Nitric oxide modulates sympathoexcitatory cardiac-cardiovascular reflexes elicited by bradykinin

Department of Medicine, University of California, Irvine, California 92697

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N. T. Phan, S. C. Tjen-A-Looi, and J. C. Longhurst. Nitric oxide modulates sympathoexcitatory cardiac-cardiovascular reflexes elicited by bradykinin. Am J Physiol Heart Circ Physiol 281:H2010–H2017, 2001.—A number of studies have demonstrated an important role for nitric oxide (NO) in central and peripheral neural modulation of sympathetic activity. To assess the interaction and integrative effects of NO release and sympathetic reflex actions, we investigated the influence of inhibition of NO on cardiac-cardiovascular reflexes. In anesthetized, sinoaortic-denervated and vagotomized cats, transient reflex increases in arterial blood pressure (BP) were induced by application of bradykinin (BK, 0.1–10 μg/ml) to the epicardial surface of the heart. The nonspecific NO synthase (NOS) inhibitor Nω-monomethyl-L-arginine (L-NMMA, 10 mg/kg iv) was then administered and stimulation was repeated. L-NMMA increased baseline mean arterial pressure (MAP) from 129 ± 8 to 152 ± 9 mmHg and enhanced the change in MAP in response to BK from 32 ± 3 to 39 ± 5 mmHg (n = 9, P < 0.05). Pulse pressure was significantly enhanced during the reflex response from 6 ± 4 to 27 ± 6 mmHg after L-NMMA injection due to relatively greater potentiation of the rise in systolic BP. Both the increase in baseline BP and the enhanced pressor response were reversed by L-arginine (30 mg/kg iv). Because L-NMMA can inhibit both brain and endothelial NOS, the effects of 7-nitroindazole (7-NI, 25 mg/kg ip), a selective brain NOS inhibitor, on the BK-induced cardiac-cardiovascular pressor reflex also were examined. In contrast to L-NMMA, we observed significant reduction of the pressor response to BK from 37 ± 5 to 18 ± 3 mmHg 30 min after the administration of 7-NI (n = 9, P < 0.05), an effect that was reversed by L-arginine (300 mg/kg iv, n = 7). In a vehicle control group for 7-NI (10 ml of peanut oil ip), the pressor response to BK remained unchanged (n = 6, P > 0.05). In conclusion, neuronal NOS facilitates, whereas endothelial NOS inhibits, the excitatory cardiovascular reflex elicited by chemical stimulation of sympathetic cardiac afferents.

Nω-monomethyl-L-arginine; 7-nitroindazole; L-arginine; nitric oxide synthase inhibition; sinoaortic denervation; vagotomy

STIMULATION OF CARDIAC AFFERENTs either mechanically or chemically can induce reflex cardiovascular responses, including changes in blood pressure (BP), heart rate (HR), and vasomotor tone (21, 35, 37). Myocardial ischemia is an important pathophysiological condition that causes both inhibitory and excitatory cardiovascular reflexes. Stimulation of vagal afferents leads to hypotension, bradycardia, and decreases in systemic vascular resistance (27). Excitatory reflexes from the ventricular myocardium result from activation of cardiac sympathetic spinal afferents (22). Activation of sympathetic afferents increases BP, HR, and myocardial contractility (4, 23). Metabolites such as prostaglandins, lactic acid, reactive oxygen species, and bradykinin (BK) released during myocardial ischemia can directly activate or sensitize cardiac afferent nerve endings (11, 30, 40, 46). We previously have shown that BK stimulates ventricular C-fiber sympathetic afferents during ischemia (43), leading to reflex activation of the cardiovascular system (37).

A number of factors potentially may modulate cardiovascular excitatory reflexes. For instance, nitric oxide (NO), which is produced from the enzymatic cleavage of L-arginine to citrulline by a family of NO synthases (NOS), including endothelial NOS (eNOS) and neuronal NOS (nNOS) (29), has been shown to play an important role in the regulation of arterial BP (1, 16). In the periphery, endothelial NO acts as a potent vasodilator by stimulating guanylyl cyclase, which then generates cGMP, inducing smooth muscle relaxation (13) and a change in myocardial contractility (3, 25). Recent evidence also suggests that endogenous NO has BP-buffering capabilities comparable to that of the baroreceptors (14).

