Hemodynamic responses to aortic depressor nerve stimulation in conscious L-NAME-induced hypertensive rats

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Hemodynamic responses to aortic depressor nerve stimulation in conscious L-NAME-induced hypertensive rats. Am J Physiol Regul Integr Comp Physiol 300: R418–R427, 2011. First published November 24, 2010; doi:10.1152/ajpregu.00463.2010.—The present study investigated whether baroreflex control of autonomic function is impaired when there is a deficiency in NO production and the role of adrenergic and cholinergic mechanisms in mediating reflex responses. Electrical stimulation of the aortic depressor nerve in conscious normotensive and nitro-L-arginine methyl ester (L-NAME)-induced hypertensive rats was applied before and after administration of methylatropine, atenolol, and prazosin alone or in combination. The hypertensive response to progressive electrical stimulation (5 to 90 Hz) was greater in hypertensive (−27 ± 2 to −64 ± 3 mmHg) than in normotensive rats (−17 ± 1 to −46 ± 2 mmHg), whereas the bradycardic response was similar in both groups (−34 ± 5 to −92 ± 9 and −21 ± 2 to −79 ± 7 beats/min, respectively). Methylatropine and atenolol showed no effect in the hypertensive response in either group. Methylatropine blunted the bradycardic response in both groups, whereas atenolol attenuated only in hypertensive rats. Prazosin blunted the hypertensive response in both normotensive (43%) and hypertensive rats (53%) but did not affect the bradycardic response in either group. Prazosin plus angiotensin II, used to restore basal arterial pressure, provided hemodynamic responses similar to those of prazosin alone. The triple pharmacological blockade abolished the bradycardic response in both groups but displayed similar residual hypertensive response in hypertensive (−13 ± 2 to −27 ± 2 mmHg) and normotensive rats (−10 ± 1 to −25 ± 3 mmHg). In conclusion, electrical stimulation produced a well-preserved baroreflex-mediated decrease in arterial pressure and heart rate in conscious L-NAME-induced hypertensive rats. Moreover, withdrawal of the sympathetic drive played a role in the reflex bradycardia only in hypertensive rats. The residual fall in pressure after the triple pharmacological blockade suggests the involvement of a vasodilatory mechanism unrelated to NO or deactivation of α1-adrenergic receptor.

electric stimulation; baroreceptors; nitric oxide; nitro-L-arginine methyl ester; autonomic nervous system

Quite recently, there has been renewed interest in using electrical stimulation of baroreceptor afferents to lower the arterial pressure of patients with refractory hypertension (13, 43, 50). Studies carried out in conscious normotensive and hypertensive dogs and patients submitted to chronic electrical stimulation of the carotid sinus have provided further insight into baroreflex regulation of arterial pressure (6, 15, 17, 22, 23, 24, 25). The hypotensive and bradycardic responses caused by electrical activation of the baroreflex under pathophysiological conditions are mainly considered a result of cardiovascular responses caused by inhibition of the sympathetic and activation of the parasympathetic nervous system.

Our laboratory has developed a means of stimulating the aortic depressor nerve (ADN) in conscious freely moving rats that permits evaluation of the acute reflex bradycardia and hypotensive responses as well as the changes in the vascular resistance of the regional beds without the undesirable effect of the anesthesia (3). In a recent study, Salgado et al. (35) demonstrated that electrical stimulation of the ADN of conscious spontaneously hypertensive rats (SHR) produced equivalent or even greater depressor responses, compared with normotensive rats, and that withdrawal of sympathetic activity contributed significantly to baroreflex-mediated bradycardia in SHR but not normotensive rats. Thus the authors suggested that conscious SHR have well-preserved baroreflex responses that reflect alterations in central baroreflex control and high resting sympathetic activity, characteristics of SHR, susceptible to inhibition by strong baroreceptor input (35).

Chronic blockade of nitric oxide synthase (NOS) by means of nitro-L-arginine methyl ester (L-NAME) provides an experimental model of arterial hypertension that is chronically sustained throughout the period of the blockade (34). It is well documented that nitric oxide (NO) plays a role in the control of arterial pressure, acting both peripherally (33) and centrally (4, 10, 45). In addition, it has been consistently demonstrated that chronic inhibition of NO causes attenuation of the reflex control of arterial pressure (38, 40, 46).

