Chronic nitric oxide inhibition with L-NAME: effects on autonomic control of the cardiovascular system

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Scrogin, Karie E., Daniel C. Hatton, Yue Chi, and Friedrich C. Luft. Chronic nitric oxide inhibition with L-NAME: effects on autonomic control of the cardiovascular system. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R367–R374, 1998.—To determine whether increased sympathetic activity contributes to the hypertensive effects of hypertension-induced by chronic exposure to moderate nitric oxide synthase (NOS) inhibition, various indexes of autonomic function were measured in rats given the NOS inhibitor N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME, 10 mg/100 ml, =16 mg·kg\textsuperscript{-1}·day\textsuperscript{-1}) in the drinking water. One week of treatment raised blood pressure (139 ± 3 vs. 106 ± 1 mmHg; \(P < 0.01\)) and lowered heart rate (319 ± 4 vs. 379 ± 6 beats/min; \(P < 0.01\)). L-NAME had no effect on cardiac sympathetic tone, but elevated cardiac parasympathetic tone (–73 ± 4 vs. –56 ± 7 beats/min; \(P < 0.05\)). Depressor responses to ganglionic blockade were greater in L-NAME-treated rats (–50 ± 5 vs. –34 ± 5 mmHg; \(P < 0.05\)), whereas resting plasma, renal, and adrenal catecholamine values did not differ between groups. Treated rats also showed evidence of reduced baroreflex sympathetic stimulation of heart rate during hypotension and reduced parasympathetic activation during hypertension. Together, these data provide only very limited, indirect evidence that sympathetic stimulation contributes to the hypertension associated with moderate NOS inhibition.

chronic N\textsuperscript{G}-nitro-L-arginine methyl ester; baroreflex

NITRIC OXIDE SYNTHASE (NOS) is the rate-limiting enzyme involved in the conversion of L-arginine to nitric oxide (17). Nitric oxide (NO) has a number of effects in various cells, the most well characterized of which involves relaxation of vascular smooth muscle cells in response to NO-mediated activation of soluble guanylyl cyclase and the subsequent formation of guanosine 3\',5\'-cyclic monophosphate (9). Chronic treatment with the orally active NOS inhibitor N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME) has recently received great interest as a model of hypertension. The inhibition of NO-mediated vasodilation is recognized as the major factor contributing to the hypertensive effects of L-NAME (5). However, the NOS enzyme has also been identified in many sites of the central and peripheral nervous system involved in autonomic control of the cardiovascular system, prompting speculation that interruption of neuronal NOS function may also contribute to the hypertensive effects of L-NAME (4, 7, 12).

Functional studies have suggested that nitric oxide acts within the central nervous system to regulate sympathetic output. Intravenous infusion of L-NAME was found to decrease the activity of baroreceptor-sensitive neurons in the nucleus of the solitary tract, whereas L-arginine infusion reversed the effect (14). In addition, NO donors were found to have sympatholytic effects, and NOS inhibitors sympathoexcitatory effects, when microinjected into pressor or depressor regions of the medullary vasomotor center (22). In contrast, within the peripheral nervous system, NO could potentially increase sympathetic activity via its ability to facilitate neurotransmission in sympathetic ganglia and suppress baroreceptor sensitivity in the carotid sinus (1, 15). Given the seemingly opposing influence of NO in the central and peripheral nervous systems, it is of interest to determine the overall effect of chronic NOS inhibition on the autonomic regulation of the cardiovascular system.

Prior studies examining the effects of chronic NOS inhibition on autonomic function have provided contradictory results. It has been suggested that a sympathoexcitatory response to chronic L-NAME treatment develops over several weeks and therefore is not an immediate response to NO inhibition (10). In contrast, other reports suggest that an initial profound sympathoexcitatory response develops within 1 wk of drug treatment (3, 23). Such contradictory results likely result from differences in drug dose as well as the parameters measured and the method of measurement. In the present study, autonomic responses were studied in rats given a moderate dose of L-NAME over a relatively short duration (1 wk) to avoid the confounding effects of hypertension-induced end-organ damage. The present study was performed to test the hypothesis that hypertension produced by a moderate dose of L-NAME administered over a relatively short duration is not supported by increased sympathetic activity. To test this hypothesis, several indexes of autonomic function were evaluated in conscious, surgically recovered rats while they remained unrestrained in their home cage to minimize the interaction between the drug regime and surgical stress. In this way, it was predicted that results would reflect more the direct effects of NO inhibition on autonomic control of cardiovascular parameters rather than secondary effects due to stress and hypertension.

