Renal Effects of Prolonged Synthesis Inhibition of Endothelium-Derived Nitric Oxide

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The aim of the present study was to investigate in conscious dogs the long-term effects of nitric oxide synthesis inhibition on glomerular filtration rate, sodium and water excretion, and plasma levels of renin and aldosterone. After a control period of 3 days, an inhibitor of endothelium-derived nitric oxide synthesis, N\textsuperscript{ω}-nitro-L-arginine-methyl ester, was infused for 3 consecutive days at a dose (50 ng/kg/min) that did not induce significant changes in arterial pressure (n=6). The inhibition of nitric oxide synthesis led to a large and sustained decrease (p<0.05) in glomerular filtration rate of approximately 35%. This change was accompanied by a decrease (p<0.05) in urinary sodium excretion from 78.9±4.6 meq/day to 49.8±6.8, 60.1±4.2, and 53.5±9.0 meq/day by days 1, 2, and 3 of nitric oxide synthesis inhibition, respectively. Changes in fractional sodium excretion failed to achieve statistical significance. Nitric oxide synthesis inhibition also induced a significant and sustained decrease in urine flow rate. The decrease in glomerular filtration rate, natriuresis, and diuresis was accompanied by a 45% increase in plasma renin activity and no change in plasma aldosterone concentration. By day 3 of the recovery period, a continuous endogenous formation of nitric oxide may play an important role in the long-term regulation of renal hemodynamic and renal excretory function. It is suggested that an increase in renin release may contribute to the renal changes induced by the inhibition of nitric oxide synthesis during 3 consecutive days. (Hypertension 1992;20:113-117)

Key Words • nitric oxide • renal function • natriuresis • renin • endothelium-derived relaxing factor

The potential importance of endothelium-derived relaxing factor (EDRF) in the regulation of arterial pressure has been suggested by different groups within the past few years (see References 1, 2, and 3 for review). EDRF has been identified as, at least in part, nitric oxide derived from the guanidino nitrogens of L-arginine (L-Arg), and the enzyme responsible for this conversion is competitively inhibited by L-Arg analogues like N\textsuperscript{ω}-monomethyl L-arginine (L-NMMA) and N\textsuperscript{ω}-nitro-L-arginine-methyl ester (L-NAME). Endothelium-derived nitric oxide (EDNO) takes part in blood pressure regulation, as indicated by studies showing that EDNO synthesis inhibition increases peripheral vascular resistance and blood pressure.5,6

Endothelium plays an important role in the regulation of renal function, as has been demonstrated only recently.2-12 The capacity of the renal vasculature to respond to known endothelium-dependent vasodilators such as acetylcholine or bradykinin suggests that this vascular bed possesses the ability to produce large quantities of EDNO.7,8 It has been demonstrated that the basal production of EDNO is required to maintain the normal level of renal blood flow (RBF) and glomerular filtration rate (GFR).9-11 The kidney seems to be more sensitive than other organs to the acute inhibition of EDNO synthesis because it has been demonstrated that RBF, GFR, natriuresis, and diuresis were reduced during the intravenous infusion of L-NAME at a dose that did not modify arterial pressure.11 These studies have analyzed the renal response to acute inhibition of EDNO synthesis and support the concept that basal synthesis of EDNO plays an important role in the acute regulation of renal function. However, it is not known whether EDNO synthesis plays an important role in the long-term regulation of GFR and renal excretory function.

The purpose of the present study was to determine if GFR and renal excretory function are affected during a long-term (3 days) intravenous infusion of L-NAME at a dose that does not induce changes in arterial pressure. This inhibitor of EDNO synthesis has been demonstrated to be active in vivo by different groups.9-12 Changes in plasma renin activity (PRA) and plasma...
Experimental Procedures

Experiments were performed in female mongrel dogs (14–22 kg body weight). All experimental procedures were designed according to the Recommendations from the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals approved by the Council of the American Physiological Society. Surgery was performed under aseptic conditions, and the animals were anesthetized with pentobarbital sodium (30 mg/kg i.v.) anesthesia for implantation of Tygon catheters into the femoral artery and vein. The tips of the catheters were placed in the aorta, distal to the origins of the renal arteries, and in the vena cava, respectively. The catheters were tunneled subcutaneously, exteriorized between the scapulae, and placed in neck collars. The dogs were allowed to recover from surgery for at least 2 weeks, during which time antibiotics were given to prevent postoperative infection.