There are other ways by which NO might modify cardiac-cardiovascular reflexes. For instance, it has been shown that NO acts as a neuromodulator and/or neurotransmitter in the central nervous system (5). A number of studies (6, 38, 50) have shown that inhibition of the synthesis of NO enhances vasoconstriction and release of norepinephrine during activation of the sympathetic nervous system. Also, there is growing evidence that a modulatory and facilitatory role for NO exists in medullary cardiovascular centers. However, it is still controversial whether NO inhibits or enhances sympathetic outflow, or causes both effects (7, 15, 26, 49).

The present study was designed to evaluate the role of NO in mediating cardiac sympathetic afferent reflexes. We hypothesized that sympathoexcitatory cardiovascular reflexes originating from the left ventricle...
are modulated by NO. In particular, we proposed that NO produced by vascular endothelium buffers (modulates) the reflex increases in BP when cardiac sympathetic afferent nerve endings are stimulated by endogenously produced ischemic metabolites such as BK. We also proposed that brain NOS plays an important role by either facilitating or modulating transmission of excitatory cardiac-cardiovascular reflexes induced by BK. To investigate these two hypotheses, we examined the influence of NO on the cardiac-cardiovascular reflex response to application of BK on the epicardial surface of the myocardium both before and after inhibition of NOS in baroreceptor-denervated and vagotomized cats. This method of stimulating cardiac chemosensitive nerve endings has been shown to activate mainly cardiac sympathetic afferents, in a manner that overrides the reflex influence of cardiac vagal afferents (12, 42). Hence, the reflex response pattern is predominately one of cardiovascular stimulation rather than inhibition. However, because there is some influence from activation of vagal afferents by BK that is directionally opposite to the reflex cardiovascular response to stimulation of sympathetic afferents (12), and because there may be secondary reflex responses resulting from stimulation of arterial baroreceptors, we performed these studies in a sinoaortic and cardiac vagally denervated preparation. A preliminary report on this work has been presented (31).

**Materials and Methods**

**Surgical Preparation**

The experimental preparations and protocols were reviewed and approved by the Institutional Animal Care and Use Committees, University of California, Davis and Irvine. The studies conformed to the American Physiology Society “Guiding Principles for Research Involving Animals.” Adult cats of either sex (2.5–5.0 kg) were anesthetized by ketamine (40 mg/kg im) followed by a bolus injection of α-chloralose (40–50 mg/kg iv). α-Chloralose (5–10 mg/kg iv) was administered as required to maintain an adequate depth of anesthesia determined by utilizing the paw pinch and eye reflex (9). The trachea was intubated and respiration was maintained artificially (model 661, Harvard pump, Ealing; South Natick, MA). Arterial blood gases and pH were measured periodically in all animals with a blood gas analyzer (model ABL-5, Radiometer; Westlake, OH) and maintained within physiological limits (P_{O_2}, 100–150 mmHg; P_{CO_2}, 28–35 mmHg; pH, 7.35–7.45) by adjusting ventilatory rate, tidal volume, or by administering 8% sodium bicarbonate (1.0 M iv). Body temperature was monitored with a rectal probe (model 41TD, Yellow Springs Instruments) and was maintained between 36 and 38°C with a heating pad and lamp. The right femoral artery and vein were cannulated to allow continuous, repeatable responses (12). Thereafter, the renal nerve was placed on a bipolar recording electrode and covered with warm mineral oil. A minimum of 1 h for stabilization of the preparation was allowed before observations were begun. In 31 cats, BK (0.1–10 μg/ml) was applied topically to the epicardium of the left ventricle using a pledget (1 cm²) soaked in the solution of BK. After maximal cardiovascular responses were evoked, the filter paper was removed and the stimulated area was washed with a continuous stream of warm Ringer solution. A significant reflex response was considered to have occurred if BP increased more than 15 mmHg. To prevent tachyphylaxis to BK, recovery periods of at least 15 min were provided between successive applications of BK; this procedure allowed consistent, repeatable responses (<20 mmHg). The same concentration of BK was used to stimulate repeated cardiovascular responses.