The reflex bradycardia elicited by the stimulation of baroreceptor afferents is mainly mediated by the parasympathetic nervous system in conscious rats (2, 12, 41). On the other hand, studies have demonstrated that the reflex hypotensive response to electrical stimulation of baroreceptor afferents is almost exclusively due to the sympathoinhibition (3, 9, 35), but there is also a small contribution of postganglionic lumbar sympathetic vasodilators fibers, which release nitrosyl factors (32). Therefore, we hypothesized that the reflex control of autonomic function is impaired when there is a deficiency in NO production. Thus we examined the reflex bradycardia and hypotension produced by electrical stimulation of the ADN in rats chronically treated with L-NAME. Furthermore, since the hemodynamic response to electrical activation of the baroreflex is classically considered a consequence of inhibition of the sympathetic activity to the heart (mediated by β1-adrenergic receptor) and vessels (mediated by α1-adrenergic receptor) and activation of parasympathetic activity to the heart (mediated by M2-muscarinic receptors), we determined the role of these actions in mediating the cardiovascular responses to baroreflex

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activation by using selective adrenergic and muscarinic antagonists.

MATERIAL AND METHODS

Animal preparation. The experiments were conducted on male Wistar rats (180–220 g) that were maintained in a controlled environment with a constant 12:12-h light-dark cycle and provided with food and water ad libitum. All procedures were reviewed and approved by the Committee of Ethics in Animal Research at the University of Sao Paulo School of Medicine at Ribeirão Preto. All drugs used were purchased from Sigma-Aldrich (St. Louis, MO).

The experimental rats received L-NAME (~70 mg/kg/day) for 2 wk, dissolved in drinking water, whereas the controls received only water. Systolic blood pressure was measured indirectly in the conscious state by means of the tail-cuff method (ITTC Life Science, Woodland Hills, CA) right before the beginning of L-NAME administration and on the 7th and 13th days of treatment. On the day before the experiment, under thiopental sodium anesthesia (40 mg/kg ip), the rats were submitted to the procedures to isolate the left ADN to implant the electrodes and to insert catheters into the femoral artery and vein (3, 35). Briefly, the rats were submitted to ventral neck surgery and, under a microscope, a 4- to 6-mm length of the left ADN was carefully isolated below its joint with the superior laryngeal nerve. The ADN was placed on a bipolar stainless steel electrode with an interelectrode distance of 2 mm. The electrodes were constructed by attaching two 40-mm stainless steel wires (0.008 in. bare, 0.011 in. Teflon coated; A-M Systems, Sequim, WA) to a small plug (GF-6; Microtech, Boothwyn, PA). The bare tips of the electrodes consisted of a 2-mm length forming a hook where the ADN was placed. The correct identification of the nerve was confirmed by its typical pattern of discharge synchronous with arterial pulse pressure. After identification of the ADN, the bipolar stainless steel electrode supporting a short segment of the ADN was carefully covered with silicone impression material (Kwik-Sil silicone elastomer; World Precision Instruments, Sarasota, FL). A 30-min period was allowed for complete polymerization of the silicone impression material, and the activity of the nerve was recorded again to confirm the integrity of the signal. Next, the fine platinum wires of the electrodes were exteriorized on the back of the rats and soldered to a small plug that was later connected with the wires from the electrical stimulator. Under the same anesthesia, the femoral artery and vein were catheterized with polyethylene tubing (Intramedic PE-50 and PE-10; Becton Dickinson, Sparks, MD) for recording pulsatile arterial pressure and for intravenous drug administration, respectively. Catheters were exteriorized on the back of the rats, and surgical incision sites were closed by sutures. Twenty-four hours after the end of the surgery, the rats were connected to the recording system, which consisted of a pressure transducer (MLT844; ADInstruments, Sydney, Australia) and an electrical stimulator (40C; AVS, Sao Paulo, Brazil). Only the rats that showed no signals of pain with electrical stimulation of the ADN were used in the study. The signal, i.e., pulsatile arterial pressure, was amplified (ML224; ADInstruments), fed to an IBM personal computer connected to a PowerLab system (ML866/P; ADInstruments) and continuously sampled (2 kHz). Mean arterial pressure (MAP) and heart rate (HR) were calculated from the arterial pulse pressure.