METHODS AND MATERIALS

Drugs. Methoxamine hydrochloride, sodium nitroprusside, atropine methyl nitrate and L-NAME were obtained from Sigma (Deisenhofen, Germany). The L-NAME was diluted in tap water (10 mg/100 ml) and provided ad libitum. Methohexital sodium, used for surgical anesthesia, was obtained from Eli Lilly (Indianapolis, IN).

Animals. All animals used in these studies were male Sprague-Dawley rats (Moellegard, Schoenwalde, Germany) weighing between 250 and 300 g. On arrival the rats were randomly assigned to one of two groups given either normal tap water or L-NAME-supplemented water. The drug solution

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was changed daily just before the beginning of the dark cycle. The animals were housed individually in plastic cages except during measurements of food and water intake, during which they were housed in individual metabolic cages. All animals were kept in the animal care facility under constant temperature (22°C) with a light-dark cycle of 12 h.

Measures taken of food and water intake and urinary excretion. To determine L-NAME consumption and to control for variations of food intake on NO metabolite excretion, animals were housed in metabolic cages for the measurement of food and water intake. Additional measurement of sodium and creatinine excretion were made for determination of renal function parameters. After 72-h habituation to the metabolic cages, food and water intake as well as urine volume and body weight were measured daily. An initial 24-h period was used as a baseline measurement, after which experimental animals were given L-NAME in their drinking water. After completion of the seventh 24-h clearance period, the animals were removed from the metabolic cage and anesthetized with ether. A 1-ml blood sample was taken via subclavian vein puncture. An identical volume of saline was given subcutaneously, and the animals were returned to a regular plastic cage. The catheter was advanced into the lower part of the ascending aorta for direct mean arterial pressure and heart rate (HR) measurements. Analog BP and HR signals were fed into an analog-to-digital conversion card (DT2801; Data Translation, Marlboro, MA), calibrated, and recorded using data acquisition software (LabTech Notebook; Laboratory Technologies, Wilmington, MA).

Measurement of blood pressure and heart rate. To determine resting baseline blood pressure (BP) and heart rate (HR), additional groups of animals were treated with either L-NAME or normal tap water for 7 days while housed in regular plastic cages. While under methohexitol sodium anesthesia (50 mg/kg ip), the rats were instrumented with femoral venous and arterial catheters constructed from PE-50 tubing filled with heparinized saline. The arterial catheter was advanced into the abdominal aorta for direct mean arterial pressure (MAP) determinations while the venous catheter was advanced into the lower part of the ascending vena cava to allow intravenous drug delivery. Both catheters were exteriorized behind the nape of the animal’s neck. After surgery, the rats were returned to the home cage for recovery. All experiments were performed at least 72 h after surgery. During the experiment, direct BP measurements were made from the arterial catheter connected with a pressure transducer (model P23XL; Viggo-Spectramed, Bilthoven, Netherlands) while the animals rested unrestrained in their home cage. The signal was amplified and calibrated with a Gould BP amplifier (Gould Electronics, Dietzenbach, Germany). HR was monitored through an internal ratemeter within the BP amplifier. Analog BP and HR signals were fed into a analog-to-digital conversion card, calibrated, and recorded using data acquisition software.

BP reactivity and HR baroreflex determinations. To determine the effect of L-NAME on BP reactivity to exogenous vasoactive drugs and to determine baroreflex HR responses to changes in BP, the rats were connected to the pressure transducer 72 h after surgery. An additional length of PE-50 tubing was connected to the venous catheter for drug administration. After at least 1 h of habituation, BP and HR responses were measured during administration of graded doses (5, 10, 15, 20, and 30 µg/kg) of methoxamine or nitroprusside while the animals remained unrestrained in their home cage. The dose of drug was administered in random order and was always given in a 100-µl volume of saline. At least 5 min were allowed between injections. Only one drug was tested per day. Responses to the alternate drug were tested the following day. The order of drug administration was randomized in both treatment groups. At the end of the second day of testing, BP and HR responses to a bolus dose of the ganglionic blocker trimethaphan (1.5 mg) were measured. We have previously found this dose of trimethaphan sufficient to achieve a maximal inhibition of postganglionic renal sympathetic nerve activity in conscious rats (21).

