Role of renal sympathetic nerve activity in hypertension induced by chronic nitric oxide inhibition

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Role of renal sympathetic nerve activity in hypertension induced by chronic nitric oxide inhibition. Am J Physiol Regul Integr Comp Physiol 292: R1479–R1485, 2007. First published January 11, 2007; doi:10.1152/ajpregu.00435.2006.—Nitric oxide levels are diminished in hypertensive patients, suggesting nitric oxide might have an important role to play in the development of hypertension. Chronic blockade of nitric oxide leads to hypertension that is sustained throughout the period of the blockade in baroreceptor-intact animals. It has been suggested that the sympathetic nervous system is involved in the chronic increase in blood pressure; however, the evidence is inconclusive. We measured renal sympathetic nerve activity and blood pressure via telemetry in rabbits over 7 days of nitric oxide blockade. Nitric oxide blockade via Nω-nitro-L-arginine methyl ester (L-NAME) in the drinking water (50 mg·kg−1·day−1) for 7 days caused a significant increase in arterial pressure (7 ± 1 mmHg above control levels; P < 0.05). While the increase in blood pressure was associated with a decrease in heart rate (from 233 ± 6 beats/min before the L-NAME to 202 ± 6 beats/min on day 7), there was no change in renal sympathetic nerve activity (94 ± 4 % baseline levels on day 2 and 96 ± 5 % baseline levels on day 7 of L-NAME; baseline nerve activity levels were normalized to the maximum 2 s of nerve activity evoked by nasopharyngeal stimulation). The lack of change in renal sympathetic nerve activity during the L-NAME-induced hypertension indicates that the renal nerves do not mediate the increase in blood pressure in conscious rabbits.

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NITRIC OXIDE (NO) levels are diminished in hypertensive patients, suggesting that NO might have an important role to play in the pathogenesis of hypertension (5, 23, 36). Chronic blockade of NO synthase leads to hypertension that is sustained throughout the period of the blockade in baroreceptor-intact animals (7, 28). It has been suggested that the sympathetic nervous system is involved in the chronic increase in blood pressure; however, the evidence is inconclusive. In studies where ganglionic blockade has been used to assess the sympathetic tone (7, 32, 34), a greater decrease in blood pressure has been observed in NO-blockade-induced hypertensive animals than controls, supporting a role of the sympathetic nervous system. Likewise, it has been shown that guanethidine-induced sympathectomy results in an attenuation of the hypertension observed in response to chronic NO blockade (31). In contrast, studies using measurement of plasma catecholamine levels found no changes in catecholamine levels with chronic NO synthase inhibition, indicating no role for the sympathetic nervous system in this model of hypertension (16, 34). No previous study has directly measured chronic levels of sympathetic nerve activity (SNA) to test the hypothesis that SNA is increased with NO synthase inhibition. Direct recordings of SNA are needed to resolve the conflicting evidence regarding the role of the sympathetic nervous system in the NO-blockade-mediated hypertension.

Recently, we explored the role of the baroreflex in the hypertension induced by chronic NO blockade. Baroreceptor-intact and sinoaortic-denervated rabbits, chronically instrumented for measurement of blood pressure and heart rate were subjected to 7 days of NO blockade via L-NAME in the drinking water (26). While blockade of NO in the baroreceptor-intact animals led to a chronic increase in pressure, blockade of NO in the sinoaortic-denervated rabbits led to only a transient increase with blood pressure returning to control levels by day 5 of the NO inhibition. This intriguing result suggested that the baroreflex played an important permissive role in the hypertension induced by chronic NO blockade. The reason behind the recovery of blood pressure in the baroreceptor-denervated animals was not determined in this previous study. Given the preeminent role of the kidneys in blood pressure regulation and the observation that renal denervation attenuates hypertension in many models of hypertension (13, 14, 41), it was speculated that renal SNA (RSNA) might have increased with chronic NO synthase inhibition.

To further clarify the role of the baroreflex and directly test the hypothesis that SNA is increased with chronic NO blockade, we measured RSNA and blood pressure chronically via telemetry in baroreceptor-intact rabbits over 7 days of NO blockade.

