L-NAME- and ADMA-induced sympathetic neural activation in conscious rats

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Augustyniak, Robert A., Ronald G. Victor, Donald A. Morgan, and Weiguo Zhang. L-NAME- and ADMA-induced sympathetic neural activation in conscious rats. Am J Physiol Regul Integr Comp Physiol 290: R726–R732, 2006. First published October 20, 2005; doi:10.1152/ajpregu.00768.2004.—Although studies in anesthetized, sino-aortic denervated animals indicate that inhibition of central nitric oxide (NO) causes an excitatory influence on efferent sympathetic nerve activity (SNA) that is normally offset by baroreflex activation, studies in conscious animals have not provided clear-cut evidence for a sympathoexcitatory effect of N\(^\text{\text{n}}\)-nitro-l-arginine methyl ester (l-NAME) or the endogenous circulating NO synthase (NOS) inhibitor asymmetric dimethylarginine (ADMA). Thus our goals were to 1) use surgical sino-aortic denervation to test for a sympathoexcitatory effect of intravenous l-NAME in conscious rats, and 2) to determine whether SNA responses to intravenous l-NAME can be extrapolated directly to intravenous ADMA. We recorded mean arterial blood pressure and renal SNA in both intact and sino-aortic-denervated conscious rats during 3 h of continuous intravenous infusion with either l-NAME or ADMA. When we eliminated the confounding influence of the sino-aortic baroreceptors, l-NAME produced a progressive increase in SNA with the peak response exceeding the baseline level of nerve firing by 150%. The same type of frank sympathetic activation was observed with intravenous ADMA. Taken together, these data offer straightforward evidence for l-NAME, as well as ADMA-induced sympathetic activation with direct recordings of SNA in conscious animals. These data confirm and extend the concept that circulating endogenous NOS inhibitors can constitute an excitatory signal to SNA.

References

1. baroreceptor reflex; neurogenic hypertension; sympathetic nervous system; sino-aortic denervation; asymmetric dimethylarginine

THE NITRIC OXIDE (NO) pathway is generally considered to be one of the most important regulatory mechanisms that defends against arterial hypertension (3, 15, 40). In the peripheral vasculature, NO is firmly established to constitute the main endothelium-derived relaxing factor (6, 27). In addition, NO has been hypothesized to act centrally to exert a tonic inhibitory influence on sympathetic nerve activity (SNA); reduction in this tonic restraint has been postulated to contribute to the hypertension caused by synthetic NO synthase (NOS) inhibitors such as N\(^\text{\text{n}}\)-nitro-l-arginine methyl ester (l-NAME) (29, 41, 49), as well as by the endogenous circulating NOS inhibitor asymmetric dimethylarginine (ADMA) (2, 40). We and others previously have found that in conscious rats, l-NAME-induced hypertension is significantly attenuated by sympathectomy (31, 32) or ganglionic blockade (4, 37, 47), providing indirect evidence that NOS inhibition increases SNA.

However, during intravenous NOS inhibition, directly recorded SNA has been reported both to increase (26) and decrease (7, 10, 51). In anesthetized rats, intravenous l-NAME (7, 10) and \(N^G\)-methyl-l-arginine (29) have been shown to increase SNA after surgical sino-aortic (baroreceptor) denervation (SAD), indicating that systemic inhibition of NO causes an excitatory influence that is normally offset by baroreflex activation. However, intravenous l-NAME has never been shown to increase SNA in conscious animals. With intravenous l-NAME, blood pressure increased and SNA decreased reflexively in both conscious rats (17) and rabbits (19). When a vasodilator drug was infused with l-NAME to clamp blood pressure and thus baroreflex activation, SNA returned toward baseline but did not exceed the baseline levels (19, 24). Thus previous studies in conscious animals have not provided clear-cut evidence for a sympathoexcitatory effect of l-NAME, raising concerns as to whether such data from anesthetized animals can be extrapolated to the conscious state.

From a translational standpoint, this is an important issue because ADMA accumulation constitutes a putative mechanism of certain forms of clinical hypertension (1, 2, 5, 34, 35, 40, 42, 44). Most of the previous animal studies have used synthetic NOS inhibitors, with the assumption being that their mechanism of action is identical to that of ADMA. However, with intracerebroventricular injection, blood pressure previously was found to increase as expected with l-NAME but decreased unexpectedly with ADMA (13).

Accordingly, the goals of this study were to 1) test for a sympathoexcitatory effect of l-NAME in conscious rats using surgical SAD to eliminate the confounding influence of the baroreceptors; and 2) determine whether SNA responses to l-NAME can be extrapolated directly to ADMA. The data herein provide straightforward evidence that in conscious rats with SAD, both l-NAME and ADMA produce unequivocal increases in renal SNA.

