, uni) N^G-nitro-L-arginine methyl ester (L-NAME), and ECF (recovery). ECF and each dose of L-NAME were dialyzed for 40 min. At the end of the 40-min dialyzing period, muscle contractions were induced. Values are means ± SE; $n = 7$ animals. *$P < 0.05$, significant difference vs. ECF dialyzing. Three of seven animals received bilateral (bi) dialysis of 20 mM L-NAME.

marked increase in arterial pressure and HR (31, 32). These afferent projecting fibers from skeletal muscle may contribute to the cardiopulmonary adjustments during muscular exercise (20). An electrophysiological study (39) has shown that electrical stimulation of the tibial nerve in the hindlimb evoked excitatory neuronal responses in the neurons of the NTS. It has also been reported that NTS neurons alter their discharge rates during static contraction of skeletal muscle (35). Moreover, c-Fos-containing neurons also were found after static muscle contraction in baroreceptor-intact and barodenervated animals (24, 26). These data suggest that neurons in the NTS are activated by inputs from skeletal muscle receptors independent of afferent inputs from arterial baroreceptors.

The distribution of NADPH-d staining has been reported to be characteristic of the distribution of NOS (15). It has been reported that neuronal cells and fibers containing NADPH-d were present in the NTS (41) in the same regions where L-arginine or L-NAME was dialyzed in present experiments. Furthermore, c-Fos-

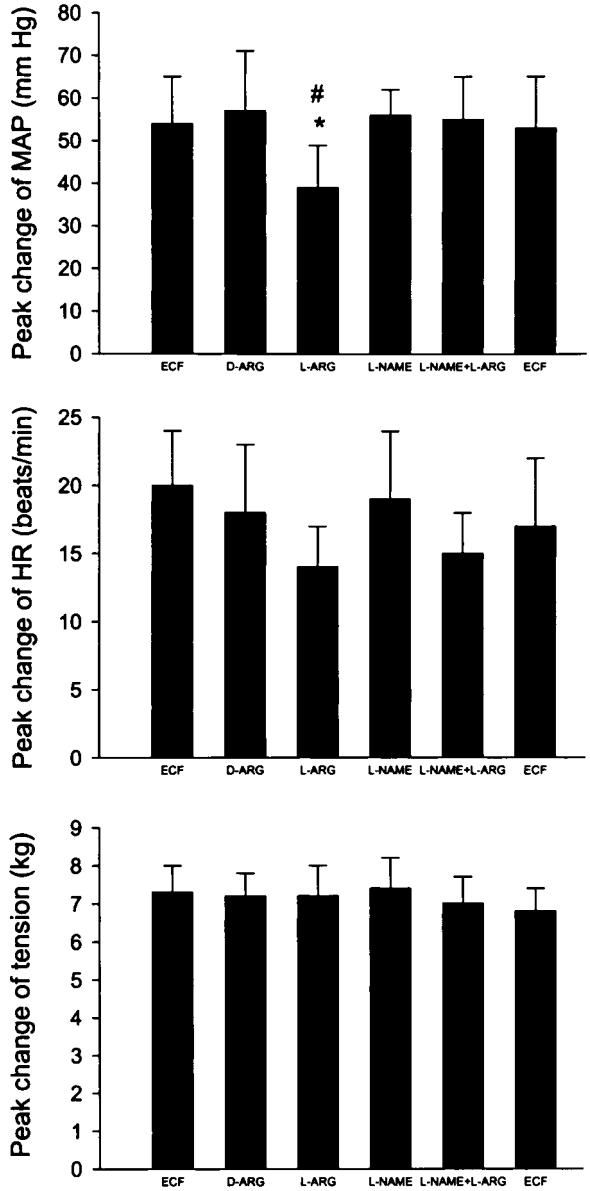

Fig. 5. Peak changes in MAP and HR in response to static muscle contraction after dialysis of ECF (control), 10 mM D-arginine (D-Arg), 10 mM L-Arg, 2 mM L-NAME, 10 mM L-Arg with predialysis of 2 mM L-NAME, and ECF (recovery) in the NTS. The cardiovascular responses to muscle contraction were obtained at the end of each dialysis period. Values are means ± SE; $n = 6$ animals. *$P < 0.05$, significant difference vs. before microdialysis of 10 mM L-Arg (control, ECF dialyzing); #$P < 0.05$, significant difference vs. L-Arg dialyzing with predialyzing 2 mM L-NAME.