METHODS

Animal preparation. Experiments were conducted in New Zealand White rabbits with initial weights of 2.4–3.5 kg. Protocols were submitted to and approved by the University of Auckland Animal Ethics Committee. The rabbits were housed individually in cages (height, 45 cm; width, 72 cm; and depth, 72 cm) with a telemetry blood pressure receiver (model RLA2000; Data Sciences, International, St Paul, MN) positioned on the roof of each cage. The rabbits were fed daily (100 g standard rabbit pellets, supplemented with hay, carrot, and apple) at 0900, and tap water was available ad libitum. The room was kept at a constant temperature (18°C) and 12:12-h light-dark cycle (lights on from 0600 to 1800).

Each animal underwent surgery to implant an arterial pressure transmitter and nerve amplifier. Anesthesia was induced by using intravenous administration of propofol (Diprivan; 10 mg/kg) followed by intubation and then maintenance with halothane. Arterial pressure
was recorded throughout the study via a radio telemetry transmitter (model PA-D70; Data Sciences). The transmitter was implanted via an abdominal incision, and the area around the iliac bifurcation was exposed. The cannula of the transmitter was inserted into a branch of the left iliac artery and advanced so that the tip of the catheter lay in the abdominal aorta 3 cm above the iliac bifurcation but well below the renal artery. The cannula was tied into position, the body of the transmitter was placed in the abdominal cavity, and the incision was closed. RSNA was recorded by using a telemetry-based implantable nerve amplifier (model 2003/01; Telemetry Research, Auckland, New Zealand). This was achieved via a flank incision with the electrodes coiled around the left renal nerve, and the electrode and nerve coated in a silicone elastomer (Kwik-sil; World Precision Instruments). To avoid movement artifacts affecting the RSNA signal, the implantable amplifier was placed as close to the nerve site as possible. After surgery the rabbits were treated prophylactically with an antibiotic (enrofloxacin, Baytril; Bayer) (5 mg/kg sc daily for 5 days) and analgesic (ketoprofen, Ketofen; Rhone Merieux, Essex, UK) (2 mg/kg sc daily for 3 days). As soon as the rabbits regained consciousness they were returned to their home cages. A heating pad was placed in the cage for 24 h after the surgery.

**Experimental protocol.** Prior to commencing experiments, the fluid and food intake was monitored to ensure each animal had resumed normal feeding patterns after the surgery. Recovery from surgery was also confirmed by the evidence of a stable circadian variation in arterial pressure and heart rate, which was usually observed after 5–7 days. Blood pressure, heart rate, and RSNA were measured 24 h a day. After animals had recovered from surgery, they were monitored for 5 days of baseline recordings before blockade of NO was induced by oral administration of L-NAME (Sigma) at a dose of 50 mg·kg⁻¹·day⁻¹. L-NAME was added to the drinking water every day and was adjusted so that the daily L-NAME intake was ~50 mg·kg⁻¹·day⁻¹ (±4 mg·kg⁻¹·day⁻¹). After administration of L-NAME for 7 days, arterial pressure, heart rate, and RSNA were monitored for a further 5 days when tap water was given to the rabbits.

**Baroreflex responses.** Baroreflex responses were determined in response to infusions of phenylephrine and sodium nitroprusside on four occasions during the protocol in each rabbit: prior to start of L-NAME, on days 3 and 7 of L-NAME and then 4 days after removal of L-NAME from the drinking water. In each case, testing of the baroreflex responses involved placing the rabbits in a small box within the home cage to allow intravenous lines to be inserted into the medial ear vein so that the vasoactive drugs could be administered. Sodium nitroprusside (1 mg/ml) was slowly infused to reduce arterial pressure down to ~45 mmHg at a rate of 0.5–1 mmHg/s. All variables were then allowed to return to baseline before phenylephrine (1 mg/ml) was infused to raise arterial pressure at a rate of 0.5–1 mmHg/s to between 120 and 140 mmHg (when SNA was silent). These sequences were repeated at least 3 times on each occasion. In addition, on each occasion, the heart rate and sympathetic responses to nasopharyngeal stimulation were assessed by exposing the rabbit to cigarette smoke dispensed by a 50-ml syringe for 2 s. Nasopharyngeal stimulation was used to assess the stability of the signal over time (4) and ensure that NO synapse inhibition did not affect signal stability.