In the time control studies, peanut oil (3 ml/kg ip) was administered to six denervated animals without 7-nitroindazole (7-NI) or L-arginine 5 min before the third application of BK. After peanut oil was injected, which was used as the vehicle because of the solubility characteristics of 7-NI (49), BK was applied four more times.

**Experimental Protocols**

To minimize the BP “buffering” action of arterial baroreceptors and because NO may play a role in these secondary reflexes (14), bilateral sinoaortic denervation was performed in all animals except those in the intact group. Also, animals were vagotomized to eliminate any influence of vagal cardiac afferents that masks the full reflex effects from stimulation of sympathetic afferents (12, 42). After a midline incision in the neck was made, the carotid sinus, aortic depressor, and vagus nerves were isolated and transected. Subsequently, the carotid bifurcations were stripped of adventitial tissue and painted with 10% phenol (Sigma; St. Louis, MO). The efficacy of baroreceptor denervation was verified by the absence of a normal decrease in HR in response to a phenylephrine-induced (10 mg/kg) increase in arterial BP. Thereafter, a midline sternotomy was performed, and the pericardium was incised longitudinally to provide access to the left ventricular epicardial surface. A small abdominal incision permitted injections of drugs intraperitoneally.

Animals were vagotomized, and the sinoaortic regions were denervated before recording sympathetic outflow. Midline sternotomy and pericardial incision were performed to expose the left ventricular epicardial surface for the stimulation of cardiac afferents with application of BK. A lateral incision below the ribs and isolation of the right renal nerve were performed. Thereafter, the renal nerve was placed on a bipolar recording electrode and covered with warm mineral oil. Hemodynamic parameters were recorded continuously (TA 4000B, Gould; Cleveland, OH). Effluent nerve action potentials were amplified and filtered (0.3–10 Hz) with a preamplifier (Grass P5) attached to a high-impedence converter probe (Grass HI P5) and were monitored with an oscilloscope (Tektronix 2201). Recordings were recorded and analyzed off-line with an enhanced graphics acquisition and analysis program (RC Electronics; Santa Barbara, CA). Action potentials were rectified, and pulse rate histograms were constructed to determine the change of sympathetic nerve efferent activity after stimulation of cardiac afferents.

**Inhibition of nNOS**

To determine the effect of nNOS on the BK-induced cardiovascular response, 7-NI (25 mg/kg ip) was administered 15 min before the third application of BK in a group of four vagal- and sinoaortic-intact animals. L-arginine was administered 15 min before the third application of BK in the group of four vagal- and sinoaortic-intact animals. L-arginine was administered 15 min before the third application of BK in the group of four vagal- and sinoaortic-intact animals. L-arginine was administered 15 min before the third application of BK in the group of four vagal- and sinoaortic-intact animals.
Because of its solubility characteristics, 7-NI was dissolved in normal saline to a concentration of 25 mg/ml and was stored at 0°C. This solution also was used within 2 wk. Because of its solubility characteristics, 7-NI was dissolved in peanut oil following sonication for 5 min (in bunts of 30 s) and was then administered intraperitoneally as described previously (46).

Data Analysis

All data are presented as means ± SE. The data were normalized with the Kolmogorov-Smirnov procedure (36). Multiple comparisons were performed by repeated-measures ANOVA on ranks followed by post hoc Student-Newman-Keuls method in the L-NMMA, 7-NI, control, and sympathetic nerve recording groups. SigmaStat (Jandel Scientific), a statistical software package, was utilized for these analyses. The level of statistical significance was selected as \( P < 0.05 \).

RESULTS

BK (0.1–10 \( \mu \)g/ml) consistently evoked a reflex pressor response. There was no change in the pressor reflex after administration of the peanut oil vehicle in six controls (Fig. 1). HR was unchanged during all of these interventions. It also has been shown previously that epicardial application of these concentrations of BK elicits pressor responses that do not diminish with time (27).