METHODS

Methods used were in accordance with institutional guidelines, and all protocols were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Texas Southwestern Medical Center.

Animals

Female Sprague-Dawley rats (225–350 g) were chosen for the present study. Although responses in male rats may be different, a previous study from our laboratory did not detect any gender differences in the sympathectomy-sensitive component to the blood pressure-raising effect of l-NAME (53). All animals were housed in a temperature-controlled room on a 12:12-h light-dark cycle and received regular rodent chow and tap water ad libitum.

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Surgical Procedures

Sino-aortic baroreceptor-intact female Sprague-Dawley rats (225 to 350 g) were anesthetized with methohexital sodium (60 mg/kg ip), and the right jugular vein and left carotid artery were cannulated. The left renal nerve was accessed through a retroperitoneal flank incision and glued to a pair of bipolar platinum electrodes, as described previously (53). Before closing, the electrode lead and catheters were tunneled subcutaneously, and exited at the nape of the neck.

Sino-aortic baroreceptor-denervated female Sprague-Dawley rats were denervated at least 2 wk before the experiment following the procedure of Krieger (16). To avoid reentering the neck region, femoral arterial and venous catheters were inserted into the left femoral artery and vein. Before beginning baseline data collection, SAD was deemed complete when a 20 mmHg rise in blood pressure with phenylephrine caused ≤20 beats/min decrease in heart rate (i.e., a gain of less than −1.0 beats/min/mmHg) (18, 25). In the baroreceptor-intact rats, a 24 ± 1 mmHg rise in blood pressure caused heart rate to decrease by −80 ± 8 beats/min. In the sino-aortic-denervated rats with the same rise in blood pressure (23 ± 1 mmHg), the bradycardic response was −11 ± 3 beats/min. Because there was still a small remaining decrease in heart rate in our sino-aortic-denervated rats, there were apparently some residual baroreceptor fibers that remained intact (36), although the reflex decrease in heart rate was attenuated by 80%. The renal nerve recording was performed as described above.

Experimental Protocols

Either 4 h or 24 h after surgery (see results), baseline data were collected in conscious, freely moving rats for 1 h, and then a continuous 3-h infusion of either a specific drug or saline was started at a rate of 0.5–1 ml/h.

Protocol 1: NOS inhibition with L-NAME in baroreceptor-intact and sino-aortic-denervated rats. In the first group of rats, the pharmacological NOS inhibitor L-NAME (2.5 mg/kg·h−1, n = 6) was infused at a dose that raised mean blood pressure 20–25 mmHg. In the second group, the α-adrenergic agonist phenylephrine (50 μg/ml, n = 6) was infused to produce the approximate hypertensive effect of L-NAME. In the third group, D-NAME (2.5 mg/kg·h−1, n = 9), the inactive enantiomer of L-NAME was infused. In the fourth group, L-NAME (2.5 mg/kg·h−1, n = 5) was infused into sino-aortic denervated rats.

Protocol 2: NOS inhibition with ADMA in baroreceptor-intact and sino-aortic-denervated rats. In the first group, the endogenous NOS inhibitor ADMA was infused at 60 mg·kg−1·h−1 (n = 8). In a second group, phenylephrine (n = 5) was infused to produce the approximate hypertensive effect of ADMA. In a third group, ADMA (60 mg·kg−1·h−1, n = 7) was infused into sino-aortic-denervated rats. In the fourth group, saline (n = 5) was infused at 1 ml/h into sino-aortic-denervated rats to control for volume and time.

Data and Statistical Analysis

Mean arterial pressure (MAP), heart rate, and renal SNA were averaged for a duration of 5 min at 30-min intervals. The nerve activity after either a bolus injection of phenylephrine to raise mean blood pressure −50 mmHg in 30 s or 30 min postmortem was considered as the systemic noise of the recording and subtracted from the calculation. In the sino-aortic-denervated rats, the remaining nerve activity after intravenous injection of the ganglionic blocker trimethaphan (5 mg/kg) was subtracted from the calculation. Basal nerve activity was normalized to 100%. All values are expressed as means ± SE. Statistical analyses were performed with either a one-way ANOVA or a two-way ANOVA for repeated measures, followed by Newman-Keuls test. A value of P < 0.05 was considered statistically significant.