In addition, to assess whether the hypertension induced by L-NAME may be mediated by the renin-angiotensin system, on each occasion prior to determination of baroreflex responses, arterial blood was sampled for measurement of plasma renin activity. Briefly, 1 ml of blood was drawn in a chilled syringe and collected in an Eppendorf tube with 50 μl of EDTA. This was immediately spun in a temperature-controlled centrifuge (–4°C) at 2,500 rpm for 10 min and stored at –20°C. The samples were sent for assay of plasma renin activity in dry ice (Dr. Steve Fisher, Endolab, Christchurch Cardioendocrine Research Group, Christchurch, New Zealand).

**Data collection.** RSNA was amplified, filtered between 50 and 5,000 Hz, full-wave rectified, and integrated using a low-pass filter with a time constant of 20 ms. Arterial pressure was recorded via telemetry, and heart rate was derived from the arterial pulse. All data were sampled at 500 Hz by using an analog-to-digital data acquisition card (model no. AT-MIO64E-3; National Instruments, Austin, TX). All subsequent data collection and analyses were performed using a data acquisition program (Universal Acquisition and Analysis, version 11; Telemetry Research). Unless otherwise stated, data presented represent the mean of the 2-s averages of heart rate and blood pressure for each treatment period, namely baseline, NO blockade, and recovery.

The 2-s averages of mean arterial pressure (MAP), heart rate, and RSNA during the baroreflex curves were collected, and a general nonlinear regression program was used to fit the collected MAP-heart rate and MAP-RSNA data to a sigmoidal logistic function to produce baroreflex curves. The noise levels for the SNA signal were taken to be the integrated value when blood pressure was high with phenylephrine and no bursts were evident in the raw SNA signal. The program uses a five-parameter nonlinear regression equation to produce the resultant baroreflex curves (29).

The equation of the model is:

\[ y = \frac{P1}{1 + f_x e^{-e_x P4}} + (1 - f_x) e^{-e_x P5} \]

where P1 is the second plateau, the value of the response at maximum blood pressure, P2 is the range of response, and P1 + P2 gives the first plateau. P3 and P5 are the two curvature parameters, which enable the curve to be nonsymmetrical. P4 corresponds to BP0, the MAP at the mid-point of the curve.

\[ f_x = \frac{1}{1 + e^{-(n_x P4)}} \]

defines a logistic weighting function (fx) varying smoothly between 0 and 1 and centered about the BP0 (29).

**Statistical analysis.** Baseline nerve activity was defined as the mean value of the 2-s averages of the 4 days prior to L-NAME administration. Daily means of RSNA were then calculated from the 2-s averages and then expressed as a percentage of the baseline. All raw data were analyzed using an analysis of variance, with planned contrasts based on baseline, 1-L-NAME, and recovery periods, and Bonferroni post hoc pairwise comparisons where appropriate. The tests were considered significant if \( P < 0.05 \). Data are shown as the means ± SE. To examine within-animal values for analysis of the baroreflex curves, all RSNA values were normalized to the maximum 2 s of RSNA evoked by the 50 ml of smoke, with the response to the smoke nominated as 100 normalized units (n.u.) (4).

**RESULTS**

**Changes in baseline variables during L-NAME.** NO blockade via L-NAME in the drinking water for 7 days caused a significant increase in arterial pressure (\( n = 7 \)). The mean increase reached 7 ± 1 mmHg above control levels (84 ± 2 mmHg on day 2 vs. 77 ± 2 mmHg on day 0, \( P < 0.05 \)) by day 2 of L-NAME intake and thereafter remained steady throughout the entire infusion period (Figs. 1 and 2). Reversal back to tap water led to a slow return of arterial pressure to pre-L-NAME levels. Addition of L-NAME to the drinking water was not associated with a change in water intake. We believe the dose of L-NAME resulted in a near-maximal blockade, as preliminary experiments showed that doubling the dose of L-NAME had no further effect on arterial pressure. NO blockade caused a significant decrease in heart rate (from 233 ± 6 beats/min before the L-NAME to 187 ± 5 beats/min on day 2 and 202 ± 6 beats/min on day 7, \( P < 0.05 \), data being the mean over the 24-h period). Blockade of NO was not accompanied by a change
in plasma renin activity levels (1.49 ± 0.5 nmol·l⁻¹·h⁻¹ before L-NAME vs. 1.3 ± 0.2 nmol·l⁻¹·h⁻¹ after L-NAME).