After recovery from surgery and 7 days before the experiments were started, the dogs were housed in individual metabolic cages and fitted with harnesses that contained blood pressure transducers mounted at heart level and connected to an amplifier (model 13-4615-71, Gould, Inc., Cleveland, Ohio). Mean arterial pressure (MAP) was recorded continuously in a Gould recorder and plasma sodium, potassium, and osmolality were drawn every 30 minutes from the paper recorder, and the single daily value was the average of the readings obtained for each 30-minute period over a 20- to 22-hour period. The femoral vein catheter was connected to a roller pump (Watson Marlow 410, Falmouth, UK) that was used to continuously infuse 20- to 22-hour period. The femoral vein catheter was connected to a roller pump (Watson Marlow 410, Falmouth, UK) that was used to continuously infuse

During the control, experimental, and recovery periods of the study, all dogs were fed a sodium-deficient diet (H/D, Hill’s Pet Products, Topeka, Kan.) that provided 5–7 meq sodium and 70 meq potassium per day. The dogs were allowed free access to tap water throughout the experiment. Isotonic saline was continuously infused via the femoral vein at a rate of 450 ml/day to maintain the total sodium intake constant at 75 meq/day, including the sodium provided in the food.

Analytical Methods

Sodium and potassium concentrations in the urine and plasma were measured by flame photometry (Corning 435, Corning Limited, Halstead Essex, UK). GFR was determined by the clearance of endogenous creatinine. Creatinine was measured using a photocolorimetric method (Boehringer Mannheim, Mannheim, FRG). Osmolality was determined by freezing-point depression (Knauer osmometer, Knauer & Co., Berlin, FRG). PRA and PAC were measured using commercially available radioimmunoassays (RIA) (Serono Labs, Milan, Italy).

Statistical Analysis

Data are presented as mean±SEM. Significance of differences in values of each day was evaluated using a one-way analysis of variance and the Duncan multiple-range test.

Experimental Groups

Group 1 (n=6): Effects of L-NAME infusion. After a 3-day control period, an inhibitor of nitric oxide synthesis, L-NAME, (Sigma, London, UK), was infused intravenously for 3 days at a continuous rate of 50 ng/kg/min. Recovery measurements were obtained for 3 days after L-NAME infusion was stopped. In preliminary experiments, it was found that this dose of L-NAME does not modify arterial pressure. A dose 5 times higher (250 ng/kg/min) produced a small but sustained increase in arterial pressure.

Group 2 (n=4): Effects of simultaneous infusion of L-NAME and L-Arg. The experimental protocol was similar to that of group 1, with the exception that L-Arg (Sigma) (5 μg/kg/min) was simultaneously infused with L-NAME (50 ng/kg/min). L-Arg infusion started 2 hours before L-NAME administration. L-Arg was infused to determine whether the renal response to the infusion of L-NAME was secondary to the inhibition of EDNO synthesis. The dose of L-Arg was larger than that of L-NAME because it has been demonstrated that an excess of L-Arg reverses the systemic effects of L-NAME by competitive binding to the nitric oxide synthetase.

Group 3 (n=4): Effects of L-Arg infusion. A similar protocol to that of group 2 was performed with the difference that, during the experimental period (3 days), only L-Arg was infused through the femoral vein.

The L-NAME and L-Arg solutions were prepared and changed daily. Twenty-four-hour urine samples, infusion volume, and water intake were measured between 9 and 10 AM each day. These measurements were used to assess daily electrolyte and water balances.