Nonspecific Inhibition of NOS

Resting mean arterial pressure (MAP) and HR were 129 ± 8 mmHg and 203 ± 7 beats/min, respectively, in nine denervated cats. Intravenous administration of L-NMMA increased resting MAP from 129 ± 8 to 152 ± 9 mmHg (\( P < 0.05 \)) at the peak response (Table 1), whereas HR remained unchanged. These changes are consistent with previous studies with the exception that HR did not decrease significantly as others have shown (1), presumably because of the sinoaortic denervation. The pressor responses evoked by BK were 32 ± 3 and 32 ± 4 mmHg during the first and second applications, respectively. The pressor response evoked by BK was increased by 22% to 39 ± 5 mmHg (\( P < 0.05 \)) in nine animals following administration of L-NMMA (Fig. 2A). The increase in resting BP following the administration of L-NMMA was transient. Compared with the response before L-NMMA, application of BK after L-NMMA significantly increased the pressor response and was accompanied by an increase in pulse pressure (6 ± 4 to 27 ± 6 mmHg, Fig. 2B).

Table 1. Effect of inhibition of eNOS on baseline and maximal MAP by application of BK on epicardium

<table>
<thead>
<tr>
<th>Condition</th>
<th>Baseline MAP, mmHg</th>
<th>Maximal MAP Response, mmHg</th>
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<tbody>
<tr>
<td>Control 1</td>
<td>121 ± 7</td>
<td>153 ± 9*</td>
</tr>
<tr>
<td>Control 2</td>
<td>129 ± 8</td>
<td>161 ± 11*</td>
</tr>
<tr>
<td>After L-NMMA</td>
<td>152 ± 9*</td>
<td>191 ± 9†</td>
</tr>
<tr>
<td>After L-Arginine</td>
<td>119 ± 9</td>
<td>143 ± 8*</td>
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</table>

Values are means ± SE; \( n = 9 \) animals. eNOS, endothelial nitric oxide synthase; MAP, mean arterial pressure; L-NMMA, \( \beta \)-monomethyl-L-arginine; BK, bradykinin. *Significant (\( P < 0.05 \)) increase in MAP comparing baseline to maximal response after application of BK. †Significant (\( P < 0.05 \)) increase in baseline and maximal MAP response comparing pre- to postinhibition of eNOS using one-way repeated-measures analysis of variance and Duncan’s post hoc test.

Drugs and Solutions

All drugs were purchased from Sigma. BK was dissolved in sterile normal saline to achieve an initial concentration of 10 mg/ml. Appropriate dilutions were made to obtain desired concentrations. The stock solution of BK was stored in a −70°C freezer and was used for no more than 2 wk after a fresh stock solution was prepared. L-NMMA (Sigma) was dissolved in normal saline to a concentration of 25 mg/ml and was stored at <0°C. This solution also was used within 2 wk. Because of its solubility characteristics, 7-NI was dissolved in peanut oil following sonication for 5 min (in bunts of 30 s) and was then administered intraperitoneally as described previously (46).

Fig. 1. Effects of peanut oil, vehicle control for 7-nitroindozole (7-NI), and time control on bradykinin (BK)-induced pressor reflexes. Bars represent increases in mean arterial pressure (ΔMAP) induced by BK applied to the epicardium. A: α-β display raw data including arterial blood pressure (BP) from an individual animal that correspond to bars in B. Arrows indicate applications of BK to the heart. Successive applications were separated by 15-min intervals. B: values under bars represent baseline MAP before application of BK. Peanut oil (10 ml ip) did not alter the pressor responses to BK (\( n = 6, P > 0.05 \)).

Arginine (300 mg/kg iv) was administered 15 min before the fifth and final application of BK.

To examine the effect of inhibition of nNOS in the BK-induced cardiovascular response in a separate group of nine denervated animals, 7-NI (25 mg/kg) was administered intraperitoneally 15 min before the third application of BK. Reflex BP responses to BK were recorded 15, 30, and 45 min following injection of 7-NI. Maximal inhibition of NOS is known to occur within this time frame (17). This protocol served as a time control for the subsequent group in which L-arginine was administered.

In another group of seven denervated animals, 7-NI (25 mg/kg ip) was administered 15 min before the third application of BK, and L-arginine (300 mg/kg iv) was administered 15 min before the fifth and final application of BK.

Sympathetic efferent activity was measured in five other denervated animals. Discharge activity was measured before and after the application of BK to the left ventricular epicardium. After vagotomy and sinoaortic denervation, BK was applied to the epicardium before and after administration of 7-NI (25 mg/kg ip) to assess the change of sympathetic efferent activity. The third and final application of BK was applied 15 min after L-arginine (300 mg/kg iv) was administered.