RESULTS

L-NAME infusion in baroreceptor-intact rats (Fig. 1 and Table 1) increased MAP 25 ± 2 mmHg by 30 min, and this pressor response was maintained throughout the experiment. Renal SNA initially decreased to ~25% of baseline during the first hour of infusion; however, it then began to progressively return such that the last three time points were not different from baseline (120, 150, and 180 min; P = NS vs. baseline). Heart rate followed a very similar response pattern. When the hypertensive effect of L-NAME was approximated with phenylephrine (Table 1), renal SNA rapidly decreased to ~25% of baseline; however, in stark contrast, renal SNA remained depressed for the remainder of the infusion. On the contrary, after initially showing a sharp decrease, heart rate tended to return toward baseline, and the response pattern was very similar to what occurred during L-NAME infusion. D-NAME had no significant effect upon any variable (Table 1). In Fig. 1. Summary data showing the mean arterial pressure, heart rate, and renal sympathetic nerve activity profile during continuous intravenous infusion of Nω-nitro-L-arginine methyl ester (L-NAME) in baroreceptor-intact rats (n = 6, left) and baroreceptor denervated rats (n = 5, right). Note that L-NAME infusion in baroreceptor-denervated rats caused an immediate and profound increase in renal sympathetic nerve activity (SNA) that was largely inhibited, whereas the baroreceptors were intact. The dotted line represents baseline nerve activity. The solid horizontal bar represents duration of infusion. For clarity, the data from two other control groups (phenylephrine infusion and the inactive enantiomer of L-NAME, D-NAME, infusion in intact rats) are omitted (see Table 1 for more information). *P < 0.05 vs. baseline; †P < 0.05 vs. baroreceptor intact.
baroreceptor-denervated rats (Fig. 1 and Table 1), baseline MAP was not different from baroreceptor-intact rats. However, in the baroreceptor-denervated rats, the effect of l-NAME upon renal SNA was directionally opposite of that in baroreceptor-intact rats with an immediate increase that was significant at 30 min and progressively rose further to 150 ± 41% above baseline at 180 min. There was no significant change in heart rate, although it tended to decrease slightly. ADMA infusion in baroreceptor-intact rats (Fig. 2 and Table 2) increased MAP 15 ± 2 mmHg by 30 min and ~20 mmHg at all time points thereafter. This hypertensive response was accompanied initially by a significant decrease in renal SNA (decreased to ~70% at 30–90 min). After 90 min of ADMA infusion, renal SNA returned toward baseline. Heart rate showed a small, but significant, decrease that was evident throughout the infusion period. Increasing MAP with phenylephrine (Table 2) to a similar magnitude as with ADMA evoked a significantly greater sympathoinhibitory response in renal SNA (60 min renal SNA = 26 ± 3% of baseline), which was maintained throughout the duration of the phenylephrine infusion (180 min renal SNA = 23 ± 8% baseline; P < 0.05 for all time points vs. ADMA). The heart rate response pattern during phenylephrine was very similar to that during ADMA infusion. In baroreceptor-denervated rats, ADMA (Fig. 2 and Table 2) caused MAP to increase to a similar extent as in baroreceptor-intact rats. In these same rats, ADMA had no significant effect upon renal SNA through 90 min, at which point there was a trend to increase. At 120, 150, and 180 min, renal SNA was significantly higher compared with ADMA infusion in baroreceptor-intact rats, and, importantly, at 180 min, renal SNA was 46 ± 17% above baseline (180 min, P < 0.05 vs. baseline). ADMA had no major effect on heart rate. Saline was infused into a separate group of baroreceptor-denervated rats (Table 2) to control for volume and time and had no significant effect upon any variable.

We were concerned that the effects of anesthesia and surgical stress in rats experimented upon 4 h after surgery could impact our results. Thus some baroreceptor-intact animals were experimented on 24 h after surgery. In the l-NAME group, at the 180-min time point, the change in blood pressure...
was 23 ± 3 mmHg (4 h, n = 4) vs. 26 ± 1 mmHg (24 h, n = 2), while the renal SNA for the 4-h group was 84 ± 14% vs. the 24-h group, which was 92 ± 8%. In the baroreceptor-intact ADMA group, at the 180-min time point, the change in blood pressure was 21 ± 1 mmHg (4 h, n = 5) vs. 23 ± 3 mmHg (24 h, n = 3), whereas the renal SNA for the 4-h group was 98 ± 26% vs. the 24-h group, which was 101 ± 32%. Thus the responses were very similar whether experimented upon 4 h or 24 h after surgery.