Determination of autonomic control of HR. To determine the individual influence of the sympathetic and parasympathetic limbs of the autonomic system on resting HR, the same animals were again connected to the BP transducer while resting unrestrained in their home cage. After at least 1 h of habituation, HR was measured 10 min after administration of either the β1-adrenergic receptor antagonist metoprolol (0.5 mg/kg iv) or the muscarinic receptor blocker atropine methyl nitrate (0.2 mg/kg iv) as described by Cunha et al. (3). Rats were then tested for HR and BP responses to 20 µg/kg methoxamine and 5 µg/kg nitroprusside to determine the sympathetic and parasympathetic contribution to each limb of the HR baroreceptor reflex. These doses of vasoactive drugs were chosen because they produce pressor and depressor effects that initiate baroreflex HR responses in the steep portion of the baroreflex curve. At least 5 min after BP and HR returned to baseline levels, the animals were given the alternate autonomic blocking agent (i.e., atropine or metoprolol) to determine the intrinsic HR. The experiment was repeated 24 h later with autonomic drugs given in the reverse order.

Plasma, renal, and adrenal catecholamine determinations. To determine the effects of L-NAME on circulating and tissue catecholamine content, separate groups of animals were treated with L-NAME as previously described. They were fitted with venous and arterial catheters as described and allowed to recover for 72 h. BP and HR were measured while the animals rested unrestrained in their home cage. Thirty minutes after the beginning of BP measurements, a 0.5-ml blood sample was drawn from the arterial line into a chilled 1.5-ml microfuge tube preinursed with heparinized saline. The plasma was immediately extracted by refrigerated centrifugation and filtration and stored at −80°C for later analysis. The following day, the animals were killed with a guillotine, and...
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their left kidney and both adrenal glands were immediately removed and frozen in liquid nitrogen.

Plasma catecholamines were extracted as previously described (6). Weighed kidneys and adrenal glands were homogenized in ice-cold volumes of 10 and 1 ml 0.3 M perchloric acid, respectively, and centrifuged at 2,400 g for 15 min at 4°C. The supernatant was frozen and treated in the same fashion as plasma. Plasma and tissue catecholamine concentration was determined by means of high-performance liquid chromatography with electrochemical detection (Bioanalytical Systems 400, West Lafayette, IN).

Data analysis. Measurements of food and water intake as well as urine volume excretion were made gravimetrically. Sodium and creatinine clearance were determined by dividing 24-h sodium or creatinine urinary excretion by plasma sodium or creatinine concentration. All data, except glomerular filtration rate (GFR), fractional sodium excretion, and NO metabolite excretion, were analyzed with repeated-measures analysis of variance (ANOVA). Data obtained at day 0 (before L-NAME treatment) and day 6 were treated as repeated measures, and the presence or absence of L-NAME in drinking water was treated as the between-group factor. Other metabolic and cardiovascular data in which only a single measurement was taken were analyzed with unpaired t-tests.

BP reactivity to nitroprusside and methoxamine was determined by repeated-measures ANOVA with drug dose as the within-group factor and L-NAME treatment as the between-group factor. One-way ANOVAs and Bonferroni post hoc tests were performed to determine at which doses BP reactivity differed between groups.

Baroreflex curves were constructed for each animal by fitting the peak BP and HR responses to bolus doses of methoxamine and nitroprusside into a logistic curve function. The curve was constructed by using a least-squares regression analysis to fit the data to the following logistic function: 

\[
Y = \frac{A}{1 + e^{(X-MAP_\text{set point})/D}}
\]

where A equals the range of HR over the resulting sigmoidal curve, B equals a parameter for determining gain, C equals MAP at the inflection point of the resulting curve, and D equals the asymptotic minimum. Best-fit curves were determined for each animal, and the resulting parameters were used to calculate the maximal gain \([A(B)/(A\times 4.56)]\) as described by Howe et al. (8). Bradycardic range was calculated as the difference between lower plateau and baseline HR. Tachycardic range was calculated by subtracting the baseline HR from the sum of the HR range and lower plateau. Instantaneous gain over the range of MAP was determined by taking the first derivative of the individual curves. Gain at the set point BP was determined for each animal by solving for Y at the set point BP in the best fit of the derivative curve according to the Gaussian expression \(Y = (k/s)\exp(-X^2/2s^2)\), where Y equals instantaneous gain, X equals the set point BP, k equals kurtosis, s equals skewness, and m equals MAP at midrange (MAP_{50}).