Experimental protocol. The experimental protocol consisted of a basal recording of cardiovascular variables (MAP and HR) for at least 10 min, followed by a series of electrical stimulations of the ADN (1-mA pulse, 2-ms duration, for 20 s) at 5, 15, 30, 60, and 90 Hz, in a random sequence, and at intervals of at least 5 min to recover the basal values. The series of electrical stimulations of the ADN were performed in normotensive control (n = 36, 330 ± 5 g) and L-NAME-induced hypertensive rats (n = 37, 302 ± 5 g) before and after the administration of drugs (experimental protocols 1–5 described below). A period of 10 min after administration of the drugs was allowed before a new series of electrical stimulations was performed. Although there is no established method to ensure that functional fiber recruitment was similar among groups right before the pharmacological protocol, the maintenance of the hemodynamic response, i.e., hypotension and/or bradycardia, when the pharmacological approach did not affect one of these variables supports the reproducibility of the preparation. The differences between the prestimulation baseline levels of MAP and HR measured over a 15- to 20-s period and the maximum changes in these variables elicited by each frequency of stimulation were quantified. In experimental protocol 1 (n = 7 normotensive rats, 325 ± 8 g; n = 7 L-NAME hypertensive rats, 320 ± 7 g), the muscarinic blocker methylylpyridine (2 mg/kg iv) was administered and the efficacy of the blockade was verified by the blunted reflex bradycardic response (control: 97 ± 2%, L-NAME: 91 ± 4%) to the pressor response induced by phenylephrine (5 μg/kg iv). The drug used in experimental protocol 2 (n = 7 normotensive rats, 307 ± 15 g; n = 7 L-NAME hypertensive rats, 284 ± 7 g) was the β1-adrenergic receptor blocker atenolol (2 mg/kg iv), and the efficacy of the blockade was verified by the blunted tachycardic response to isoproterenol (1 μg/kg iv) in control (82 ± 3%) and L-NAME rats (82 ± 4%). In experimental protocol 3 (n = 8 normotensive rats, 341 ± 9 g; n = 8 L-NAME hypertensive rats, 283 ± 5 g), the α1-adrenergic receptor blocker prazosin (1 mg/kg iv) was administered and the efficacy of the blockade was verified by the blunted hypertensive response to phentolamine (control: 97 ± 1%, L-NAME: 90 ± 2%). To circumvent the fall in MAP caused by prazosin administration, experimental protocol 4 (n = 7 normotensive rats, 336 ± 8 g; n = 7 L-NAME hypertensive rats, 330 ± 14 g) was carried out. In protocol 4, after prazosin administration (1 mg/kg iv), an intravenous infusion of angiotensin II (500 ng·kg⁻¹·min⁻¹) was used to return the MAP back toward basal levels. In the absence of a fall in MAP, this maneuver permitted a comparison of baroreflex-mediated reductions in MAP during α1-adrenergic receptor blockade without the confounding influence of reduced base levels of MAP (protocol 3). A combination of the three blockers was used in experimental protocol 5 (n = 8 normotensive rats, 300 ± 17 g; n = 7 L-NAME hypertensive rats, 274 ± 17 g), i.e., prazosin, methylylpyridine, and atenolol, and their efficacy was verified by the blunted response to phenylephrine (control: 90 ± 3%, L-NAME: 95 ± 1%) and to isoproterenol (control: 73 ± 4%, L-NAME: 74 ± 4%) before the triple pharmacological blockade and at the end of the experiment.

