Temporal Influence of the Renal Nerves on Renal Excretory Function During Chronic Inhibition of Nitric Oxide Synthesis

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Abstract To determine whether the sympathetic nervous system contributes to the hypertension induced by long-term suppression of nitric oxide synthesis, we determined the neurally induced changes in renal excretory function during chronic administration of N\textsuperscript{\textnd}-nitro-L-arginine methyl ester (L-NAME). Studies were carried out in six conscious chronically instrumented dogs subjected to unilateral renal denervation and surgical division of the urinary bladder into two hemi-urinary bladders to allow separate 24-hour urine collection from denervated and innervated kidneys. Animals were studied during acute (100 minutes) and chronic (5 days) intravenous infusion of L-NAME at 37.1 nmol/kg per minute (10 μg/kg per minute). During the first 100 minutes of L-NAME, there were no significant changes in mean arterial pressure (control 96±3 mm Hg), but heart rate fell from 66±6 to 55±7 beats per minute. Changes in glomerular filtration rate were not significant, but renal plasma flow and urinary sodium excretion decreased to ~75% and 50% of control values, respectively, however, these changes were comparable in both kidneys. In association with these responses, plasma concentrations of norepinephrine (control 887±130 pmol/L or 150±22 pg/mL) and epinephrine (control 691±192 pmol/L or 108±30 pg/mL) tended to decrease. In contrast to the acute responses, mean arterial pressure increased from 92±3 to 106±3 mm Hg and heart rate decreased from 72±4 to 57±5 beats per minute by day 5 of L-NAME infusion, while renal plasma flow and glomerular filtration rate were not significantly different from control values. Most importantly, there were no significant differences in urinary sodium excretion between innervated (control 31±2 mmol/d) and denervated (control 33±2 mmol/d) kidneys during chronic L-NAME infusion or during the recovery period. These results indicate that the renal sympathetic nerves do not play an important role in promoting sodium retention during either acute or chronic inhibition of nitric oxide synthesis in conscious dogs. Thus, increased renal sympathetic nerve activity does not contribute significantly to L-NAME-induced hypertension. (Hypertension. 1997;29[part 2]:199-204.)

Key Words • nitric oxide • hypertension • renal nerves • sympathetic nervous system • L-NAME

Nitric oxide has a number of effects that impact on the regulation of renal function and arterial pressure. NO is a highly labile substance formed in a number of tissues by enzymatic conversion of the endogenous substrate L-arginine. The conversion of L-arginine to NO is mediated by a family of related enzymes known as NOS. The influence of endogenous NO production on physiological processes has been assessed by the systemic administration of L-arginine analogues such as L-NAME, which competitively inhibit NO synthesis. Acute administration of L-NAME increases arterial pressure and vascular resistance, actions attributed to diminished production of endothelial NO. Chronic treatment with L-NAME leads to sustained hypertension, but the mechanisms that mediate this response have not been fully elucidated.

NOS isoforms are present in the brain and NO appears to be an important neural modulator in brain regions involved in the autonomic regulation of circulatory and renal function. Specifically, acute studies in anesthetized animals have demonstrated that injection of NO synthesis antagonists directly into either the rostral ventrolateral medulla or the nucleus tractus solitarius causes increments in arterial pressure and marked activation of the efferent renal sympathetic nerves. Similarly, either intravenous or intracisternal inhibition of NO increases arterial pressure and efferent renal sympathetic nerve activity. Therefore, since acute activation of the renal nerves promotes sodium retention, a number of investigators have hypothesized that the renal nerves play an important role in mediating the hypertension induced by chronic inhibition of NO synthesis. Indeed, recent studies in the rat have shown that either chronic renal denervation or chemical sympathectomy delays or reduces the severity of L-NAME-induced hypertension.

Studies in conscious chronically instrumented dogs have not elucidated the role of the renal nerves in mediating L-NAME-induced hypertension. Whereas one study indicates that the sympathetic nervous system contributes importantly to the acute antinatriuretic effects of L-NAME infusion, another fails to support the concept advanced in rat studies that the renal nerves contribute importantly to the hypertension caused by chronic blockade of NO synthesis. One interpretation of the data from experiments in dogs is that L-NAME administration produces acute but not chronic activation of the sympathetic nervous system. However, there have been no studies in any species that have determined the time-dependent changes in neurally induced sodium retention during L-NAME administration.