RSNA in two of the seven rabbits showed increasing noise levels with time, and thus the SNA recordings from these rabbits were excluded. In the five rabbits analyzed, there was no change in RSNA during L-NAME infusion (Fig. 2). RSNA was at 94 ± 4% of baseline levels on day 2 and 95 ± 5% of baseline levels on day 7 of L-NAME. There was no change in RSNA values when the animals were reverted back to tap water. The response to the nasopharyngeal stimulus (smoke) remained consistent within each rabbit, with the maximum nerve activity reached not varying on the four different days it was assessed (the mean RSNA response to the smoke stimuli was 121 ± 9 au (arbitrary units) before L-NAME, 122 ± 10 au on day 3, 105 ± 6 au on day 7 of L-NAME, and 105 ± 5 au during recovery) (Fig. 3).

Changes in the arterial baroreflex during NO blockade. The baroreflex relationship between the MAP-RSNA, (Fig. 4) was during recovery (Fig. 3). During L-NAME (from 30 days to 7 days of L-NAME) and 105 ± 5 au during recovery) (Fig. 3).

DISCUSSION

Our study is the first to continuously measure SNA to the kidney during chronic inhibition of NO. While our previous results (26) suggested that the arterial baroreflex plays an important role in NO synthase inhibition-based hypertension, the lack of change in RSNA throughout the 7 days of NO blockade in conscious rabbits indicates that the hypertension is not mediated by increased renal nerve activity.

Role of the renal nerves. There is considerable controversy as to the role of the renal nerves in mediating the elevation in blood pressure seen with NO blockade. While this question has been previously addressed, the results have been conflicting. Bilateral renal denervation in rats appeared to delay and attenuate the hypertension with L-NAME (60 mg·kg⁻¹·day⁻¹ oral administration) (20, 41), suggesting the renal nerves did play a role in the hypertension induced by chronic NO synthase inhibition. In contrast, Granger et al. (11) found L-NAME administration (25 µg·kg⁻¹·min⁻¹ iv) similar responses in control and bilateral renal-denervated dogs, suggesting no contribution of renal nerves to the L-NAME-induced hypertension. Likewise, by using a split hemibladder preparation in dogs, Reinhart et al. (27) also observed no contribution of the renal nerves to the hypertension induced by chronic NO synthase inhibition (10 µg·kg⁻¹·min⁻¹ iv). While it is possible that the difference between these studies can be attributed to a species or dose difference, the use of renal denervation is at best a crude method to determine the role of the renal nerves. Several studies have used other indirect measures of SNA, such as ganglionic blockade to explore the role of SNA in hypertension. The decrease in blood pressure with ganglionic blockade is greater in rats made hypertensive with NO blockade (80 mg·kg⁻¹·day⁻¹ in drinking water) compared with controls (32, 34), suggesting an increase in SNA is responsible for the hypertension induced by chronic NO synthase inhibition. In addition, the hypertension induced by NO synthase inhibition is also attenuated in guanethidine-induced sympathectomized rats (31). However, ganglionic blockade is a method that only indirectly assesses SNA and cannot distin-
guish between an increase in nerve activity and an increase in responsiveness of the vasculature nor the possibility of differential changes in SNA.

In our study, the lack of change in RSNA during the hypertension induced by NO synthase inhibition indicates that the renal nerves do not mediate the increase in blood pressure. One criticism of long-term nerve recordings is the potential for the nerve signal to deteriorate with time. In this study, nasopharyngeal stimulation was used to monitor the stability of the SNA signal (4), and only rabbits in which the nerve signal was stable with time \((n = 5)\) were analyzed. In addition, the baroreflex responses in the five rabbits were also consistent over time with the curves achieved before and after recovery from the L-NAME treatment being not significantly different (Table 1). While in this study, no change in RSNA was observed with the L-NAME treatment, we have previously shown by using a similar experimental model, that angiotensin II-induced hypertension does result in a significant sustained decrease in RSNA (2, 3), suggesting that the lack of change in RSNA with L-NAME treatment is real and not due to an inability to detect changes in RSNA.