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Data Analysis

All data are presented as means ± SE. The data were normalized with the Kolmogorov-Smirnov procedure (36). Multiple comparisons were performed by repeated-measures ANOVA on ranks followed by post hoc Student-Newman-Keuls method in the L-NMMA, 7-NI, control, and sympathetic nerve recording groups. SigmaStat (Jandel Scientific), a statistical software package, was utilized for these analyses. The level of statistical significance was selected as \( P < 0.05 \).

RESULTS

BK (0.1–10 \( \mu \)g/ml) consistently evoked a reflex pressor response. There was no change in the pressor reflex after administration of the peanut oil vehicle in six controls (Fig. 1). HR was unchanged during all of these interventions. It also has been shown previously that epicardial application of these concentrations of BK elicits pressor responses that do not diminish with time (27).

Nonspecific Inhibition of NOS

Resting mean arterial pressure (MAP) and HR were 129 ± 8 mmHg and 203 ± 7 beats/min, respectively, in nine denervated cats. Intravenous administration of L-NMMA increased resting MAP from 129 ± 8 to 152 ± 9 mmHg (\( P < 0.05 \)) at the peak response (Table 1), whereas HR remained unchanged. These changes are consistent with previous studies with the exception that HR did not decrease significantly as others have shown (1), presumably because of the sinoaortic denervation. The pressor responses evoked by BK were 32 ± 3 and 32 ± 4 mmHg during the first and second applications, respectively. The pressor response evoked by BK was increased by 22% to 39 ± 5 mmHg (\( P < 0.05 \)) in nine animals following administration of L-NMMA (Fig. 2A). The increase in resting BP following the administration of L-NMMA was transient. Compared with the response before L-NMMA, application of BK after L-NMMA significantly increased the pressor response and was accompanied by an increase in pulse pressure (6 ± 4 to 27 ± 6 mmHg, Fig. 2B). Resting BP
was restored within 10 min. Augmentation of MAP was mediated by a disproportionately large increase in systolic BP (SBP, 37±6 to 57±8 mmHg, P < 0.05) compared with the small change in diastolic BP (DBP, 30±4 to 31±5 mmHg). The increases in resting BP, the pressor reflex, SBP, and pulse pressure were reversed by L-arginine (Table 1 and Fig. 2, A and B).

**Inhibition of nNOS**

Resting MAP and HR were 121±7 mmHg and 238±17 beats/min, respectively, and were unchanged by treatment with 7-NI in nine denervated cats (Fig. 3). The pressor response induced by application of BK to the heart was 32±4 and 31±4 mmHg during the first and second control period, respectively. The BP reflex was reduced to 17±3, 18±3, and 15±4 mmHg at 15, 30, and 45 min, respectively, after 7-NI (Fig. 3). In this group tested with 7-NI, low-dose (30 mg/kg) L-arginine did not reliably reverse the response to blockade of nNOS.

In the vagus- and sinoaortic-intact group, the first and second pressor responses were 19±3 and 20±2 mmHg, respectively. The pressor responses were decreased to 5±6 and 7±2 mmHg at 15 and 30 min, respectively, following administration of 7-NI (P < 0.05 compared with controls). L-Arginine (300 mg/kg) reversed the attenuated reflex pressor response to 23±4 mmHg (P < 0.05, Fig. 4A). HR did not change significantly.

Therefore, in seven denervated animals L-arginine was administered intravenously at a high dose to determine whether the response to 7-NI was reversible. In this group of cats subjected to blockade of nNOS, resting MAP and HR before the blockade were 113±3 mmHg and 238±17 beats/min, respectively. The pressor response induced by application of BK to the epicardial surface was 37±3 mmHg during the first control period and 37±1 mmHg during the second control period. The pressor reflex response was decreased to 18±3 and 15±4 mmHg at 15 and 30 min, respectively, after 7-NI (all P < 0.05 compared with controls, Fig. 4B). L-Arginine (300 mg/kg) reversed the attenuated reflex pressor response caused by 7-NI from 15±4 to 34±3 mmHg (P < 0.05).