The parasympathetic control of resting HR was determined by subtracting the HR observed after \(\beta_1\)-adrenergic receptor blockade with metoprolol from that determined after blockade with both atropine and metoprolol. Sympathetic tone was determined by subtracting measurements taken after vagal blockade from basal HR measurements determined after combined metoprolol and atropine administration. Sympathetic and parasympathetic control of baroreflex HR responses were determined by calculating the change in HR divided by the change in BP produced by methoxamine or nitroprusside after atropine or metoprolol, respectively.

RESULTS

Intake and excretion data are shown in Table 1. Exposure to L-NAME in the drinking water did not influence either 24-h food intake or body weight. By the end of 6 days, there was a significant decrease in sodium intake and an increase in water intake in rats treated with L-NAME in their drinking water. There was no change in urinary sodium excretion, and no significant change in body weight in rats treated with or without L-NAME (Table 1).

Table 1. Body weight, 24-h food and water intake, and urinary excretion data before and after 6 days of exposure to L-NAME or normal tap water

<table>
<thead>
<tr>
<th></th>
<th>L-NAME (n = 12)</th>
<th>Control (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>Day 6</td>
<td>Day 0</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>266 ± 3</td>
<td>266 ± 3</td>
</tr>
<tr>
<td>24-h Food intake, g</td>
<td>23.9 ± 1.0</td>
<td>23.6 ± 1.0</td>
</tr>
<tr>
<td>24-h Water intake, ml</td>
<td>44.6 ± 2.5</td>
<td>39.2 ± 3.1</td>
</tr>
<tr>
<td>24-h Urine volume, m</td>
<td>11.6 ± 1.7</td>
<td>11.3 ± 0.5</td>
</tr>
<tr>
<td>GFR, ml/min</td>
<td>1.32 ± 0.09</td>
<td>1.13 ± 0.08</td>
</tr>
<tr>
<td>Na\text{E}, mg/mg</td>
<td>0.0063 ± 0.0003*</td>
<td>0.0076 ± 0.000</td>
</tr>
</tbody>
</table>

Values are means ± SE. GFR, glomerular filtration rate; Na\text{E}, fractional sodium excretion; L-NAME, N 3- nitro-L-arginine methyl ester. Control rats were given normal tap water. Symbols represent significant differences between groups. Separate between-group comparisons were made for days 0 and 6: *P < 0.05, †P < 0.01.

Table 2. Blood pressure, HR, and catecholamine data in rats treated for 7–10 days with L-NAME or normal tap water