Most of the studies that support a role for the sympathetic nervous system in mediating the hypertension induced with NO blockade have typically used high doses of NO synthase inhibitors and have achieved increases in blood pressures of between 33 and 55 mmHg. In both of the studies in dogs (11, 27) and in the present study, in which there was no evidence for a change in SNA, the changes in blood pressure were more modest (7 to 14 mmHg). Thus, it is possible that the different doses of NO synthase inhibitors used in the studies could have played an important role in the differing responses observed. However, in preliminary experiments, we found that doubling the dose of L-NAME used in our experiments did not lead to further hypertension, suggesting that NO synthase was maximally inhibited. Previous studies in rabbits have used lower doses of L-NAME and confirmed the inhibition of NO synthase by observing inhibition of endothelium-dependent relaxation and impaired cGMP accumulation in response to acetylcholine (6, 15, 24). Interestingly, none of the previous studies in rabbits observed an increase in blood pressure levels with NO synthase inhibition.

It seems highly unlikely that the difference between our results and those previously reported in the rat is a species-

Fig. 2. Mean responses to chronic blockade of nitric oxide over 7 days via L-NAME (50 mg·kg\(^{-1}\)·day\(^{-1}\)) in the drinking water \((n = 7)\) for arterial pressure and heart rate; \(n = 5\) rabbits for RSNA). Data are presented from the mean value for each day for each rabbit. Error bars represent the mean ± SE. L-NAME was added to drinking water on day 0 with reversal back to tap water on day 7. *\(P < 0.05\) from control values.

Fig. 3. Raw RSNA showing the first 20 s after administration of cigarette smoke on 4 different days (days 12, 16, 20, and 23 after electrode implantation) in the same animal. In the rabbit shown, days 16 and 20 correspond with days 3 and 7 of L-NAME treatment, respectively. This figure illustrates that the baseline noise level remained stable, and distinct bursts of nerve activity (which were in synchrony with the arterial pulse) were clearly observed throughout the protocol. In all rabbits, cigarette smoke evoked a decrease in heart rate and an increase in RSNA, which was consistent with time. arb units, Arbitrary units.
dependent phenomenon, as experiments in rats in which plasma catecholamine levels have been used to estimate sympathetic activity (16, 34) also support the notion that there is no change in sympathetic activity during NO blockade. Indeed, Scrogin et al. (34) reported that \( \text{L-NAME} \) administration resulted in a greater responsiveness to ganglionic blockade, but no change in catecholamine levels in the same rat, suggesting it is not a dose or species phenomenon but rather, that method by which SNA is estimated that has in the past influenced the conclusion of whether or not the renal nerves are contributing to the hypertension. The discrepancies between the measurement of catecholamines and the ganglionic blockade studies are difficult to explain at present. One possible explanation is that the vascular reactivity to the same level of plasma catecholamines may be increased leading to the enhanced depressor responses to ganglionic blockade. Indeed Vials et al. (40) showed that the vasoconstrictor responses to transmural electrical nerve stimulation are significantly enhanced in the presence of \( \text{L-NAME} \), suggesting NO inhibits vasoconstrictor responses to nerve stimulation. In addition, Nase and Boegehold (25) have also observed augmented responses of mesenteric arteries to sympathetic stimulation following NO synthase inhibition, strengthening the argument for a role of NO in modulating vasoconstrictor responses to sympathetic stimulation. Thus, while plasma catecholamines or SNA do not change during NO blockade, an augmented vascular response to the presence of sympathetic activity may play a role in the maintenance of the hypertension.

While an increased vascular response to the same level of nerve activity remains a possibility, an alternative explanation is that NO may differentially regulate nerve activity to different vascular beds. The observation that the drop in blood pressure with ganglionic blockade is higher in animals made hypertensive with inhibition of NO synthase (7, 32) implies that sympathetic activity to at least some of the vascular beds may be increased. Differential control of SNA to different vascular beds is an important characteristic of the sympathetic nervous system that has been previously demonstrated (1, 22).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>79±3</td>
<td>86±3*</td>
<td>86±3*</td>
<td>79±2</td>
</tr>
<tr>
<td>Renal sympathetic nerve activity, nu</td>
<td>13±3</td>
<td>13±3</td>
<td>14±3</td>
<td>13±2</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>207±7</td>
<td>163±9*</td>
<td>177±7*</td>
<td>194±9</td>
</tr>
</tbody>
</table>