Renal sympathetic efferent activity was enhanced by stimulation of cardiac afferents in five denervated animals. Similar to the change in the hemodynamic re-

Fig. 2. Effect of Nω-monomethyl-L-arginine (L-NMMA) and L-arginine (L-Arg) on BK-induced pressor reflexes. Bars display the increase in ΔMAP induced by BK applied to the epicardial surface of the heart (A). A: a–d represent raw data of arterial BP (inset). Arrows indicate application of BK to the heart. Successive applications were separated by 15-min intervals. Values under bars represent baseline MAP before application of BK. L-NMMA (10 mg/kg iv) significantly increased the pressor reflex from 32±4 to 39±5 mmHg (n = 9, *P < 0.05) after L-Arg (30 mg/kg iv) reversed the increase in the pressor responses. B: after administration of L-NMMA, BK application resulted in significant increases in the change in pulse pressure (ΔPP) and systolic BP (ΔSBP) from 6±4 to 27±6 mmHg and 37±6 to 57±8 mmHg (n = 9, *P < 0.05), respectively. These changes were reversed by L-Arg. Mean change in diastolic BP (ΔDBP) was not affected.

Fig. 3. Effect of 7-NI, a specific brain nitric oxide synthase inhibitor, on BK-induced pressor reflexes. Bars display the increase ΔMAP induced by BK. A: a–e display raw data of arterial BP from an individual animal that correspond to the bars in B. Arrows indicate application of BK to the heart. Repeated applications were separated by 15-min intervals. B: values under bars represent baseline MAP before application of BK. 7-NI (25 mg/kg ip) significantly inhibited the pressor reflex (n = 9, *P < 0.05).
DISCUSSION

This is the first study to show that nonspecific inhibition of NOS with L-NMMA significantly potentiates the cardiac-cardiovascular pressor reflex response to BK, whereas 7-NI, a brain-specific NOS inhibitor, significantly attenuates the response. We observed that L-NMMA, which inhibits the synthesis of NO, enhanced the pressor reflex during stimulation of cardiac afferents by application of BK on the epicardium. On the other hand, 7-NI, the brain-specific inhibitor of NOS, decreased the pressor reflex and renal sympathetic efferent activity following BK stimulation of cardiac sympathetic (spinal) afferents. Because L-NMMA is a nonspecific inhibitor of NOS, it initially was unclear whether its actions were through a central neural or a peripheral (endothelial) mechanism. From our results we suggest that L-NMMA mainly acts at the level of the endothelium because 7-NI, which acts centrally, caused the opposite response. Our data, which pertain to the global peripheral (endothelial) and central neural actions of NO, demonstrate a complex modulatory role for NO in the reflex response to cardiac afferent stimulation. On the one hand, nNOS facilitates the sympatoexcitatory cardiac-cardiovascular reflex, whereas, on the other hand, eNOS modulates it.

We chose to apply BK to the epicardial surface of the heart for several reasons. First, this technique preferentially stimulates sympathetic afferents, which are more selectively accessed by epicardial than by transmural chemical stimulation (2). Second, BK is produced during myocardial ischemia, particularly during conditions of sympathetic stimulation (4, 40, 43, 46). Third, our laboratory recently has shown that BK is particularly relevant during ischemia. In this regard, through a BK2 receptor mechanism, endogenous BK activates cardiac afferents during myocardial ischemia (43). Finally, BK application to the heart activates sympatoexcitatory neurons in the ventrolateral medulla (19). Thus BK applied to the epicardial surface of the myocardium is a particularly relevant chemical stimulus for activating cardiac sympathetic afferents.

Numerous studies indicate that the NO system plays an important role in regulating arterial BP (38, 51). Its mechanism may be through vasodilation (34), peripheral nerve transmission (6, 46a), and/or through central neural regulation by interacting with excitatory and inhibitory amino acids (44, 45). Consistent with previous work (6, 34, 38, 50), we observed an increase in baseline BP after inhibition of NOS with L-NMMA.