<table>
<thead>
<tr>
<th></th>
<th>L-NAME</th>
<th>Control</th>
</tr>
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<tbody>
<tr>
<td>Baseline MAP, mmHg</td>
<td>139 ± 3†</td>
<td>106 ± 3</td>
</tr>
<tr>
<td>Baseline HR, beats/min</td>
<td>319 ± 4†</td>
<td>379 ± 6</td>
</tr>
<tr>
<td>MAP after ganglionic blockade, mmHg</td>
<td>90 ± 7</td>
<td>72 ± 5</td>
</tr>
<tr>
<td>%ΔMAP, mmHg</td>
<td>50 ± 5*</td>
<td>34 ± 5</td>
</tr>
<tr>
<td>%ΔMAP, %</td>
<td>36 ± 4</td>
<td>32 ± 5</td>
</tr>
<tr>
<td>Plasma NE, pg/ml</td>
<td>354 ± 69</td>
<td>366 ± 21</td>
</tr>
<tr>
<td>Renal NE, ng/mg tissue</td>
<td>1281 ± 6.4</td>
<td>1237 ± 10.6</td>
</tr>
<tr>
<td>Adrenal NE, ng/mg tissue</td>
<td>96 ± 7</td>
<td>120 ± 17</td>
</tr>
<tr>
<td>Adrenal Epine, ng/mg tissue</td>
<td>366 ± 28</td>
<td>334 ± 49</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8. MAP, mean arterial pressure; HR, heart rate; NE, norepinephrine; Epi, epinephrine; Δ, change. Comparisons were made between groups: *P < 0.05, †P < 0.01.
sixth day of L-NAME exposure, control animals showed a significant decrease in urinary output compared with treated rats. This was due to a progressive decline in urine output among the control group, while urine output of the L-NAME-treated group remained constant at the higher level across the 6 days (data not shown). There was a tendency for increased GFR in L-NAME-treated rats ($P < 0.07$) and a significant decrease in fractional sodium excretion compared with controls. In treated animals, water intake provided an average daily dose of 1.59 mg L-NAME per 100 g body wt, or $16 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$. Figure 1 shows the individual values for combined urinary excretion of nitrite and nitrate normalized for creatinine excretion in control and L-NAME-treated animals. Treated animals showed a 37% decrease in NO metabolite excretion compared with controls ($2.8 \pm 0.2$ vs. $4.5 \pm 0.2 \mu\text{mol/mg creatinine}$, $P < 0.01$). This was not due to a difference in creatinine excretion because both groups excreted the same amount of creatinine over the 24-h measurement period ($7.72 \pm 0.33$ vs. $7.28 \pm 0.42 \mu\text{g/24 h}$).

As shown in Table 2, L-NAME treatment elevated BP and reduced resting HR. Ganglionic blockade caused a larger absolute decrease in BP among L-NAME-treated animals compared with controls. Although the difference in percent decrease after ganglionic blockade was still larger in L-NAME-treated rats, this difference did not reach significance. Resting measurements of plasma, renal, and adrenal catecholamines did not differ between treatment groups.

Figure 2 depicts the BP responses to increasing doses of sodium nitroprusside and methoxamine. Rats treated with L-NAME showed exaggerated BP responses to both drugs. Figure 3 depicts the baroreflex curves relating the change in HR as a response to changes in BP for the two groups and the instantaneous gain over the range of MAP for each group. Values of parameters used to determine the average curves are shown in Table 3. The baroreflex response was reset to the higher resting baseline BP in L-NAME-treated rats, as indicated by the increased MAP$_{50}$. L-NAME-treated rats also had a reduced maximal reflex gain as well as a reduced gain at the set point BP compared with controls. Although the range of the reflex response did not differ between groups, there was a significant shift of the curve downward, as indicated by the significantly reduced lower plateau in L-NAME-treated rats.

Figure 4 demonstrates typical HR and BP responses of individual control and L-NAME-treated rats following β-adrenergic receptor blockade with metoprolol and subsequent muscarinic receptor blockade with atropine. As demonstrated in Fig. 4, the L-NAME-treated
rat had a lower baseline HR, as did the treated group as a whole. Both control and L-NAME animals responded similarly to metoprolol, but subsequent atropine administration caused a larger HR increase in L-NAME-treated animals (Fig. 4). The gain of the bradycardic reflex response to methoxamine was attenuated in L-NAME-treated rats, likely because of the already low baseline HR. The difference in intrinsic HR (i.e., HR after both atropine and metoprolol) and HR observed after either β1-adrenergic blockade (parasympathetic tone) or muscarinic blockade (sympathetic tone) alone. Comparisons were made between groups: *P < 0.05, †P < 0.01.

Reflex HR responses to changes in BP before or after either parasympathetic blockade with atropine or sympathetic blockade with metoprolol are shown in Table 5. The gain of the bradycardic reflex response to methoxamine was attenuated in L-NAME-treated rats, likely because of the already low baseline HR. The difference was normalized after parasympathetic, but not sympathetic, blockade, suggesting that excessive parasympathetic tone was responsible. When both autonomic reflex limbs were intact, the gain of reflex HR responses to nitroprusside did not differ between groups. However, blockade of the parasympathetic limb with atropine uncovered an attenuated sympathetic stimulation of HR in L-NAME-treated rats.