We tested the hypothesis that increments in renal sympathetic nerve activity decrease renal excretory function both acutely and chronically during L-NAME adminis-
Kidneys would be attributable to the direct influence of renal excretion between the innervated and denervated blood pressure and circulating factors, any difference in bladder to allow the separate collection of urine from each kidney. Since the innervated and denervated kidneys of each animal were subjected to the same level of arterial blood pressure and circulating factors, any difference in renal excretion between the innervated and denervated kidneys would be attributable to the direct influence of the renal nerves. Finally, to provide insight into global changes in activity of the sympathetic nervous system during NO synthesis inhibition, circulating levels of plasma catecholamines were determined during acute and chronic L-NAME administration.

Methods

Animal Preparation

Studies were carried out in six female mongrel dogs and all procedures were carried out in accordance with institutional and NIH guidelines regarding the use of laboratory animals. Dogs were sedated with acepromazine (0.2 mg/kg) and anesthetized with pentobarbital sodium (30 mg/kg IV). Using aseptic techniques, we implanted Tygon catheters (0.05 m ID, 0.09 m OD, Norton Plastics) in the femoral arteries and veins and exteriorized with pentobarbital sodium (30 mg/kg IV) using aseptic technique. Dogs were sedated with acepromazine (0.2 mg/kg) and anesthetized with trilmethoprim (400 mg) and sulfamethoxazole (80 mg) combination (Sulfadran, BID, Schein Pharmaceutical, Inc) were given prophylactically.

Throughout the study, arterial pressure was recorded from a femoral arterial catheter connected to the pressure transducer and a Grass polygraph (model 7D, Grass Instruments). A microcomputer and customized software were used to sample the signal from the Grass recorder at 200 Hz for a duration of 12 seconds, once a minute, 24 hours a day. The digitized data for each 12-second burst were immediately processed to compute MAP and heart rate. The daily values for MAP and heart rate were determined from the average of the 1110 sample points collected during the 18 5-hour period between 1 AM and 7:30 AM. The hours excluded from the 24-hour analysis included the time required for calibration of pressure transducers, measurement of renal function (in selected days), feeding, and cleaning of cages.

Experimental Protocol

Before the control period, the dogs were conditioned to the daily regimen during a training and equilibration period that lasted 7 days. During this time, the dogs were housed on the cage floor for the measurement of renal function and for the collection of arterial blood samples. After a 3-day control period, the dogs were continuously infused with L-NAME for 5 days at 37.1 nmol/kg per minute (10 μg/kg per minute) to inhibit synthesis of NO. Renal hemodynamics and renal excretory function were assessed twice during the control period, on day 5 of L-NAME, and several days after the infusion was terminated. Additionally, the transient renal response to L-NAME was measured for an additional five clearance periods during the first 100 minutes of L-NAME infusion.

GFR and RPF were estimated from the clearances of [125I]lothalamate (Glofil, Isotex Diagnostics) and [131I]iodohippurate (Hippuran, Syncon International Corp), respectively, as previously described. During each experiment, the results of three consecutive 20-minute clearance periods were averaged to determine the GFR and RPF. At the end of the clearance periods, each hemibladder was flushed twice with a total of 20 mL of sterile distilled water and the wash was added to the urine collected. A 1.5-mL arterial blood sample was taken at the midpoint of each clearance period for determination of the plasma concentrations of [125I]lothalamate and [131I]iodohippurate. Before each renal clearance study and on intermittent days throughout the control, experimental, and recovery periods, 8-mL arterial blood samples were collected for measurement of hematocrit, PRA, and the plasma concentrations of norepinephrine, epinephrine, sodium, potassium, and protein. Blood samples were taken after the dogs had been in a recumbent position for approximately 45 minutes, and after MAP and heart rate were stable.

Analytical Methods

PRA was measured by radioimmunoassay. Plasma and urine concentrations of sodium and potassium were determined by flame photometry (IL 943, Instrumentation Laboratories), plasma protein concentration by refractometry (American Optical), and hematocrit by a micrometer (Auto crit II, Clay Adams). The plasma concentrations of norepinephrine and epinephrine were determined by high-performance liquid chromatography as previously described. Additionally, renal norepinephrine concentration was determined in four dogs by methods previously employed in our laboratory.

Statistical Analysis

Results are expressed as mean±SEM. Experimental and recovery data were compared with control data using analysis of variance with Dunnett's t test for repeated measures. Control values were averaged to calculate a single control value.
Acute Responses to Infusion of L-NAME

During the first 100 minutes of L-NAME infusion, there were no significant changes in MAP but heart rate decreased. Moreover, there were progressive reductions in RPF and urinary sodium excretion, however, the responses were quantitatively similar in both kidneys. Maximum reductions in RPF and urinary sodium excretion (to ~75% and 50% of control, respectively) occurred during the last clearance period (after 80 to 100 minutes of L-NAME infusion) and are illustrated in the Table. Although RPF decreased acutely, there were no significant changes in GFR during the first 100 minutes of L-NAME infusion, and consequently increments in filtration fraction were comparable in each kidney. Potassium excretion tended to decrease in both kidneys (~20%), but the changes were not statistically significant. Finally, there were no significant changes in PRA or in the plasma concentrations of norepinephrine, epinephrine, protein (control 6.7 ± 0.4 mg/dL), sodium (control. 145 ± 1 mmol/L), potassium (control 4.4 ± 0.1 mmol/L), or hematocrit (control 38 ± 3%) during the first 100 minutes of L-NAME infusion. Plasma levels of catecholamines did tend to fall during acute infusion of L-NAME, but these responses were not quite statistically significant (P = 0.07).