Statistical analysis. Data are means ± SE. Baseline values of MAP and HR before and after each drug treatment and between normotensive control and L-NAME-induced hypertensive rats were compared by paired and unpaired t-tests. Systolic blood pressure data and the frequency response to ADN stimulation in L-NAME-induced hypertensive and normotensive control rats were analyzed by linear regression model with mixed effects (random and fixed effects) followed by orthogonal contrasts (SAS software 9.1, PROC MIXED) (37). Differences were considered significant when P < 0.05.

RESULTS

Basal hemodynamics. Chronic oral administration of L-NAME induced an increase in systolic arterial pressure as measured by the tail-cuff method (from 122 ± 2 to 166 ± 3 and 173 ± 3 mmHg after 1 and 2 wk of L-NAME treatment, respectively), whereas in normotensive control rats, arterial pressure remained stable during the same period (from 126 ± 2 to 124 ± 2 mmHg). L-NAME administration caused a significant increase in MAP and HR (accessed directly from the femoral artery) after 2 wk of treatment compared with normotensive control rats (146 ± 2 vs. 105 ± 2 mmHg, P < 0.01, and 391 ± 6 vs. 360 ± 5 beats/min, P < 0.01).
Hemodynamic responses to electrical stimulation of the ADN. Figure 1 shows the hypotensive and bradycardic response to electrical stimulation (60 Hz) of the ADN in conscious normotensive control and L-NAME-induced hypertensive rats. The group data for changes in MAP and HR elicited by electrical stimulation of the ADN from all normotensive control and L-NAME-induced hypertensive rats under basal conditions are summarized in Fig. 2. ADN stimulation caused significant frequency-dependent decreases in MAP and HR in both groups (Fig. 2). The absolute decrease in MAP was significantly larger in L-NAME-induced hypertensive rats than in normotensive controls at all frequencies of stimulation. The frequency-dependent decreases in HR in response to ADN stimulation were not different between the groups (Fig. 2). Nevertheless, the relative decreases (%) in MAP (normotensive: \(-16 \pm 1\) to \(-44 \pm 2\) mmHg vs. L-NAME: \(-18 \pm 1\) to \(-44 \pm 2\) mmHg) and HR (normotensive: \(-6 \pm 1\) to \(-22 \pm 2\) beats/min vs. L-NAME: \(-9 \pm 1\) to \(-23 \pm 2\) beats/min) were similar in L-NAME-induced hypertensive rats and normotensive controls.

Effects of methylatropine on hemodynamic responses to electrical stimulation of the ADN. Methylatropine increased basal HR in both L-NAME-induced hypertensive and normotensive control rats without changing basal MAP (Table 1). The group data for changes in MAP and HR elicited by electrical stimulation of the ADN before and after methylatropine are shown in Fig. 3. Methylatropine did not influence baroreflex-mediated decreases in MAP in either normotensive control or L-NAME-induced hypertensive rats (Fig. 3). Methylatropine almost abolished the bradycardic response to ADN stimulation in both groups, but a similar residual bradycardic response remained in the two groups (Fig. 3).

Effects of atenolol on hemodynamic responses to electrical stimulation of the ADN. Atenolol significantly decreased basal HR in L-NAME-induced hypertensive and normotensive control rats but did not change basal MAP in either group (Table 1). The group data for changes in MAP and HR elicited by electrical stimulation of the ADN before and after atenolol are summarized in Fig. 4. In the normotensive control rats, ADN stimulation produced frequency-dependent decreases in both MAP and HR, and atenolol did not affect these responses (Fig. 4). As observed in the normotensive control rats, atenolol did not influence baroreflex-mediated decreases in MAP in the L-NAME-induced hypertensive group. On the other hand, atenolol significantly attenuated the reflex decreases in HR in the L-NAME-induced hypertensive rats (Fig. 4).

Effects of prazosin on hemodynamic responses to electrical stimulation of ADN. Prazosin administration reduced basal MAP to a significantly larger extent in L-NAME-induced hypertensive rats than in normotensive control rats (Table 1). The fall in MAP produced by prazosin caused reflex tachycardia in both groups (Table 1). The group data for changes in MAP and HR elicited by electrical stimulation of the ADN before and after prazosin are summarized in Fig. 5. Prazosin attenuated the hypotensive response to ADN stimulation in both normotensive control (43% for 90 Hz) and L-NAME-induced hypertensive rats (53% for 90 Hz). Nevertheless, after \(\alpha_1\)-adrenergic receptor blockade, a similar residual fall in MAP was observed in the two groups (Fig. 5). The reflex bradycardic response was not affected by prazosin administration in either group (Fig. 5).