Previous in vivo investigations have suggested an important role for NO in regulation of sympathetic nerve activity in several regions of the brain, including the nucleus tractus solitarii (NTS) (7, 24, 45), paraventricular nucleus (PVN) (10, 53), and rostral ventral lateral medulla (RVLM) (15, 45). For example, microinjection of L-NMMA or N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME) into the PVN increases resting renal sympathetic nerve activity (RSNA), BP, and HR (53). Investigation of the role of NO in the NTS and RVLM has yielded conflicting results. For example, Harada et al. (7) showed that inhibition of NO in the NTS, following microinjection of L-NMMA, increased arterial pressure and RSNA in intact as well as in sinoaortic-denervated and vagotomized rabbits and inhibited the depressor and bradycardiac effects of the excitatory amino acids L-glutamate and N-methyl-D-aspartate. Similar conclusions were reached by Tseng et al. (45), who demonstrated a depressor effect with the NO precursor L-arginine administered into the NTS and RVLM and a pressor and tachycardia response to L-NMMA administered into the cerebral ventricle. In contrast, Matsumura et al. (24) observed decreases in

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**Fig. 4. Effect of 7-NI on BK-induced pressor reflexes.** Bars display the increase in \( \Delta \text{MAP} \) induced by BK. Values under the bars indicate baseline MAP before application of BK. 7-NI (25 mg/kg ip) significantly inhibited the pressor reflex \(( \ast P < 0.05 \)) and L-Arg (300 mg/kg iv) significantly reversed the effect of 7-NI \(( n = 7 \)) significantly reversed the effect of 7-NI \(( n = 7 \)) in intact \(( A \)) and in baroreceptor-denervated \(( B \)) animals \(( n = 4 \), \( \ast P < 0.05 \)). 7-NI decreased the percent change in sympathetic efferent activity in denervated animals \(( n = 5 \), \( \ast P < 0.05 \); B, inset B). L-Arg (300 mg/kg iv) reversed the inhibitory effect of 7-NI on sympathetic nerve activity.

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arterial pressure and RSNA in rats during inhibition of NOS in the NTS with L-NMMA. Likewise, Hirooka et al. (8) reported reduced arterial pressure and RSNA following microinjection of L-NAME into the RVLM. Thus the responses to inhibition of NOS in the NTS and RVLM are variable, consisting of either increased or decreased sympathetic activity depending on the preparation and experimental paradigm.

Investigations of the importance of NO in the central nervous system, with regard to cardiovascular control, have utilized either regional stimulation or inhibition or, as in the present study, a more global approach involving systemic administration of NOS inhibitors. It is difficult to compare studies utilizing these two approaches because cardiac-cardiovascular sympathoexcitatory reflexes may be integrated to a variable extent in a number of regions influenced by NO. Other than our previous study of the NTS (42), little information currently is available on the integrative role of these CNS regions in cardiovascular reflexes originating from stimulation of cardiac spinal afferents. The value of studies like the present one involving assessment of the influence of NOS system on the CNS is that they provide an integrated evaluation of the overall importance of NOS in cardiac-cardiovascular reflex control.

In contrast to observations resulting from localized microinjection of nonspecific NOS inhibitors into specific regions of the brain, previous studies have demonstrated that systemic administration of 7-NI, a relatively specific inhibitor of nNOS, does not affect baseline BP (18, 26). In line with these studies, our data demonstrated that brain NOS does not tonically modulate sympathetic activity to any significant extent.

nNOS is located in both peripheral nerves and in the central nervous system. Inhibition of NOS activity potentially could alter function at either or both locations. For example, nNOS has been found in the macula densa of the kidney. In this regard, several groups of investigators have reported a role for nNOS in the control of renal hemodynamic function, including the glomerular filtration rate in animals (39, 41, 48). nNOS activity also has been identified in perivascular nerves surrounding the mesenteric artery (28). Furthermore, blockade of nNOS with 7-NI inhibits rat hindlimb arterial vasodilatation produced by electrical stimulation of the superior laryngeal nerve (33). These studies indicate that nNOS in the perivascular nerves surrounding peripheral vessels plays a vasodilatory or depressor role. On the other hand, our study has shown that inhibition of NOS reduces cardiac-cardiovascular excitatory reflexes in both intact and vagotomized sinoaortic-denervated animals. Still other studies have found that intraperitoneal administration of 7-NI reduces cortical brain NOS activity (47), indicating that 7-NI crosses the blood-brain barrier to inhibit NOS. Given the fact that 7-NI crosses the blood-brain barrier and causes the opposite response to that observed when peripheral NOS is inhibited, we believe that our observations with 7-NI predominantly result from inhibition of the activity of brain NOS. Thus the net effect of nNOS in the integrative cardiac sympathetic-cardiovascular response is to facilitate sympathetic excitatory reflexes, most likely through its action in the brain.