**DISCUSSION**

In the present study it was found that 1 wk of exposure to a moderate dose of the NOS inhibitor L-NAME was sufficient to provide a partial blockade of NO production, as evidenced by the 37% decrease in NO metabolite excretion. Reduced nitrite and nitrate excretion observed with L-NAME treatment was not due to lower arginine intake because both groups were found to eat similar amounts of food and to increase body weight at the same rate. The lower urinary volume observed in controls compared with L-NAME rats after 1 wk was due to a progressive decline in excretion among control rats, while L-NAME-treated rats maintained a consistent volume excretion. It is not clear why urinary excretion decreased in control animals because they drank and ate the same amount consistently throughout the week. Although difficult to explain, the drop in urine excretion likely did not result from stress, because rats gained weight at a normal rate over the week of metabolic measurements. A similar drop in urinary excretion in the L-NAME-treated could have been counteracted by pressure-induced diuresis, resulting in maintenance of the same excretion rate throughout the week.

Indications of a pressure-dependent diuresis in L-NAME-treated rats is provided by data demonstrating a tendency for a larger GFR in L-NAME-treated rats compared with controls. However, sodium clearance was reduced despite the tendency for a larger GFR, resulting in the lower sodium filtration fraction with L-NAME treatment. This finding suggests that tubular sodium reabsorption was increased in L-NAME-treated rats. The mechanism for elevated sodium reabsorption was not clear, although the lack of difference in renal catecholamine content between groups would suggest that increased renal sympathetic tone did not account for the difference in sodium reabsorption.
known whether the effects of anesthesia or surgical
above studies tested rats only 6 h after surgery. It is not
used or differences in methodology. In our studies,
suggests further that sympathetic tone in general was
difference in plasma, renal, or adrenal catecholamines
study.

L-NAME-treated rats theoretically could have been
lessened parasympathetic cardiac tone. The contradic-
tive vasoconstrictor tone after only 1 wk.

L-NAME treatment also increased the BP respon-
sitivity to adrenergic-receptor stimulation. Therefore,
abnormal HR recovers because of increased
L-NAME, whereas continued daily treatment increased
plasma catecholamines after 28 days. In the same
study, ganglionic blockade did not completely abolish
the difference in BP between groups 12 days after the
beginning of treatment whereas it totally abolished the
difference after 28 days, suggesting that only prolonged
NOS inhibition had sympathostimulatory effects.

The present study does provide evidence that NO
may modulate cardiac parasympathetic tone. L-NAME-
treated rats showed a decreased basal HR compared
with control rats as well as an exaggerated increase in
HR with atropine administration after β-adrenergic
blockade. Although the bradycardic effect found in this
study could have been due exclusively to hypertension-
induced baroreflex activation, resetting mechanisms
would be expected to have returned HR back to normal
within a few days, as has been demonstrated in other
experimental models of hypertension (18). In the pre-
cent study, set point HR in L-NAME-treated rats was
situated on the lower portion of the baroreflex curve,
suggesting that bradycardia was partially mediated by
baroreflex activation. However, the obvious shift of
the entire baroreflex curve downward suggests that addi-
tional changes in autonomic function were responsible
for the persistent bradycardia. Previous reports have
suggested that the effects on HR disappear with more
prolonged exposure to L-NAME. Rats treated daily with
seven times the dose of L-NAME used in this experi-
ment showed a lower HR throughout the 4-wk study,
although the difference between treated and untreated
groups was only significant through the first week of
treatment (16). Similarly, a 20 mg/kg dose of L-NAME
lowered HR in rats 24 h after treatment, but HR was
normalized by the end of the study 25 days later (11).

Given the delayed onset of sympathostimulation specu-
lated to occur with continued NOS inhibition (10), it is
possible that normal HR recovers because of increased
cardiac sympathetic tone, although it is also possible
that increased parasympathetic tone wanes with time.
In a previous study from our laboratory, L-NAME given
at the same dose did not alter HR after either 1 or 5 wk

for an increased sodium reabsorption in L-NAME-
treated rats. Indeed, Reinhart et al. (19) found that
decreased sodium reabsorption observed during acute
L-NAME infusion was not influenced by renal denerva-
tion in the dog. Moreover, the same study indicated that
renal denervation had no influence on sodium excretion
during prolonged (5 day) L-NAME infusion at a dose (14
mg·kg⁻¹·day⁻¹) comparable to that used in the present
study.