Effects of prazosin plus angiotensin II on hemodynamic responses to electrical stimulation of ADN. Prazosin administration reduced basal MAP in L-NAME-induced hypertensive rats (from 136 ± 3 to 94 ± 8 mmHg, \(\Delta\text{MAP} = -43 \pm 6\) mmHg, \(P < 0.01\)) and in normotensive control rats (from 102 ± 2 to 80 ± 3 mmHg, \(\Delta\text{MAP} = -22 \pm 2\) mmHg, \(P < 0.01\)), whereas angiotensin II infusion returned the MAP back toward basal values (Table 1). The fall in MAP produced by prazosin caused reflex tachycardia in both groups (L-NAME: from 389 ± 14 to 480 ± 10 beats/min, \(\Delta\text{HR} = 91 \pm 15\) beats/min, \(P < 0.01\); normotensive: from 333 ± 8 to 385 ± 23 beats/min, \(\Delta\text{HR} = 52 \pm 21\) beats/min, \(P < 0.05\)). The group data for changes in MAP and HR elicited by electrical stimulation of the ADN before and after prazosin plus angiotensin II are shown in Fig. 6. The hypotensive response to ADN stimulation was attenuated in both the L-NAME-induced hypertensive and normotensive control rats.
ever, even after α1-adrenergic receptor blockade plus the recovery of the MAP to basal levels, a similar residual fall in MAP was still observed in the two groups (Fig. 6). The reflex bradycardic response was not affected by prazosin plus angiotensin II in either group.

Effects of triple pharmacological blockade (methylatropine, atenolol, and prazosin) on hemodynamic responses to electrical stimulation of ADN. After administration of all three antagonists, basal MAP decreased in both the L-NAME-induced hypertensive rats compared with normotensive control rats. Studies carried out in conscious or anesthetized rats with different types of arterial hypertension (i.e., SHR and 2K1C) not involving the inhibition of NO production in L-NAME-induced hypertensive rats might interfere with the peripheral and/or central processing of the afferent input from electrical stimulation of the ADN. Nevertheless, in the present study, it was observed that ADN stimulation evoked greater hypotensive response and similar bradycardia in L-NAME-induced hypertensive rats compared with normotensive control rats. Studies carried out in conscious or anesthetized rats with different central site of baroreflex regulation (4, 28, 42, 45) and that L-NAME can cross the blood-brain barrier and inhibit NOS in important nuclei responsible for cardiovascular regulation (44, 51) suggest that inhibition of NO production in L-NAME-induced hypertensive rats might interfere with the peripheral and/or central processing of the afferent input from electrical stimulation of the ADN. Nevertheless, in the present study, it was observed that ADN stimulation evoked greater hypotensive response to electrical stimulation of baroreceptors afferents (8, 35, 52). On the other hand, it has been demonstrated that systemic inhibition of NOS can have an effect on the reflex control of HR, but the results are inconsistent. During chronic inhibition of NOS in rats, some authors have reported reduced sensitivity of the baroreflex control of HR (38, 40, 46) or no change (1). In contrast, Liu et al. (21) demonstrated that acute infusion of N\textsuperscript{G}-nitro-L-arginine, another nonspecific NOS inhibitor, potentiated the reflex bradycardia elicited by electrical stimulation of the ADN in anesthetized sinoaortic-dener- vated rabbits, but it did not affect the renal sympathetic nerve activity. Nevertheless, in the present study, the reflex brady-

![Figure 2](https://example.com/image2.png)

**Fig. 2.** Frequency-dependent changes in mean arterial pressure (ΔMAP) and heart rate (ΔHR) in response to electrical stimulation of the aortic depressor nerve (1-mA pulses, 2-ms duration) in normotensive control and L-NAME-induced hypertensive rats. Data are means ± SE. *P < 0.01 compared with the respective control value.