Table 4. *Effect of 10 mM L-Arg dialyzed into the NTS on baseline MAP and HR and reflex responses evoked by static muscle contraction after dialysis of 2 mM L-NAME*

Dialysis	ECF	L-Arginine	L-NAME	L-NAME + L-Arginine	ECF
			MAP, mmHg		
Control	96 ± 4	95 ± 7	95 ± 5	90 ± 6	92 ± 8
Peak	150 ± 13*	134 ± 15*	151 ± 10*	145 ± 12*	145 ± 15*
			HR, beats/min		
Control	197 ± 10	190 ± 12	188 ± 9	182 ± 10	190 ± 14
Peak	217 ± 9*	204 ± 14*	207 ± 10*	197 ± 9*	207 ± 10*
			Tension, kg		
Control	0.8	0.8	0.8	0.8	0.8
Peak	8.1 ± 0.7*	8.0 ± 0.8*	8.2 ± 0.8*	7.8 ± 0.7*	7.6 ± 0.6*

Values are means ± SE; $n = 6$ animals. Peak tension is the difference between the absolute maximum and resting tension. *Significant increase above control of baseline ($P < 0.05$).

labeled cells in the NTS were shown to receive close appositions from NADPH-d-positive fibers (25). These findings provide strong neuroanatomic evidence that NO-containing neurons in the NTS are involved in the expression of the cardiovascular responses evoked by skeletal muscle contraction.

NO is known to be a widespread transmitter in the central nervous system, and it has been shown that the NO system in the central nervous system may play a role in the regulation of cardiovascular responses (13, 14, 19, 27, 36, 40). We found that increasing NO formation by dialysis of L-arginine in the NTS attenuated the pressor response to muscle contraction in this study. The cardiovascular effects by administration of NO donors into the NTS or ventrolateral medulla (VLM) has been reported to be blocked by methylene blue (14, 23), suggesting the effects of NO result from the activation of soluble guanylate cyclase. At present, it is believed that NO activation of a cGMP-dependent mechanism via guanylate cyclase may facilitate the release of glutamate from presynaptic nerve terminals in response to neuronal activation (6, 11). Increased calcium influx then activates excitatory amino acid receptors on postsynaptic neurons. In this experiment, the increased formation of NO in the NTS may activate excitatory amino receptors and attenuate the pressor response evoked by muscle contraction by facilitating baroreceptor neurotransmission. Other studies (10, 11, 18, 42) have suggested the potential importance of such physiological interactions of NO with the excitatory amino acid glutamate. Recently, NO-containing artificial cerebrospinal fluid was dialyzed directly into the paraventricular nucleus of hypothalamus to cause a decrease in ABP and an increase in glutamate in this region (16). It was assumed that glutamate is the neurotransmitter released from presynaptic neurons to activate postsynaptic receptors in the NTS causing this cardiovascular modulation during muscle contraction. NO may facilitate the release of glutamate from presynaptic nerve terminals in response to neuronal activation (6, 11). Furthermore, c-Fos-labeled neurons that receive close appositions from NADPH-d-positive fibers within the NTS (25) suggest the possibility that presynaptic release of NO may alter the characteristics of the activated neurons during static muscle contraction. It has been reported that L-arginine-derived NO can affect the spontaneous discharge rate of the NTS neurons (28).

Because the baroreceptor reflex is also activated by the increasing of ABP during static muscle contraction, barosensitive cells as well as muscle afferent-sensitive cells in the NTS may be modulated by the NO system. It has been shown that sensory inputs from carotid sinus baroreceptors and mechanically and metabolically sensitive skeletal muscle receptors interact to inhibit sympathoexcitation (34). Furthermore, NO may modulate baroreceptor reflex (19, 27). Results from the present study show that increasing NO synthesis by microdialysis of L-arginine in the NTS attenuates the pressor response to static muscle contraction. Therefore, it is possible that the effect of NO on the cardiovascular responses evoked by muscle contraction may have been mediated through an alteration in the arterial baroreceptor reflex. This arrangement may contribute to the integration of the cardiovascular responses to static muscle contraction.