In the present study, NOS inhibition did not appear
to influence sympathetic cardiac tone. The lack of
difference in plasma, renal, or adrenal catecholamines
suggests further that sympathetic tone in general was
not altered with L-NAME treatment. However, these
findings cannot rule out the possibility that L-NAME
had regional sympathoexcitatory effects that could not
be discerned by measurement of circulating, renal, or
adrenal catecholamines. Indeed, the larger drop in BP
after ganglionic blockade among L-NAME-treated rats
suggests that increased sympathetic vasoconstrictor
tone may have contributed to the hypertension. How-
ever, L-NAME treatment also increased the BP respon-
siveness to adrenergic-receptor stimulation. Therefore,
the larger decrease in BP with ganglionic blockade in
L-NAME-treated rats theoretically could have been
due, at least in part, to the removal of normal neuro-
genic vascular tone from more responsive vessels.

The lack of substantial evidence for an L-NAME-
dependent increase in sympathetic tone is at odds with
other studies. Cunha et al. (3) found that 1 wk of
exposure to L-NAME in drinking water at a dose 10
times that given in the present study significantly
increased sympathetic control of HR and virtually
abolished parasympathetic cardiac tone. The contradic-
tory results observed between their study and our own
may be due either to differences in the dose of L-NAME
used or differences in methodology. In our studies,
animals were allowed at least 3 days of recovery before
cardiovascular parameters were tested, whereas the
above studies tested rats only 6 h after surgery. It is not
known whether the effects of anesthesia or surgical

stress may be exaggerated by L-NAME treatment, but
the higher incidence of hindlimb ischemia at the site of
catheter placement in L-NAME-treated animals ob-
served by us (unpublished observation) suggests that
there may be a confounding effect of stress on cardiovas-
cular measurements in this model of hypertension.
However, an additional study (20) showed that chemi-
cal sympathectomy substantially attenuated the hyper-
tensive effect of 1 wk of exposure to a high dose of
L-NAME (80 mg·kg⁻¹·day⁻¹) in animals given 5 days to
recovery from catheter implantation. This additional
study lends further support to the notion that very high
doses of L-NAME will significantly increase sympa-
thetic vasoconstrictor tone after only 1 wk.

Increasing evidence suggests that the sympa-
thostimulatory effect of more mild NO inhibition devel-
ops only after prolonged exposure to the inhibitor and is
therefore not likely a direct effect of NOS inhibition.
Joblonski and Howe (10) showed that plasma catechol-
amines were reduced 24 h after a 37 mg/kg dose of
L-NAME, whereas continued daily treatment increased
plasma catecholamines after 28 days. In the same
study, ganglionic blockade did not completely abolish
the difference in BP between groups 12 days after the
beginning of treatment whereas it totally abolished the
difference after 28 days, suggesting that only prolonged
NOS inhibition had sympathostimulatory effects.

Table 5. Sympathetic and parasympathetic
coronary contribution to baroreflex control

<table>
<thead>
<tr>
<th></th>
<th>L-NAME (n = 10)</th>
<th>Control (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MX (20 µg/kg)</td>
<td>NP (5 µg/kg)</td>
</tr>
<tr>
<td>without drug</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atropine (0.2 g/kg)</td>
<td>-0.65 ± 0.06†</td>
<td>-3.02 ± 0.21</td>
</tr>
<tr>
<td>Metoprolol (0.5 g/kg)</td>
<td>-0.37 ± 0.08</td>
<td>-1.27 ± 0.04*</td>
</tr>
<tr>
<td>Metoprolol (0.5 g/kg)</td>
<td>-0.43 ± 0.04*</td>
<td>-1.49 ± 0.33</td>
</tr>
</tbody>
</table>

Values are means ± SE. Values represent the maximal change in
HR (beats/min) divided by maximal change in MAP (mmHg) after a
bolus dose of methoxamine (MX) or sodium nitroprusside (NP)
without prior drug, after muscarinic-receptor blockade with atropine,
or after β-adrenergic-receptor blockade with metoprolol. Separate
between-group comparisons were made for responses to MX and NP:
*P < 0.05, †P < 0.01.
of treatment. It is likely that the contradictory findings in our two studies relate to the method of measurement. In our prior study, measurements were made in restrained rats 6 h after surgery (21). The HR of control rats in the present study were comparable to those in our previous study, but rats treated with L-NAME in the present study had a much lower HR in comparison to those examined in the past report, suggesting that L-NAME treatment may render animals more reactive to stresses such as surgery or restraint.