### Table 1. Effect of autonomic blockers on basal hemodynamics of normotensive and L-NAME hypertensive rats

<table>
<thead>
<tr>
<th>Blockades</th>
<th>Normotensive MAP, mmHg</th>
<th>L-NAME Hypertensive MAP, mmHg</th>
<th>HR, beats/min</th>
<th>HR, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>∆MAP</td>
<td>Before</td>
</tr>
<tr>
<td>Methylatropine</td>
<td>101 ± 5</td>
<td>104 ± 4</td>
<td>3 ± 3</td>
<td>353 ± 13</td>
</tr>
<tr>
<td>Atenolol</td>
<td>107 ± 5</td>
<td>108 ± 4</td>
<td>1 ± 2</td>
<td>364 ± 7</td>
</tr>
<tr>
<td>Prazosin</td>
<td>108 ± 2</td>
<td>85 ± 4*</td>
<td>-23 ± 4</td>
<td>362 ± 7</td>
</tr>
<tr>
<td>Prazosin + ANG II</td>
<td>102 ± 2</td>
<td>99 ± 3</td>
<td>-3 ± 1</td>
<td>333 ± 8</td>
</tr>
<tr>
<td>Triple blockade</td>
<td>107 ± 3</td>
<td>93 ± 3*</td>
<td>-14 ± 4</td>
<td>383 ± 11</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.01 compared with value before drug administration. †P < 0.05 compared with normotensive rats. L-NAME, N-nitro-L-arginine methyl ester; MAP, mean arterial pressure; HR, heart rate; ∆MAP and ∆HR, changes in basal MAP and HR due to drug administration.
cardia evoked by ADN stimulation was similar in both groups, suggesting that systemic inhibition of NOS did not alter the central/efferent processing of baroreflex control of HR in L-NAME-induced hypertensive rats. Since electrical stimulation of ADN bypasses the site of baroreceptor mechanosensory transduction, our findings suggest that if there is some alteration in the baroreflex control of HR in L-NAME-induced hypertensive rats, it might be in the arterial pres-

Fig. 3. Frequency-dependent ΔMAP and ΔHR in response to electrical stimulation of the ADN (1-mA pulses, 2-ms duration) before and after methylatropine in normotensive control (left) and L-NAME-induced hypertensive rats (right). Data are means ± SE. *P < 0.01 compared with respective basal values.

Fig. 4. Frequency-dependent ΔMAP and ΔHR in response to electrical stimulation of the ADN (1-mA pulses, 2-ms duration) before and after atenolol in normotensive control (left) and L-NAME-induced hypertensive rats (right). Data are means ± SE. *P < 0.05 compared with respective basal values.
sure-sensing step of baroreflex circuit. It should be pointed out that several studies have demonstrated that NO is present in the baroreceptor afferents endings (14, 19) and that NO is involved in the modulation of baroreceptor resetting and sensitivity (18, 27, 29, 36), but, to our knowledge, there have been no previous studies that have examined the role played by NO on baroreceptor function in L-NAME-induced hypertensive rats.

Fig. 5. Frequency-dependent ΔMAP and ΔHR in response to electrical stimulation of the ADN (1-mA pulses, 2-ms duration) before and after prazosin in normotensive control (left) and L-NAME-induced hypertensive rats (right). Data are means ± SE. *P < 0.01 compared with respective basal values.

