Sympathetic Baroreceptor Responses After Chronic $N^G$-Nitro-L-Arginine Methyl Ester Treatment in Conscious Rats

Kari E. Scrogin, Roland Veelken, Friedrich C. Luft

Abstract  Blood pressure elevations after nitric oxide inhibition may result in part from increased sympathetic tone. In this study arterial baroreceptor reflex control of heart rate, renal sympathetic nerve activity (RSNA), and adrenal sympathetic nerve activity (ASNA) were compared in rats given normal tap water or a 3.7 mmol/L (10 mg%) solution of $N^G$-nitro-L-arginine methyl ester (L-NAME) for 1 or 5 weeks. L-NAME raised blood pressure after 5 weeks of treatment (153±3 versus 130±3 and 124±2 mm Hg, 5 weeks versus 1 week and control). The sensitivity of arterial baroreceptor reflex control of RSNA was reduced after both 1 and 5 weeks of treatment (−5.05±0.63% and −4.46±0.2% versus −6.43±0.39% baseline activity per millimeters of mercury). Set point gain of ASNA was attenuated after 5 weeks of treatment compared with controls (−1.7±3% versus −3.3±3% baseline activity per millimeters of mercury). Maximal inhibition of ASNA was attenuated in groups treated 1 and 5 weeks (60±3% and 66±3% versus 34±4% baseline activity). The maximal increase in both RSNA and ASNA was elevated in rats treated 5 weeks (RSNA: control, 263±19%; 1 week, 224±17%; 5 weeks, 324±20%; ASNA: control, 272±29%; 1 week, 252±31%; 5 weeks, 361±28% baseline activity). The data indicate that chronic L-NAME treatment alters arterial baroreceptor reflexes in part before the onset of hypertension. (Hypertension. 1994;23[part 2]:982-986.)

Key Words  • nitric oxide  • sympathetic nervous system  • pressoreceptors

Increased blood pressure after nitric oxide (NO) blockade may involve the sympathetic nervous system. NO blockade has been shown to increase renal sympathetic nerve activity (RSNA) in anesthetized rats.1 Similarly, microinjection of NO inhibitors in the nucleus tractus solitarius raised RSNA in anesthetized rabbits,2 and injection of nitroprusside, an NO donor, or the NO synthase substrate L-arginine into vasopressor medullary nuclei decreased RSNA in cats.3 The presence of NO synthase has been verified in the nucleus tractus solitarius,4 vasopressor medullary nuclei,5 spinal intermediolateral nuclei containing preganglionic sympathetic nerve cell bodies,6 and peripheral sympathetic ganglia,7 each of which are sites of integration that contribute to arterial baroreceptor reflex (ABR) control of sympathetic tone8 and thus could be involved in the sympathostimulatory response to NO inhibition. In the present study we examined the effect of chronic NO inhibition with $N^G$-nitro-L-arginine methyl ester (L-NAME) on ABR control of heart rate, RSNA, and adrenal sympathetic nerve activity (ASNA) in conscious rats. We hypothesized that L-NAME treatment would reduce the sensitivity of baroreceptor reflexes.

Methods

Male Sprague-Dawley rats (90 to 110 g) (Ivanovas) were given either normal tap water or an L-NAME solution (3.7 mmol/L)10 for 1 or 5 weeks. L-NAME is known to be orally active and at this dose alters blood pressure and renal function over 4 to 6 weeks.9 Daily water intake and thus drug dose were calculated gravimetrically. After treatment the animals were anesthetized with a bolus dose of methohexital sodium (50 mg/kg IP) followed by a maintenance infusion (0.50 to 1 mg/kg per minute) for placement of femoral venous and arterial catheters and stainless steel bipolar recording electrodes around renal and adrenal sympathetic fiber bundles. All experiments were carried out in accordance with guidelines for the care and use of laboratory animals of the American Association for Accreditation of Laboratory Animal Care.

Nerve activity was amplified (20,000 to 50,000 times) and filtered by a Universal signal conditioner (Gould Electronics) with bandwidth high- and low-pass filters of 100 and 1000 Hz, respectively. Activity was displayed on an oscilloscope (Hameg Instruments) and integrated over 1-second intervals with a Gould integrator.

Renal nerve preparations showing at least a 50% decline in voltage after intravenous methoxamine (20 μg) were included for analysis. Preganglionic adrenal activity was verified by the presence of increased activity after ganglionic blockade with trimethaphan (1.5 mg/kg). Viable nerves were embedded in silicon (Bisco). Voltages measured 30 minutes after rats were killed were subtracted from experimental data to factor out electrical noise.