The lowered baseline HR observed with L-NAME treatment in this study reflected a shift of the total HR baroreflex curve downward. Alterations in baroreflex function associated with hypertension are typically characterized by an attenuation of reflex vagal HR slowing, resulting in a blunted reflex range and gain (8). However, baroreflex changes associated with NOS inhibition do not follow this pattern. In the present study, moderate inhibition of NOS caused changes in both limbs of the autonomic system, resulting in the downward shift of the baroreflex curve. After parasympathetic blockade, tachycardic responses to hypotension were impaired in L-NAME-treated rats indicating a reduced baroreflex-mediated sympathetic activation. In contrast, the tachycardic response did not differ between groups after sympathetic blockade, indicating normal parasympathetic withdrawal. In contrast, bradycardic responses to increased pressure were reduced after sympathetic blockade, indicating reduced parasympathetic stimulation in L-NAME-treated rats. This observation was likely due to the impaired capacity to lower HR during increased pressure as indicated by the limited bradycardic reflex range noted in L-NAME-treated rats. Overall, these changes resulted in reduced maximal and set point gain. These results stand in marked contrast to those of Vasquez et al. (23). In their study, L-NAME treatment (>60 mg·kg\(^{-1}\)·day\(^{-1}\) over 6 days) caused a significant increase in the HR range and reflex gain, apparently due to an exaggerated sympathetic cardiac tone. This discrepancy between studies may reflect a dose-dependent effect of L-NAME on sympathetic activation or differences in postsurgical recovery time. In another report, rats given 50 times the dose of L-NAME as given in the present study reduced reflex gain without changes in basal HR after 4 wk (13). It may be that the longer duration of treatment used in their study allowed for full resetting of the HR baroreflex concomitant with structural changes in the vasculature that may have reduced baroreceptor sensitivity.

It should be noted that physiological responses to L-NAME treatment are typically assumed to result from the specific effects of NOS inhibition. However, researchers have noted that some NOS inhibitors, particularly L-NAME, show antagonistic effects on muscarinic receptors that could potentially confound interpretation of results using these compounds. Nonetheless, the same report indicated that L-NAME had an affinity of between 65 and 235 µM for muscarinic receptor subtypes in various rat tissues. In the present study, rats drank ~55 µmol·kg\(^{-1}\)·day\(^{-1}\). Given the relatively short half-life of L-NAME, it would seem unlikely that plasma L-NAME levels would have ever reached concentrations sufficient to have any effect on muscarinic receptors at the dose given in this study.

The majority of reports addressing the role of NO in autonomic function have utilized acute inhibition of the NOS enzyme. Results of these acute studies suggest that NO acts within the central nervous system to tonically inhibit sympathetic tone. Despite these findings, the present study indicates that continued exposure to NOS inhibition sufficient to significantly reduce NO formation by one-third does not have direct cardiac sympathostimulatory effects, but rather enhances parasympathetic cardiac tone. Furthermore, this study provides only very limited indirect evidence that moderate L-NAME-induced hypertension is mediated by sympathovasconstrictor tone.

**Perspectives**

This study provides new data regarding the ability of NOS inhibition to modify the parasympathetic control of the heart. Although in this study the response of the cardiac parasympathetic nervous system was described, it is quite possible that parasympathetic control of other functions are also affected by NOS inhibition. As yet, studies of the effects of chronic NOS inhibition have focused predominantly on the control of the cardiovascular system. Because the majority of neural control of the cardiovascular system comes from the sympathetic innervation, little emphasis has been paid to the influence of NO on the control of the parasympathetic nervous system. As suggested by data in this study, it appears that NO may have a more profound inhibitory influence on the parasympathetic nervous system. However, such an effect has yet to be carefully examined.

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