Fig. 6. Frequency-dependent ΔMAP and ΔHR in response to electrical stimulation of the ADN (1-mA pulses, 2-ms duration) before and after prazosin administration plus angiotensin II (prazosin + ANG II) infusion in normotensive control (left) and L-NAME-induced hypertensive rats (right). Data are means ± SE. *P < 0.01 compared with respective basal values.
It has been consistently demonstrated that the reflex bradycardia evoked by increases in arterial pressure is mediated primarily by rapid parasympathetic activation (i.e., ~1 s after the arterial pressure reaches its peak) (2, 11, 12, 41). Nonetheless, some authors have demonstrated that the reflex withdrawal of sympathetic activity also makes a small but somewhat slower contribution to reflex bradycardia (2, 41). In the present study, the period of electrical stimulation was 20 s. Therefore, the reflex bradycardia elicited by ADN stimulation was due not only to parasympathetic activation but also to reflex suppression of sympathetic activity. Thus, after the blockade of the parasympathetic system by means of methylnaltropane, the bradycardia response was almost abolished. However, after methylnaltropane administration, there was a residual bradycardia in both groups, which was caused by the reflex withdrawal of sympathetic activity to the heart. On the other hand, attenuation of the reflex bradycardia did not influence the baroreflex-mediated decrease in MAP in normotensive control and in L-NAME-induced hypertensive rats, in agreement with studies demonstrating that complete autonomic blockade of the heart or vagotomy does not affect the reflex decrease in MAP in anesthetized rabbits or rats (3, 9, 20, 26). Thus these results indicated that the hypertensive response to ADN stimulation is independent of the baroreflex-mediated decrease in arterial pressure in normotensive rats or SHR. In addition, atenolol did not affect the reflex bradycardia due to ADN stimulation in normotensive rats. This result is in line with the primary role of the parasympathetic drive in mediating reflex bradycardia (2, 11, 41). Because atenolol attenuated the baroreflex-mediated bradycardia in L-NAME-induced hypertensive rats, this observation indicates that withdrawal of sympathetic activity contributes significantly to baroreflex-mediated bradycardia in L-NAME-induced hypertensive rats, contrasting with normotensive control rats. Similar results were found by Salgado et al. (35), who also demonstrated attenuation of baroreflex-mediated bradycardia after atenolol administration in SHR. The fact that atenolol substantially attenuated the reflex bradycardia in this hypertensive model indicates enhanced adrenergic stimulation of the heart. In this regard, previous studies have demonstrated that administration of NOS inhibitors in anesthetized rabbits (39) and rats (47, 48) results in a sympathetically-mediated tachycardia. In the present study, there was a greater fall in MAP in L-NAME-induced hypertensive than in normotensive control rats in response to prazosin. This greater fall in basal MAP during L-NAME-induced hypertension might be an indirect evidence of sympathetic overactivity in this model of hypertension. However, a recent study from our laboratory demonstrated that the arterial hypertension induced by L-NAME treatment over the course of 2 and 14 days is not associated with sympathetic overactivity (5). One possible explanation for this larger decrease in MAP may be an enhanced vascular reactivity to the normal sympathetic drive, since previous studies demonstrated an increase in vascular responsiveness to
phenylephrine and norepinephrine in rats chronically treated with l-NAME (30, 31).

The results from electrical stimulation of the ADN showed that after prazosin, the baroreflex-mediated hypotension was markedly attenuated in both normotensive control and l-NAME-induced hypertensive rats, whereas the reflex bradycardia was not affected in either group. The attenuation of the baroreflex-mediated hypotension after prazosin might simply be attributed, in part, to the lower levels of arterial pressure in both groups. However, when MAP was returned to basal levels with angiotensin II after prazosin administration, the same diminished response to ADN stimulation was observed. Therefore, the attenuation of baroreflex-mediated hypotension observed after prazosin reflected withdrawal of α1-adrenergic vasoconstrictor tone prior to ADN stimulation. Nevertheless, after α1-adrenergic receptor blockade, electrical stimulation of the ADN produced a residual decrease in MAP that was similar in both groups. One possible explanation for this residual fall in MAP might be attributed to a vasodilation of the hindlimb vasculature resulting from activation of postganglionic lumbar sympathetic vasodilator fibers that release nitrosyl factors, as proposed by Possas et al. (32). However, because the residual fall in MAP due to ADN stimulation was still present in l-NAME-induced hypertensive rats (in which NO production was impaired), it is unlikely that activation of postganglionic lumbar sympathetic vasodilator fibers was responsible for the further decrease in arterial pressure after α1-adrenergic receptor blockade. Therefore, it is likely that some other vasodilatory mechanism unrelated to NO or deactivation of α1-adrenergic receptors was responsible for this residual fall in MAP elicited by ADN stimulation.