After surgery the animals recovered for 4 to 6 hours while loosely restrained and were given a rehydrating infusion of saline (12 μL/min).

Mean arterial blood pressure and heart rate were measured with a P23XI pressure transducer (Viggo-Spectromed) and Gould pressure-processing unit. Mean arterial pressure, heart rate, and integrated ASNA and RSNA data were digitized with a computer card (DT2801 series, Data Translation) and sampled at 3 Hz with acquisition software (LabTech Notebook).

Responses to bolus injections of nitroprusside (20 μg) and the ganglionic blocker trimethaphan (1.5 mg/kg) were deter-
minded in each group. ABRs were assessed by measuring changes in heart rate, ASNA, and RSNA during slow ramp increases or decreases in blood pressure by slowly increasing the speed of intravenous administration of 4.0 μmol/L (0.1%) methoxamine or 3.4 μmol/L (0.1%) nitroprusside (1 μL to 10 μL/min). The order of drug infusion was randomized within each group.

For analyses of response to bolus drug injection, baseline mean arterial pressure, heart rate, ASNA, and RSNA were subtracted from peak responses. Because variable numbers of nerve fibers are found in individual whole-nerve preparations, nerve activity was expressed as percent baseline. Baroreceptor curves relating the percent change from baseline for heart rate, RSNA, and ASNA were constructed by combining the pressor and depressor responses for each animal and fitting the data into the following formula: Y = A + B/(1 + exp(-MAPl); where Y equals heart rate or nerve activity; A, the lower plateau of Y; B, the range in Y; C, the mean arterial pressure (MAP) at midrange of B (MAPa); and D, a coefficient for the determination of gain. Maximal gain (G) was determined for each curve with the equation G = -B / D/4. Gain curves were produced by taking the first derivative of logistic function.10 Curve fitting was performed via computer (SIGMA PLOT 4.1, Jandel Scientific).

All parameters were averaged over treatment groups. Treatment effects for all data were tested by one-way ANOVA. Specific group differences were determined by least-squares difference multiple range tests. Probability values of less than .05 were considered significant. All statistical analyses were performed with STATGRAPHICS software (Version 5).

**Results**

There was no difference in water intake between L-NNAME–treated and control animals (15.4±0.9 versus 16.1±4 mL/100 g body wt). In L-NNAME–treated rats this provided a daily dose of 15.4 mg/100 g body wt. L-NNAME increased blood pressure in animals treated 5 weeks compared with those treated 1 week and control rats (control, 124±2; 1 week, 130±3; 5 weeks, 153±3 mm Hg). Neither heart rate (control, 392±26; 1 week, 399±14; 5 weeks, 372±14 beats per minute) nor body weight (control, 320±10; 1 week, 300±9; 5 weeks, 304±15 g) differed among groups. Both L-NNAME–treated groups showed a larger depressor response to nitroprusside compared with controls, whereas rats treated 5 weeks had a larger response than those treated 1 week (control, -58±2; 1 week, -71±4; 5 weeks, -83±2 mm Hg). Ganglionic blockade lowered blood pressure more in 5-week L-NNAME rats than in either 1-week or control rats (control, -59±3; 1 week, -51±4; 5 weeks, -76±6 mm Hg).

The Table shows the group means of the parameters used to determine the sigmoidal functions in Figs 1 through 3. As seen in Fig 1 (top) the sensitivity of the response was attenuated in the group treated 5 weeks due to the reduced slope. The bottom portion of Fig 1 depicts the instantaneous heart rate gain over a range of blood pressure values. In this figure the reduced slope is clearly depicted by the lowered maximal gain of the group treated 5 weeks. Although the range was increased in animals treated 1 week, the slope of the response was slightly reduced, thus precluding any change in maximal gain.

ABR control of RSNA is shown in Fig 2 (top). A resetting of the baroreceptor response was accompanied by a reduced sensitivity in animals treated 5 weeks. Both L-NNAME–treated groups had an attenuated maximal gain compared with controls (Fig 2, bottom). In the group treated 1 week the reduced maximal gain was due to the smaller range of RSNA. However, in the rats treated 5 weeks the slope of the response rather than the range was significantly reduced.