Chronic Responses to L-NAME

Changes in MAP, heart rate, and daily urinary sodium excretion during chronic infusion of L-NAME are summarized in Fig 1. Although MAP failed to increase during the first 100 minutes of L-NAME, elevations in MAP occurred later in the day. On day 1, MAP increased from 92 ± 3 to 107 ± 4 mm Hg, and this increase was sustained throughout the 5-day infusion period of L-NAME. The hypertension was associated with a pronounced bradycardia; on day 5, heart rate was reduced from a control value of 72 ± 4 to 57 ± 5 beats per minute. Although urinary sodium and potassium excretion decreased during acute administration of L-NAME, there were no significant changes in the urinary excretion of these electrolytes (control sodium, 64 ± 5 mmol/d; potassium, 55 ± 3 mmol/d) on day 1 or on any subsequent day of L-NAME infusion.

Most importantly, the renal nerves did not influence sodium excretion during L-NAME-induced hypertension. As shown in Fig 2, rates of urinary sodium excretion were comparable in innervated and denervated kidneys during chronic infusion of L-NAME, as well as during the control and recovery periods. This is also reflected by similar denervated-to-innervated kidney ratios for urinary sodium excretion throughout the study (Fig 2). The apparent increase in the ratio on day 2, suggesting neurally induced sodium retention, was not statistically significant and was primarily influenced by one dog that excreted low amounts of sodium (~8 mmol) on this day.

The chronic effects of L-NAME on renal hemodynamics are summarized in Fig 3. Although RPF decreased and filtration fraction increased during acute infusion of L-NAME (Table), these changes were not sustained chronically; GFR, RPF, and filtration fraction were not sig-
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FIG 3 Effects of chronic L-NAME infusion (5 days) on GFR, RPF, and filtration fraction. Values are mean ± SEM, n=6.

Significantly different from control values on day 5 of L-NAME or during the recovery period.

Fig 4 illustrates that there were no significant changes in either plasma norepinephrine (control: 845 ± 136 pmol/L or 143 ± 23 pg/mL) or epinephrine (control: 557 ± 115 pmol/L or 87 ± 18 pg/mL) concentration, suggesting a lack of general sympathetic stimulation during chronic L-NAME infusion. Also, PRA (control 0.40 ± 0.13 nmol angiotensin I per liter per hour or 0.6 ± 0.2 ng angiotensin I per milliliter per hour), hematocrit (control 38 ± 3), and the plasma concentration of protein (control 6.7 ± 0.4 mg/dL), sodium, and potassium were unchanged during chronic L-NAME infusion.

Finally, as in a recent investigation from our laboratory employing the exact same experimental preparation, there was more than a 30-fold difference in norepinephrine content between innervated and denervated kidneys, indicating pronounced depletion of norepinephrine in the denervated kidneys. In accordance with this earlier study, norepinephrine concentration in the denervated kidneys (112.5 ± 53 fmol/mg tissue or 19 ± 9 pg/mg tissue) was <118 fmol/mg tissue (<20 pg/mg tissue).

**Discussion**

Although the mechanisms have not been fully elucidated, numerous studies have shown that chronic blockade of NO synthesis produces hypertension. Our results confirm these observations, but they provide no support for the concept that the hypertension is mediated by the sympathetic nervous system. Plasma levels of catecholamines, an index of global sympathetic activity, did not increase during chronic L-NAME-induced hypertension and, more importantly, the renal nerves did not promote sodium retention.

Acute blockade of NO synthesis produces a number of changes in renal function which could contribute to the development of chronic hypertension, however, the importance of these effects in mediating L-NAME-induced hypertension is unclear. Several studies, including the present, have shown that acute blockade of NO synthesis decreases sodium excretion in association with reductions in renal blood flow and increments in filtration fraction. While such hemodynamic responses may impair pressure natriuresis acutely, in the present study they were not sustained chronically. As reported by others, RPF and filtration fraction returned to control levels during chronic L-NAME infusion. Thus, although the maintenance of sodium balance despite sustained elevations in arterial pressure indicates a hypertensive shift in the pressure natriuresis relationship during L-NAME administration, the causal mechanisms that contribute to impaired renal excretory function are not apparent.