The findings from the triple pharmacological blockade (i.e., prazosin, atropine, and atenolol) lent support to the hypothesis that some other vasodilatory mechanism, independent of both NO and removal of the α1-adrenergic mediated vasoconstriction, contributes to fall in MAP elicited by ADN stimulation. As expected, after the triple pharmacological blockade, the baroreflex-mediated bradycardia was abolished, whereas the baroreflex-mediated hypotension was still partially attenuated in both groups. Still, there was a remnant fall in MAP in normotensive control and l-NAME-induced hypertensive rats. The present results are consistent with those obtained by Lohmeier et al. (24), who demonstrated that suppressing central sympathetic outflow by chronic electrical stimulation of the carotid baroreflex had sustained effects to lower arterial pressure even in the presence of complete blockade of α1- and β1,2-adrenergic receptors. These authors (24) suggested that the extra fall in pressure elicited by electrical activation of baroreceptor afferents after prazosin and propranolol administration was probably caused by diminishing attendant activation of postjunctional α2-adrenergic receptors. Thus, in the current study, the mechanism involved in the further decrease of MAP elicited by ADN stimulation after combined blockade of the autonomic nervous system and NO production might involve deactivation of postjunctional α2-adrenergic receptors as proposed by Lohmeier et al. (24). Nonetheless, the possibility that other neuronal and/or humoral mechanisms are involved in this response cannot be ruled out. These neuronal mechanisms may involve other neurotransmitters or cotransmitters present in the sympathetic terminals nerves, such as ATP and neuropeptide Y, which produce smooth muscle cell contractions (49). Thus, when sympathetic activity is reflexly inhibited, the diminished release of these neurotransmitters (P2X receptor for ATP and Y2 receptor for neuropeptide Y) and the reduced stimulation of their receptors may also contribute to vasodilatation. Furthermore, Iliescu and Lohmeier (16), using computer simulations closely reproducing empirical data, demonstrated the potential importance of hormonal, i.e., atrial natriuretic peptide, and renal hemodynamic mechanisms in contributing to lowering arterial pressure during long-term baroreflex activation.

In conclusion, baroreflex-mediated stimulation decreases in MAP and HR in conscious l-NAME-induced hypertensive rats were well preserved during ADN stimulation. Withdrawal of the sympathetic drive plays a role in the reflex bradycardia in l-NAME-induced hypertensive rats but not in normotensive control rats. However, there was no differential hypotensive response to sympathetic inhibition between normotensive control and l-NAME-induced hypertensive rats. The residual fall in pressure after the triple pharmacological blockade suggests the involvement of a vasodilatory mechanism unrelated to NO and diminished stimulation of α1- and β-adrenergic receptors.

**Perspectives**

In the current study, acute electrical stimulation of the ADN in conscious l-NAME-induced hypertensive rats produced a residual fall in pressure after the triple pharmacological blockade (atropine, atenolol, and prazosin), suggesting the involvement of a vasodilatory mechanism unrelated to NO and α1- and β-adrenergic receptors. Similarly, recent observations in chronically instrumented dogs indicated that mechanisms unrelated to diminished activation of α1- and β-adrenergic receptors contribute to the chronic blood pressure-lowering effects of prolonged electrical stimulation of carotid baroreceptor afferents. Therefore, the assessment of the hemodynamic responses to acute electrical stimulation of the ADN in conscious rats has the potential to elucidate previously undefined mechanisms that lower blood pressure in response to inhibition of central sympathetic outflow by activation of the baroreflex. These mechanisms may include not only reduced NE release and diminished activation of postjunctional α2-adrenergic receptors (24) but also decreased release of cotransmitters such as ATP and neuropeptide Y (49). Mechanistic insight into how suppression of central sympathetic outflow lowers arterial pressure is particularly important and timely in light of recent clinical trials indicating that electrical stimulation of baroreceptor afferents represents a safe and effective therapeutic option for the treatment of patients with severe refractory hypertension resistant to pharmacological therapy (13, 43, 50).

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**DISCLOSURES**

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