In the present study, we found that inhibition of NOS in the brain attenuated rather than potentiated the pressor and sympathetic efferent responses. The reason for the differences between our observations and those of others employing nonselective blockers are uncertain. However, in contrast with other studies (7, 15, 24, 45, 51, 53), we did not locally apply the NOS inhibitor to a specific region of the brain but instead administered it intraperitoneally. Presumably the latter method of application provides a broad influence on multiple regions of the brain. Furthermore, ours is the only study to examine the role of NO in the brain during a physiological input, namely cardiac chemoreceptor stimulation. Thus the use of different types of NOS inhibitors, different routes of administration, and a focus on reflex rather than the baseline of cardiovascular responses make it difficult to compare the results of our studies with those of others.

A single study published by Zanzinger et al. (52) examined the role of nNOS during activation of somatosympathetic reflexes. They observed increased responses of RSNA and arterial BP during electrical stimulation of the greater sciatic nerve in pigs following intracerebroventricular injection of the NOS inhibitor 7-NI (52). These results contrast with our observations in which inhibition of nNOS attenuates the reflex response induced by the activation of cardiac sympathetic afferents. In contrast to the previous study, which used a nonphysiological method to activate somatic afferents, we employed BK, an important chemical mediator that stimulates cardiac sympathetic afferents during myocardial ischemia and reperfusion (43). Thus the current model used a different, but perhaps a pathophysiologically more relevant, means to activate spinal afferents to induce sympathoexcitatory responses. Finally, we used a different route of administration of the NOS inhibitor than Zanzinger and co-workers (52) used. These procedural differences may explain the disparate observations between the two studies.

The present study demonstrates that neuronal NO plays an important role in the excitation of pressor reflexes both in the intact and in baroreceptor-denervated animals. Compared with the denervated group, animals with intact vagi and arterial baroreceptors displayed smaller changes in BP responses following epicardial application of BK. Furthermore, relative to the initial increase in BP before inhibition of NOS, the pressor responses in the intact and denervated animals dropped 66% and 57%, respectively, 30 min after administration of 7-NI. Because both intact and denervated animals demonstrated a similar profile of responses after NOS inhibition, we suggest that baroreceptor input does not interact with the sympathetic cardiac reflex mediated by NO. Similar directional changes in the pressor responses to 7-NI in both groups and subsequent restoration by L-arginine in the
intact and denervated animals confirm that nNOS facilitates the excitatory cardiovascular reflex elicited by chemical activation of cardiac sympathetic afferents.

There are several implications of our study. eNOS promotes vasodilation, whereas nNOS, particularly that found in the brain (8, 24), promotes vasoconstriction. These two systems appear to compensate for each other. These counterbalancing modulatory roles presumably limit the extent of the NO response in any one of the regions. They facilitate cardiovascular homeostasis in a manner similar to the action of the diametrically opposite reflexes that result from activation of cardiac vagal and sympathetic afferents (42). When cardiac sympathetic afferents are activated, for example during myocardial ischemia, neuronal NO in the brain facilitates the sympathoexcitatory reflex, an effect that would help maintain coronary perfusion pressure. Conversely, endothelial NO restrains the systemic vasoconstriction response, thereby limiting the imbalance that might result between myocardial oxygen supply and demand (20). It is interesting that compensatory mechanisms have developed that modulate the reflex actions of the sensory system of the heart, both between different functional components (vagal vs. sympathetic afferents in their reflex effects) and within the same component (influence of nNOS vs. eNOS on sympathetic reflex response). Such modulatory effects likely serve to reduce the magnitude of reflex hemodynamic responses during activation of cardiac sensory nerves.

In conclusion, our results suggest that endothelial NO plays an important role in the tonic and reflex regulation of arterial BP, whereas neuronal NO facilitates the sympathoexcitatory cardiovascular reflex elicited by chemical stimulation of sympathetic cardiac afferents. Thus full expression of the cardiac-cardiovascular pressor reflex induced by epicardial application of BK is dependent on an intact central NO mechanism. Additional studies are needed to determine the exact mechanisms by which NO acts to support or inhibit short-term reflex responses of the cardiovascular system.

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