Previous acute studies in anesthetized animals have advanced the concept that neuronal as well as endothelial NOS may contribute to the tonic regulation of vasomotor tone and arterial pressure. Injection of NO synthesis antagonists directly into either the rostral ventrolateral medulla or the nucleus tractus solitarius causes increments in arterial pressure and marked activation of the effluent renal sympathetic nerves, while intravenous or intracranial inhibition of NO leads to similar changes in arterial pressure and renal nerve activity. These acute studies and others have led to the hypothesis that increments in sympathetic outflow mediate, at least in part, the hypertensive response to chronic L-NAME administration. If sustained increases in sympathetic outflow were directed to the kidneys during chronic inhibition of NO synthesis, then a neurogenic basis reduction in renal excretory function could contribute to the hypertensive shift in the pressure natriuresis relationship. Indeed, a recent study by Matsuoka et al in the rat suggests that renal denervation delays the onset of L-NAME-induced hypertension, at least as assessed by indirect tail-cuff measurements of blood pressure. However, direct measurements of arterial pressure from arterial catheters indicated that the chronic hypertensive response to L-NAME was not significantly influenced by the renal nerves after 5 weeks of treatment. A more recent study in which chronic chemical sympathectomy markedly attenuated L-NAME-induced hypertension in rats supports a more general role for the sympathetic ner-
ward the body was decreased.

The results of the present study suggest that the sympathetic nervous system and more specifically the renal nerves do not contribute appreciably to either the acute or chronic phases of L-NAME-induced hypertension. In the present study, there were no significant increments in plasma levels of catecholamines at any time during chronic L-NAME administration, suggesting that the hypertension was not sympathetically mediated. Moreover, there were no significant differences in urinary sodium excretion between innervated and denervated kidneys in the present study during acute L-NAME infusion, our findings indicate that the renal nerves were not selectively activated at a time when sympathetic activity to the remainder of the body was decreased.

The possibility should be considered that potential differences in sodium excretion between innervated and denervated kidneys could be caused by subtle changes in arterial pressure or circulating factors. This is not the case in experiments comparing responses in either renal denervated or sympathetically blocked animals to responses in intact controls.

The present study in conscious chronically instrumented dogs fails to support the notion that NO normally restrains central sympathetic outflow. During acute infusion of L-NAME, there was no significant increase in arterial pressure, but there were reductions in heart rate and in plasma levels of catecholamines. Although the decrease in plasma norepinephrine concentration did not quite achieve statistical significance \( (P \approx 0.07) \), taken together our results suggest that acute increments in arterial pressure were not discernible because of reflex mechanisms that inhibited central sympathetic outflow. Thus, our findings are consistent with the results of Hansen et al. These investigators recorded muscle sympathetic nerve activity in humans during a 15-minute infusion of the NO synthase inhibitor, \( N^G \)-monomethyl-L-arginine. In this study, blockade of NO synthesis did increase arterial pressure 10 mm Hg, but this response was associated with bradycardia and a decrease in sympathetic nerve activity.

Furthermore, since there were comparable reductions in urinary sodium excretion in innervated and denervated kidneys, the renal nerves were not selectively activated at a time when sympathetic activity to the remainder of the body was decreased. The possibility should be considered that potential differences in sodium excretion between innervated and denervated kidneys could be caused by subtle changes in arterial pressure or circulating factors. This is not the case in experiments comparing responses in either renal denervated or sympathetically blocked animals to responses in intact controls.

had no effect on the relative excretion rates of sodium between innervated and denervated kidneys. Thus, it is unlikely that at the prevailing plasma levels of norepinephrine in the present study, supersensitivity of the renal vasculature or tubules of chronically denervated kidneys accounts for the fact that there were no differences in sodium excretion between the two kidneys.

In conclusion, these studies performed in conscious chronically instrumented dogs indicate that the hypertension produced by chronic blockade of NO synthesis is not associated with long-term changes in whole-kidney hemodynamics. Moreover, these studies fail to support the hypothesis that the sympathetic nervous system, and more specifically, the renal nerves contribute significantly to the long-term increments in arterial pressure produced by the chronic administration of L-NAME.

Acknowledgments

This research was supported by National Heart, Lung, and Blood Institute grant HL-51971. Dr. Reinhardt is a recipient of a National Research Service Award from the National Institutes of Health, Bethesda, Md.

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Hypertension. 1997;29:199-204
doi: 10.1161/01.HYP.29.1.199

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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