**DISCUSSION**

Circulating NOS inhibitors are hypothesized to act centrally and reduce the tonic restraint on SNA, but previous conscious animal studies have been unable to show that SNA exceeds the baseline level of nerve firing during intravenous infusion of l-NAME, a potent NOS inhibitor. Similarly, in conscious rats with intact baroreceptors, we found that intravenous l-NAME produced a rapid and sustained increase in blood pressure, whereas SNA decreased initially and then returned slowly toward, but never exceeded, the baseline level, suggesting but not proving a late relative sympathoexcitation. Our major new findings are twofold. First, when we eliminated the confounding influence of the sino-aortic baroreceptors with surgical SAD, in conscious rats, l-NAME produced a progressive and unequivocal increase in SNA, with the peak response exceeding the baseline level of nerve firing by 150%. Second, the same type of frank sympathetic activation was observed with intravenous ADMA, the endogenous NOS inhibitor. Taken together, these data offer straightforward evidence for l-NAME, as well as ADMA-induced sympathetic activation with direct recordings of SNA in conscious animals. The data confirm and extend the concept that the circulating endogenous NOS inhibitor can constitute an excitatory signal to SNA.

Our initial experiments with intravenous l-NAME or ADMA demonstrate the difficulty in testing this hypothesis in rats with intact baroreceptors. That renal SNA initially decreased sharply and to the same level when blood pressure was increased with either of the NOS inhibitors or phenylephrine suggests that the initial sympathoinhibition is a simple baroreflex. That renal SNA slowly returned to baseline over the next 2 h after l-NAME or ADMA, but remained suppressed with phenylephrine, is consistent with an l-NAME- or ADMA-induced relative sympathoexcitation. This biphasic response in renal SNA during systemic NOS inhibition has been previously reported in conscious rats (17, 24). However, this initial set of experiments was not conclusive for several reasons. First, by the end of either the l-NAME or ADMA infusion, renal SNA had returned to the baseline level of nerve firing, but never exceeded it. So, there was no frank sympathetic excitation. Second, phenylephrine may not be the ideal negative internal control for the blood pressure-raising effect of l-NAME or ADMA in these experiments. Prolonged intravenous infusion of phenylephrine and other α-adrenergic agonists has been shown to cause central potentiation of baroreflex suppression of renal SNA (11). Although the increase in blood pressure with phenylephrine closely matched that of the l-NAME or ADMA groups, we cannot exclude the possibility that the baroreflex-mediated inhibition of renal SNA was greater with phenylephrine than with either NOS inhibitor. So, these experiments do not distinguish whether the differentiated pattern of renal SNA response to these pressor agents is explained by a special property of l-NAME or ADMA (i.e., central sympathoexcitation) or by a special property of phenylephrine (i.e., central potentiation of the inhibitory baroreflex). In contrast to renal SNA, the heart rate response patterns for either l-NAME or ADMA were nearly identical to their respective phenylephrine controls. This is most logically explained by baroreflex resetting of heart rate to the prevailing level of blood pressure.

One method that has been used to avoid the confounding effects of systemic hypertension and baroreflex activation during intravenous infusion of l-NAME has been concurrent intravenous infusion of the vasodilator hydralazine. Liu et al. (19) recorded renal SNA in conscious rabbits and found that intravenous l-NAME increased blood pressure and reflexively decreased renal SNA, whereas the return of blood pressure to baseline with hydralazine caused renal SNA to return to, but not exceed, baseline. This suggested that l-NAME alone did not exert sympathoexcitatory effects upon renal SNA. On the contrary, McKeogh et al. (24) recently suggested that the predominant effect of systemic NOS inhibition is to decrease SNA to both the heart and kidneys. That interpretation was
based upon findings in conscious rats, in which there was an initial reduction in renal SNA and a progressive decrease in heart rate during intravenous infusion of l-NAME, as well as hydralazine, which was used to clamp blood pressure (24). Although both studies were elegantly performed, there are potential limitations to the use of hydralazine to normalize blood pressure. First, it is difficult to know the extent to which baroreflex activation was prevented or normalized, and even residual baroreflex activation could have obscured the hypothesized l-NAME-induced sympathetic excitation. In addition, although hydralazine would be expected to cause reflex sympathetic activation, previous studies have shown that intravenous hydralazine itself can decrease renal SNA and heart rate in rats (52), as well as humans (23). Thus the use of hydralazine to avoid baroreflex activation could partially offset or even eliminate an l-NAME-induced increase in SNA.