Results

Experimental Groups

Group 1: Effects of L-NAME infusion. Figure 1 illustrates the effect of 3 days L-NAME infusion on MAP, GFR, urinary sodium excretion (U\textsubscript{Na}V), and urine flow
rate (UV). Changes in MAP during L-NAME infusion failed to achieve statistical significance. GFR decreased from 58.9±9.6 to 39.3±8.2 ml/min (p<0.05) on day 1 of L-NAME infusion. The decrease in GFR was also significant (p<0.05) during the second (42.3±8.1 ml/min) and third (40.5±6.3 ml/min) day of L-NAME infusion and then returned toward control levels during the 3-day recovery period. The long-term inhibition of EDNO synthesis also induced a significant decrease in UNaV (p<0.05) during the 3-day administration of L-NAME. Cumulative sodium balance was 73.2±14.7 meq sodium during the L-NAME infusion. During the recovery period, UNaV increased (p<0.05) to levels that were not significantly different from those found in the control period. It should be noted that fractional excretion of sodium was not significantly altered throughout the experiment. Changes in UV exhibited similar directional trends as those of UNaV (Figure 1). It can be seen that inhibition of EDNO synthesis produced a decrease in UV from 0.62±0.03 ml/min to 0.45±0.03, 0.51±0.03, and 0.50±0.03 ml/min by days 1, 2, and 3 of L-NAME infusion, respectively. One day after L-NAME infusion stopped, UV increased to a value (0.74±0.08 ml/min) that was significantly higher (p<0.05) than that observed during the control period and then gradually returned to control levels by day 3 of the recovery period (0.63±0.04 ml/min) (Figure 1). Both urine and plasma osmolality and potassium excretion did not change significantly throughout the experiment.

Average values for PRA and PAC are illustrated in Figure 2. PRA increased from 1.62±0.16 ng angiotensin I (Ang I)/ml/hr to 2.32±0.39 and 2.35±0.20 ng Ang I/ml/hr on days 1 and 3, respectively, of L-NAME infusion, but the increment was only significant (p<0.05) by day 3 of EDNO synthesis inhibition. During the recovery period, PRA decreased to 1.18±0.26 ng Ang I/ml/hr (p<0.05). Changes in PAC failed to achieve statistical significance (Figure 2).

Group 2: Effects of simultaneous infusion of L-NAME and L-Arg. Effects of the simultaneous infusion of L-NAME and L-Arg during 3 consecutive days are illustrated in Figure 3. It can be observed that L-NAME infusion did not induce significant changes in MAP, GFR, UNaV, and UV when L-Arg was simultaneously infused at a dose (5 µg/kg/min) that was 100 times higher than the dose of L-NAME used (50 ng/kg/min). There were no significant changes in potassium excretion, plasma and urine osmolality, PRA, or PAC during either the simultaneous infusion of L-NAME and L-Arg or the recovery period.
Group 3: Effects of L-Arg infusion. The intravenous infusion of L-Arg (5 μg/kg/min) for 3 consecutive days did not induce significant changes in any of the parameters analyzed in the present study.

Discussion

The present study demonstrates that the infusion of an EDNO synthesis inhibitor (L-NAME) during 3 consecutive days induces a sustained decrease of GFR, UNaV, and UV. The effect of L-NAME infusion was inhibited by the simultaneous administration of L-Arg. In addition, we have demonstrated that renal function is much more sensitive than arterial pressure to the effects of prolonged inhibition of EDNO synthesis because a low dose of L-NAME, while having no systemic pressor effects, caused a significant reduction in GFR, UNaV, and UV. These findings clearly indicate that the kidney has a basal synthesis of EDNO because the decreases in GFR, UNaV, and UV can be attributed to the inhibition of nitric oxide synthesis inhibitor (L-NAME) during 3 consecutive days. The present study demonstrates that the infusion of an EDNO synthesis inhibitor (L-NAME) during 3 consecutive days induces a sustained decrease of GFR, UNaV, and UV. The effect of L-NAME infusion was inhibited by the simultaneous administration of L-Arg. In addition, we have demonstrated that renal function is much more sensitive than arterial pressure to the effects of prolonged inhibition of EDNO synthesis because a low dose of L-NAME, while having no systemic pressor effects, caused a significant reduction in GFR, UNaV, and UV. These findings clearly indicate that the kidney has a basal release of EDNO, which substantially contributes to the long-term regulation and maintenance of renal function. The renal effects induced by the infusion of L-NAME can be attributed to the inhibition of nitric oxide synthesis because the decreases in GFR, UNaV, and UV were prevented by the simultaneous administration of the nitric oxide precursor L-Arg. This hypothesis is supported by "in vitro" studies showing that the mechanism of action of L-NAME is by competitive inhibition, which is specifically reversed by L-Arg. A major assumption underlying our results is that the dose of L-NAME used does not completely inhibit the systemic synthesis of EDNO because MAP did not change. However, one purpose of the present study was to determine if renal function is affected by the prolonged infusion of L-NAME during 3 consecutive days at a dose that does not produce changes in arterial pressure. It is not known whether the infusion of L-NAME in our study could affect natriuresis and diuresis for a much longer period than 3 days, but it is hypothesized that an increase in MAP would be required to restore normal sodium excretion. The lack of L-NAME infusion to affect MAP may be attributed to the dose used in our study, which is smaller than those used in previous studies performed by other groups.6-12