Renal Sympathetic Nerve Activity Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower plateau, nu</td>
<td>9.5±4</td>
<td>8.3±3</td>
<td>10.1±3</td>
<td>10.0±3</td>
</tr>
<tr>
<td>Range, nu</td>
<td>30.4±5</td>
<td>33.7±3</td>
<td>27.6±5</td>
<td>25.7±4</td>
</tr>
<tr>
<td>Lower plateau curvature, nu/mmHg</td>
<td>-0.33±0.1</td>
<td>-0.19±0.2</td>
<td>-0.21±0.02</td>
<td>-0.23±0.02</td>
</tr>
<tr>
<td>( \text{BP}_{50} ), mmHg</td>
<td>73±2</td>
<td>78±2*</td>
<td>76±3*</td>
<td>70±3</td>
</tr>
<tr>
<td>Upper plateau curvature, nu/mmHg</td>
<td>-0.40±0.1</td>
<td>-0.28±0.04</td>
<td>-0.31±0.02</td>
<td>-0.31±0.07</td>
</tr>
<tr>
<td>Maximum gain, nu/mmHg</td>
<td>-3.16±1</td>
<td>-2.41±0.5</td>
<td>-1.85±0.5*</td>
<td>-2.10±0.5</td>
</tr>
</tbody>
</table>

Heart Rate Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower plateau, beats/min</td>
<td>102±20</td>
<td>111±8</td>
<td>103±13</td>
<td>121±7</td>
</tr>
<tr>
<td>Range, beats/min</td>
<td>300±30</td>
<td>222±11</td>
<td>266±32</td>
<td>237±13</td>
</tr>
<tr>
<td>Lower plateau curvature, beats·min(^{-1})·mmHg(^{-1} )</td>
<td>-0.14±0.08</td>
<td>-0.10±0.01</td>
<td>-0.11±0.03</td>
<td>-0.13±0.02</td>
</tr>
<tr>
<td>( \text{BP}_{50} ), mmHg</td>
<td>74±7</td>
<td>76±2</td>
<td>77±3</td>
<td>74±1</td>
</tr>
<tr>
<td>Upper plateau curvature, beats·min(^{-1})·mmHg(^{-1} )</td>
<td>-0.10±0.02</td>
<td>-0.17±0.02</td>
<td>-0.13±0.02</td>
<td>-0.11±0.03</td>
</tr>
<tr>
<td>Maximum gain, beats·min(^{-1})·mmHg(^{-1} )</td>
<td>-6.73±1</td>
<td>-7.16±1</td>
<td>-5.89±1</td>
<td>-6.56±1</td>
</tr>
</tbody>
</table>

Data are means ± SE, nu, normalized units; \( \text{L-NAME} \), \( \text{N}^\text{\textdegree} \)-nitro-\( \text{l} \)-arginine methyl ester; \( \text{BP}_{50} \), mean arterial pressure at the midpoint of the curve. *\( P < 0.05 \), where the data are compared with before start of \( \text{L-NAME} \). Note the resting values were taken at the time of determining baroreflex curves.
we did not observe any changes in the mean levels of RSNA, it is possible that SNA to other beds may have been increased. Indeed, NO in the nucleus tractus solitarius (NTS) has been shown to differentially affect sympathetic outputs to different vascular beds (33). Further studies looking at differential long-term regulation of sympathetic activity to different vascular beds are needed to explore this possibility.

**Role of the baroreflex.** The role of the baroreflex in long-term blood pressure control has been revisited recently after some key studies pointed toward nonresetting of the baroreflex in chronic hypertension (3, 18, 38). Our results clearly show baroreflex resetting of RSNA with NO blockade. We have previously shown evidence for baroreflex nonresetting with angiotensin II-induced hypertension in baroreceptor-intact rabbits (3). It is important to note that the hypertension induced with NO blockade was of a lesser magnitude than that achieved with angiotensin II infusion (increase in blood pressure of 7 ± 1 mmHg with NO synthase inhibition vs. 18 ± 3 mmHg with angiotensin II). The time taken to reach the new pressure was also longer with NO synthase inhibition compared with the angiotensin II infusion and may be related to the different routes of administration in the two studies (drinking water vs. intravenous). We cannot rule out the possibility that the responses in nerve activity might be specific to angiotensin II or NO as opposed to a baroreflex-mediated response to an increase in blood pressure. In this regard, Barrett et al. (2) examined the blood pressure responses in baroreceptor-intact and sinoaortic-denervated rabbits to angiotensin II infusion. They observed similar arterial pressure responses in the intact and sinoaortic-denervated animals, which are in contrast to inhibition of NO synthase (26). This suggests that the sympathoinhibition observed with angiotensin II infusion may be specific to angiotensin II.