There are at least three possible mechanisms by which NO may inhibit the pressor response during static muscle contraction. First, in terms of the central pathways of the arterial baroreceptor reflex, NTS neurons receive primary baroreflex input and project to the caudal VLM (cVLM, glutamate projections) where they activate GABAergic neurons, which project to the rostral VLM (rVLM) to inhibit preganglionic sympathetic neurons in the intermediolateral columns of the spinal cord. This hypothesis has been well established by studies (37, 38). Thus one of possibilities is that activation of the neural pathways from the NTS to the cVLM evoked by baroreflex was increased by NO by dialysis of L-arginine in the NTS to attenuate the pressor response during muscle contraction. Second, in terms of the central pathways of muscle afferents during the exercise pressor reflex, it has not yet been clarified. In our previous work, we (26) demonstrated that the number of c-Fos-labeled cells evoked by muscle contraction in the NTS is reduced after barodener-

vation. However, the number of c-Fos-labeled neurons in the rVLM was not altered after barodenervation (26). Furthermore, the excited neurons evoked by static muscle contraction have been recorded in the rVLM, and these neurons were activated possibly by a direct projection from the spinal cord (3, 4). Therefore, we think that there exist independent neuronal pathways for skeletal muscle afferents to activate the rVLM to excite sympathetic neurons. Third, a previous study has shown that the NTS projects directly to the rVLM (12), and there exist excitatory connections from the NTS to rVLM (1). Thus sympathoexcitatory neurons projecting directly from the NTS to the rVLM may be one of those pathways activated by muscle afferents during muscle contraction. Increasing NO in the NTS may attenuate activity of sympathoexcitatory projections from the NTS to the rVLM to inhibit the pressor response to muscle contraction.

In conclusion, the increase of NO formation by microdialyis of L-arginine in the NTS significantly attenuated the pressor response to static muscle contraction, and this effect was reduced by L-NAME. Bilateral dialysis of L-NAME into the NTS to inhibit NO synthesis increased the pressor response to muscle contraction. It is postulated that characteristics of NTS neurons activated by arterial baroreceptors or/and skeletal muscle receptors during static muscle contraction may be modulated by the NO system, and this may contribute to the attenuated pressor response by contraction of skeletal muscle. Thus these findings suggest that the NO system in the NTS plays a role in mediating the cardiovascular responses to muscle afferent inputs during static muscle contraction.

The authors express gratitude to Dr. Jere H. Mitchell for generous support and encouragement in this study. The authors also express gratitude to James Jones, Julius Lamar, Jr., and Maggie Robeldo for expert technical assistance.

This study was supported by American Heart Association Texas Affiliate Grant 9960088Y (to J. Li).