Baroreceptor reflex control of ASNA is shown in Fig 3 (top). Although maximal gain did not differ between L-NNAME–treated or control rats, animals treated 5 weeks did show reduced gain at the set point blood pressure compared with controls (-1.7±3% versus -3.3±0.4% baseline activity per millimeters of mercury), indicating a lack of baroreceptor resetting. This is
Discussion

We sought to determine whether chronic NO blockade with L-NAME alters ABRs independent of hypertension. Although 5 weeks of treatment was required to produce hypertension, just 1 week was sufficient to alter ABR responses. Maximal sensitivity of the renal sympathetic reflex was reduced in rats treated both 1 and 5 weeks, whereas only those treated 5 weeks exhibited decreased sensitivity of reflex heart rate control. Although L-NAME had no effect on maximal gain of the adrenal reflex, gain at the set point pressure was reduced in animals treated 5 weeks. Rats treated both 1 and 5 weeks showed an attenuation of adrenal sympathetic inhibition, whereas the maximal increase in renal and adrenal activity was higher in rats treated 5 weeks.

The data indicate that L-NAME altered baroreceptor control of heart rate, RSNA, and ASNA by attenuating sensitivity and the capacity to buffer against increases in pressure.

The hypertensive effect of chronic L-NAME treatment was first described by Baylis et al.9 who found that 2 months of treatment increased blood pressure and renal vascular resistance and produced glomerular hypertension in conjunction with glomerular sclerosis. Increased plasma renin activity is observed during chronic NO blockade,11,12 whereas the hypertensive response to chronic NO inhibition is prevented by concurrent angiotensin II receptor blockade.12,13 Furthermore, angiotensin II blockade prevents the decrease in glomerular filtration and renal plasma flow caused by acute NO inhibition.14 Renal denervation attenuates the decreased glomerular filtration rate and renal plasma flow associated with acute L-NAME administration,15 suggesting a role for the sympathetic system in the renal effects of NO inhibition.

The increased depressor responses to nitroprusside of rats treated 1 and 5 weeks indicate indirectly that NO was inhibited in treated groups. This probably reflects the upregulation of the NO receptor, soluble guanylate cyclase, in response to lowered NO levels.16 Results in
blood pressure, which could lead to more frequent and mals treated 1 week indicate that L-NAME reduces the and the attenuated renal sympathetic reflex gain in ani-

adrenal sympathetic and heart rate baroreceptor reflexes influence the hypertensive response to NO inhibition. The notion that reduced baroreceptor responsiveness may

arterial sympathetic and heart rate baroreceptor reflexes to buffer increasing pressure. The differing responses of the renal and adrenal nerve reflexes to treatment may reflect a differential influence of NO on the volume regulatory (renal) and sympathoadrenomedullary reflexes. Furthermore, the elevated maximal renal and adrenal sympathetic responses to hypotension demonstrate that the treatment depresses the capacity of the sympathetic nervous system to buffer against pressor responses but enhances its ability to respond to depressor changes.

This study is the first to examine the effects of chronic NO inhibition on ABRs. Earlier investigations showed that acute NO blockade in the nucleus tractus solitarius had no effect on reflex renal sympathetic responses in anesthetized rats. Furthermore, acute intravenous NO blockade increased renal sympathetic baroreceptor gain in conscious rats. These results diverge from our own probably because of the differing affects of chronic and acute NO blockade on baroreceptor reflexes. NO inhibits mitogenesis and proliferation of vascular smooth muscle cells in culture. Chronic but probably not acute inhibition of such a process, if occurring in vivo, could affect vascular distensibility and thus baroreceptor responses. Moreover, chronic loss of soluble guanylate cyclase stimulation causes an upregulation of the receptor, which could alter second messenger activity in neural tissue involved in sympathetic transmission. Such responses would not be expected to occur during acute NO inhibition.

The attenuated maximal and set point gain of the baroreceptor reflexes as well as the elevated minimum and maximum plateaus of RSNA and ASNA observed in treated groups probably resulted from the direct effects of L-NAME rather than indirectly via its hypertensive effects. Prolonged exposure to high blood pressure is thought to alter baroreceptor reflexes by reducing the distensibility of vessels housing the baroreceptors. However, recent data indicate that 6 weeks of exposure to an L-NAME dose fourfold greater than that used in our study did not alter carotid artery distensibility in Wistar rats. Thus, it is unlikely that the lesser dose and shorter exposure to L-NAME used in the present study produced vascular stretch-mediated baroreceptor activation.

In summary, we showed that chronic L-NAME treatment altered baroreceptor reflexes regulating heart rate, RSNA, and ASNA and reduced the capacity of sympathetic reflexes to buffer increasing pressure.

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