In this regard, surgical sino-aortic denervation has an advantage in that it removes the influence of the baroreflex. When we infused l-NAME in baroreceptor-denervated rats, the initial sympathoinhibitory effect that was observed in baroreceptor-intact rats was abolished, proving that it was baroreflex mediated, and in fact, we observed an initial rise in renal SNA, which after 60 min, increased progressively to nearly 150% above baseline. During ADMA infusion, there was not the initial significant decrease that was seen in baroreceptor-intact rats, but rather a delayed rise in renal SNA that reached nearly 50% above baseline. Thus the present SAD experiments provide unequivocal evidence that either l-NAME or ADMA increases SNA in conscious SAD rats. That heart rate remained relatively unchanged in our experiments suggests that systemic l-NAME and ADMA have a smaller effect on SNA to the heart than to the kidneys.

These data confirm and extend the current body of literature in two important ways. First, previous findings in anesthetized animals, which demonstrated that NOS inhibitors administered either centrally (9, 48, 50) or via intravenous infusion in sino-aortic-denervated animals (7, 10, 26, 29, 41) caused an increase in SNA above the baseline level of neural firing, can now be extrapolated to the conscious state. Second, although it was previously shown in anesthetized rats that intracerebroventricular administration of l-NAME and ADMA exerted directionally opposite effects (hypertension with l-NAME and hypotension with ADMA) (13), our data in conscious rats demonstrate that intravenous l-NAME and ADMA exert qualitatively similar hypertensive and sympathetic neural effects. There are two potential explanations for these discrepant findings: 1) the former study (13) was performed under pentobarbital sodium anesthesia and 2) with intravenous infusion (as done in our study), NOS inhibitors may not gain access to the same central neuronal pools that they do with intracerebroventricular injection.

Interestingly, the delay in the onset of this increase in renal SNA is entirely consistent with several previous studies from our laboratory suggesting that the neurogenic component of the blood pressure effect to systemic NOS inhibition is delayed from 60 to 90 min after the onset of infusion (30, 31, 33). Presumably, this is the time required for the systemic inhibitors to cross the blood-brain barrier and inhibit NOS in the relevant neuronal pools (43). In this regard, one previous study found that intravenous infusion of N\textsuperscript{G}-nitro-l-arginine in conscious, sino-aortic denervated rabbits had no effect upon renal SNA; however, responses were only observed for 40 min after NOS inhibition (20). In our experiments, in which ADMA was infused into sino-aortic-denervated rats, we would have arrived at the same conclusion had we stopped our observation period at 40 min.

There are limitations to our study that should be addressed. First, because we infused the drugs intravenously, we cannot specify the site(s) of NOS inhibition that led to the rise in renal SNA. In this regard, afferent, central, or efferent neural mechanisms could all be involved (8, 9, 21, 28, 38, 39, 45, 48, 50). Second, although SAD interrupts baroreceptor influence, chronic SAD may also lead to alterations in central neural transmission within brainstem nuclei, such as the nucleus tractus solitarius, which could modulate cardiovascular responses (12). With that in mind, our results are entirely consistent with the idea that intravenous NOS inhibition leads to a sympathoexcitatory response that is normally offset by baroreflex activation. Third, although the rats in our current study were conscious, they were not yet fully recovered from surgery. However, we do not believe this affected our results because in our former study (31), the sympathectomy-sensitive component to l-NAME was found to accrue over a period of days in unstressed rats. Therefore, with longer-term recordings of renal SNA, it would likely either remain elevated or continue to rise in response to l-NAME or ADMA, even while potential effects of surgical stress wane. Finally, it may appear unusual that the late increase in renal SNA during NOS inhibition, particularly in the SAD groups, were not accompanied by a further rise in blood pressure. In our previous study (31), the sympathectomy-sensitive component to l-NAME-induced hypertension after 3 h of l-NAME accounted for only 18% of the total hypertensive response; however, after 6 days, it accounted for 61%. Therefore, because the time frame of our current study was only 3 h, the sympathetic component to the rise in blood pressure in either the baroreceptor-intact or the SAD rats would be expected to be small and difficult to resolve in experiments that were not designed to do so. It is conceivable that the main mechanism through which the rise in renal SNA contributes to the blood pressure raising effect of NOS inhibition is not through adrenergically mediated vasoconstriction but rather through increased sodium reabsorption at the distal tubule. This would occur over a period of days, not hours. In this regard, l-NAME-induced hypertension is known to be salt-sensitive (3, 46).

Taken together, the data from these experiments with intravenous l-NAME, and particularly with intravenous ADMA, suggest that a circulating endogenous inhibitor of NOS can exert an excitatory influence on SNA. In some clinical settings such as renal failure (22, 54), atherosclerosis (14), and preeclampsia (14), elevated plasma ADMA levels have been implicated in the pathogenesis of hypertension. Further studies are needed to determine whether ADMA-induced sympathetic activation is a mechanism that contributes to hypertension in such patients.

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GRANTS

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