REFERENCES

1. **Agarwal SK and Calaresu FR.** Reciprocal connections between nucleus tractus solitarii and rostral ventrolateral medulla. *Brain Res* 523: 305–308, 1990.
2. **Aicher SA, Kurucz OS, Reis DJ, and Milner TA.** Nucleus tractus solitarius efferent terminals synapse on neurons in the caudal ventrolateral medulla that project to the rostral ventrolateral medulla. *Brain Res* 693: 51–63, 1995.
3. **Bauer RM, Iwamoto GA, and Waldrop TG.** Discharge patterns of ventrolateral medullary neurons during muscular contraction. *Am J Physiol Regulatory Integrative Comp Physiol* 259: R606–R611, 1990.
4. **Bauer RM, Waldrop TG, Iwamoto GA, and Holzwarth MA.** Properties of ventrolateral mudullary neurons that respond to muscular contraction. *Brain Res Bull* 28: 167–178, 1992.
5. **Berman AL.** *The Brain Stem of the Cat.* Madison, WI: University of Wisconsin Press, 1968.
6. **Bredt DS and Snyder SH.** Nitric oxide, a novel neuronal messenger. *Neuron* 8: 3–11 1992.
7. **Craig AD and Mense S.** The distribution of afferent fibers from the gastrocnemius-soleus muscle in the dorsal horn of the cat, as revealed by the transport of horseradish peroxidase. *Neurosci Lett* 41: 233–238, 1983.
8. **Dampney RAL.** Functional organization of central pathways regulating the cardiovascular system. *Physiol Rev* 74: 323–364, 1994.
9. **Dampney RAL, Czachurski J, Dembowsky K, Goodchild AK, and Seller H.** Afferent connections and spinal projections of the pressor region in the rostral ventrolateral medulla of the cat. *J Auton Nerv Syst* 20: 73–86, 1987.
10. **Fujimori H and Pan-Hou H.** Effect of nitric oxide on L-[^{3}H]glutamate binding to rat brain synaptic membranes. *Brain Res* 554: 355–357, 1991.
11. **Garthwaite J, Charles SL, and Chess-Williams R.** Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. *Nature* 336: 385–388, 1988.
12. **Hancock MB.** Evidence for direct projections from the nucleus of the solitary tract onto medullary adrenaline cells. *J Comp Neurol* 276: 460–467, 1988.
13. **Harada S, Tokunaga S, Momohara M, Masaki H, Tagawa T, Imaizuma T, and Takeshita A.** Inhibition of nitric oxide formation in the nucleus tractus solitarius increases renal sympathetic nerve activity in rabbits. *Circ Res* 72: 511–516, 1993.
14. **Hirooka Y, Polson JW, and Dampney RAL.** Pressor and sympathoexcitatory effects of nitric oxide in the rostral ventrolateral medulla. *J Hypertens* 14: 1317–1324, 1996.
15. **Hope BT, Michael GJ, Knigge KM, and Vincent SR.** Neuronal NADPH-diaphorase is a nitric oxide synthase. *Proc Natl Acad Sci USA* 88: 2811–2814, 1991.
16. **Horn T, Smith PM, McLaughlin BE, Bauce L, Marks GS, Pittman QJ, and Ferguson AV.** Nitric oxide actions in paraventricular nucleus: cardiovascular and neurochemical implications. *Am J Physiol Regulatory Integrative Comp Physiol* 266: R306–R313, 1994.
17. **Iwamoto GA, Wappel SM, Fox GM, Buetow KA, and Waldrop TG.** Identification of diencephalic and brainstem cardiorespiratory areas activated during exercise. *Brain Res* 726: 109–122, 1996.
18. **Izumi Y, Clifford DB, and Zorumski CF.** Inhibition of long-term potentiation by NMDA-mediated nitric oxide release. *Science* 257: 1273–1276, 1992.
19. **Jimbo M, Suzuki H, Ichikawa M, Kumagai K, Nishizawa M, and Saruta T.** Role of nitric oxide in regulation of baroreceptor reflex. *J Auton Nerv Syst* 50: 209–219, 1994.
20. **Kalia M, Mei SS, and Kao FF.** Central projections from ergoreceptors (C fibers) in muscle involved in cardiopulmonary responses to static exercise. *Circ Res* 48: I49–I62, 1981.
21. **Kumada M, Terui N, and Kuwaki T.** Arterial baroreceptor reflex: its central peripheral neural mechanisms. *Prog Neurobiol* 35: 331–361, 1990.
22. **Lawrence AJ and Jarrott B.** Neurochemical modulation of cardiovascular control in the nucleus tractus solitarius. *Prog Neurobiol* 48: 21–53, 1996.
23. **Lewis SJ, Ohta H, Machado B, Bates JN, and Talman WT.** Microinjection of *S*-nitrosocysteine into the nucleus tractus solitarii decreases arterial pressure and heart rate via activation of soluble guanylate cyclase. *Eur J Pharmacol* 202: 135–136, 1991.
24. **Li J, Hand GA, Potts JT, Wilson LB, and Mitchell JH.** c-Fos expression in the medulla induced by static muscle contraction in cats. *Am J Physiol Heart Circ Physiol* 272: H48–H56, 1997.
25. **Li J, Potts JT, and Mitchell JH.** Role of nitric oxide in the nucleus tractus solitarii on the pressor response to static muscle contraction (Abstract). *FASEB J* 11: A48, 1997.
26. **Li J, Potts JT, and Mitchell JH.** Effect of barodenervation on c-Fos expression in the medulla induced by static muscle contraction in cats. *Am J Physiol Heart Circ Physiol* 274: H901–H908, 1998.
27. **Liu JL, Murakami H, and Zucker IH.** Effects of NO on baroreflex control of heart rate and renal nerve activity in conscious rabbits. *Am J Physiol Regulatory Integrative Comp Physiol* 270: R1361–R1370, 1996.
28. **Ma S, Abboud FM, and Felder RB.** Effect of L-arginine-derived nitric oxide synthesis on neuronal activity in nucleus tractus solitarius. *Am J Physiol Regulatory Integrative Comp Physiol* 268: R487–R491, 1995.