There was a significant decrease in the MAP-RSNA baroreflex gain with blockade of NO (Table 1). Previously, Scroggin et al. (35) measured renal nerve activity 4–6 h after surgery in different groups of animals after 1 and 5 wk of L-NAME and observed a reduction in the gain of the MAP-RSNA baroreflex response after both 1 and 5 wk of NO blockade. They found that 5 wk of NO blockade was needed to cause hypertension, suggesting the reduction in gain preceded the hypertension. One potential complication with recording SNA 4–6 h after surgery is that the stress of the surgery may lead to an increased mean level of SNA. In our animals, all cardiovascular variables were recorded well after the animals had recovered from surgery (~5 days). Our results confirm that L-NAME administration results in a reduced gain of the MAP-RSNA baroreflex response. The decreased gain suggests NO may play an important role in baroreceptor afferent processing. Indeed, previous studies have suggested a neurotransmitter role for NO in the NTS (9, 10). It appears that NO is involved in mediating the effects of glutamate released from baroreceptor afferents at the level of the NTS (17, 37). Given that L-NAME is nonselective for all isoforms of NO synthase and intravenous administration of L-NAME has been shown to reduce NO synthase catalytic activity in the brain (12, 39), it is possible that the decrease in gain might be a result of inhibition of the neuronal isoform of NO synthase in the NTS.

NO has been implicated as one of the factors involved in the regulation of baroreceptor resetting (21). Previous studies have indicated that administration of NO donors (19) and adeno viral vector-induced increases in NO synthase activity in the carotid sinus (21) are both associated with an inhibition of baroreceptor activity. Recent results suggest acute inhibition of NO synthase attenuates resetting of aortic baroreceptors to a sustained increase in arterial pressure (30). In contrast, our results indicate that chronic inhibition of NO synthase does not attenuate baroreceptor resetting. It is worth noting that in a separate study, a ramp increase in arterial pressure during NO synthase inhibition results in a greater extent of resetting to hypertensive levels (8). It is unclear whether the chronic nature of the stimulus in our study or the slow increase in pressure is a factor in the different responses observed. Clearly, more studies need to explore the role of NO in resetting of baroreceptor afferent activity.

The absence of any change in plasma renin activity during NO synthase inhibition in our study suggests the hypertension is not mediated by the renin-angiotensin system. The role of the renin-angiotensin system in the hypertension induced by chronic inhibition of NO synthase has been controversial with both increases in plasma renin levels (42), as well as no change (27) being reported. An increase in blood pressure would be expected to decrease plasma renin levels, and as such we cannot discount the possibility that the increase in blood pressure may have masked a direct stimulation of renin release by inhibition of NO synthase. It is possible that the absence of any change in RSNA, as well as plasma renin activity, seen in the present study may be related to the fact that we only observed a modest increase in blood pressure with NO synthase inhibition. We cannot rule out the possibility that a higher increase in blood pressure may have led to changes in RSNA and plasma renin activity.

The lack of a change in RSNA in response to the L-NAME-induced hypertension suggests the nerve activity is not the cause of the elevation in blood pressure. It could be argued that the RSNA is inappropriately high during L-NAME-induced hypertension and is effectively aiding in maintaining the elevated pressure. We do not believe that this is the case. The finding that bilateral denervation in dogs does not attenuate NO synthase inhibition hypertension (11) supports our finding. If the renal nerves were maintaining the hypertension, one would expect plasma renin levels to be elevated and the resulting increase in angiotensin II to mediate this increase in blood pressure. We did not observe any changes in plasma renin activity, which further suggests that the renal nerves are not playing a role in the hypertension with chronic NO synthase inhibition.

In summary, the lack of change in RSNA during the L-NAME-induced hypertension indicates that the renal nerves do not mediate the increase in blood pressure.

**GRANTS**

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**REFERENCES**


RENAL NERVE ACTIVITY AND NITRIC OXIDE INHIBITION