29. **Menetrey D and Basbaum AI.** Spinal and trigeminal projections to the nucleus of the solitary tract: a possible substrate for somatovisceral and viscerovisceral reflex activation. *J Comp Neurol* 255: 439–450, 1987.
30. **Mense S and Craig AD Jr.** Spinal and supraspinal termination of primary afferent fibers from the gastrocnemius-soleus muscle in the cat. *Neuroscience* 26: 1023–1035, 1988.
31. **Mitchell JH, Kaufman MP, and Iwamoto GA.** The exercise pressor reflex: its cardiovascular effects, afferent mechanisms, and central pathways. *Annu Rev Physiol* 45: 229–242, 1983.
32. **Mitchell JH, Reardon WC, and McCloskey DI.** Reflex effects on circulation and respiration from contracting skeletal muscle. *Am J Physiol Heart Circ Physiol* 233: H374–H378, 1977.
33. **Nyberg G and Blomqvist A.** The central projection of muscle afferent fibers to the lower medulla and upper spinal cord: an anatomical study in the cat with the transganglionic transport method. *J Comp Neurol* 230: 99–109, 1984.
34. **Potts JT, Hand GA, Li J, and Mitchell JH.** Central interaction between carotid baroreceptors and skeletal muscle receptors inhibits sympathoexcitation. *J Appl Physiol* 84: 1158–1165, 1998.
35. **Potts JT, Li J, and Waldrop TG.** Extracellular recordings from nucleus tractus solitarii (NTS) neurons: Response to arterial baroreceptor and skeletal muscle receptor inputs (Abstract). *FASEB J* 12: A61, 1998.
36. **Shapoval LN, Sagach VF, and Pobegailo LS.** Nitric oxide influences ventrolateral medullary mechanisms of vasomotor control in the cat. *Neurosci Lett* 132: 47–50, 1991.
37. **Spyer KM.** Central nervous mechanisms contributing to cardiovascular control. *J Physiol* 474: 1–19, 1994.
38. **Sun M-K and Guyenet PG.** GABA-mediated baroreceptor inhibition of reticulospinal neurons. *Am J Physiol Regulatory Integrative Comp Physiol* 249: R672–R680, 1985.
39. **Toney GM and Mifflin SW.** Time-dependent inhibition of hindlimb somatic afferent input to nucleus tractus solitarius. *J Neurophysiol* 72: 63–71, 1994.
40. **Tseng CJ, Liu HY, Lin HC, Ger LP, Tung CS, and Yen MH.** Cardiovascular effects of nitric oxide in the brain stem nuclei of rats. *Hypertension* 27: 36–42, 1996.
41. **Vincent SR and Kimura H.** Histochemical mapping of nitric oxide synthase in the rat brain. *Neuroscience* 46: 755–784, 1992.
42. **Zhang C and Wong-Riley MTT.** Do nitric oxide synthase, NMDA receptor subunit R1 and cytochrome oxidase co-localize in the rat central nervous system? *Brain Res* 729: 205–215, 1996.