Our results suggest that long-term regulation of GFR appears to be EDNO dependent. These results do not allow us to define the specific mechanism by which the prolonged L-NAME infusion induces a decrease of GFR. However, results of previous studies suggest that the effect of nitric oxide synthesis inhibition on GFR could be due to an increase of resistance in the renal afferent arteriole15 or secondary to the contraction of glomerular mesangial cells.16,17 In an in vitro preparation, Ito et al15 have provided evidence that local production of EDNO is an important determinant of the renal afferent arteriole tone. Other in vitro studies16,17 have suggested that nitric oxide induces the relaxation of mesangial cells. On the other hand, several in vivo studies have demonstrated that filtration fraction increases during nitric oxide synthesis inhibition,11,12,18 suggesting that this inhibition induces an efferent contraction. In our present study, an increase of efferent arteriole resistance in the face of declines in GFR only could be explained if glomerular capillary ultrafiltration coefficient (Kf) decreases during nitric oxide synthesis inhibition. An increase in glomerular blood pressure and a decrease in Kf during blockade of nitric oxide synthesis has been reported previously by Zatz and de Nucci.19

The effects of EDNO synthesis inhibition on GFR in this study could be potentiated by the inappropriately high PRA levels found during the L-NAME infusion. Although it was not significant, PRA increased during the first 24 hours of L-NAME infusion despite a sodium retention of 29±9 meq. During the third day of L-NAME infusion, PRA increased significantly despite a sodium retention of 73.2±14.7 meq. Similar sodium retentions to those found on days 1 and 3 of L-NAME infusion are usually followed by significant decreases in PRA.20

The hypothesis that angiotensin II (Ang II) could contribute to the renal effects of L-NAME is supported by the results recently obtained by our group in conscious dogs (unpublished observations). It has been found that intrarenal Ang II administration at
doses that do not change GFR induces a significant decrease in GFR when nitric oxide synthesis is previously inhibited. Furthermore, it has been demonstrated that pretreatment with an Ang II receptor antagonist blunted the decreases in GFR and RBF induced by the acute infusion of an EDNO synthesis inhibitor.\(^{18}\)

The rise in renin release found in our study, despite sodium retention during the 3-day period, suggests that EDNO may be one of the long-term modulators of renin secretion. However, it cannot be excluded that the increase in PRA is secondary to some other event rather than a direct response to nitric oxide synthesis inhibition. An increase of PRA during acute EDNO synthesis inhibition has been demonstrated previously,\(^{14}\) but this is the first time that an increase of PRA has been reported during long-term inhibition of EDNO synthesis. An increase of intrarenal Ang II levels during EDNO inhibition would be expected to increase postglomerular resistances leading to an increase in filtration fraction.\(^{21}\) Such an effect has been reported in acute experiments in rats during the intrarenal infusion of L-NMMA\(^{18}\) and during the intravenous infusion of L-NAME.\(^{11,11}\) In contrast to the antinatriuretic effect of L-NAME found by our group and by Lahera et al,\(^{11}\) Baylis et al\(^{12}\) observed that L-NAME infusion induced an increase in natriuresis. This natriuretic effect of L-NAME was most probably due to a significant increase in arterial pressure because the infusion of subpressor doses of L-NAME usually induces a decrease in natriuresis and diuresis.\(^{11}\)

A direct tubular effect of EDNO synthesis inhibition has been suggested by Lahera et al,\(^{11}\) who demonstrated that the decrease of EDNO production in rats affects renal function, first by decreasing natriuresis and diuresis and second by decreasing renal blood flow and glomerular filtration rate. However, fractional sodium excretion (\(\text{Fe}_\text{Na}\)) did not change in our study, an indication that renal function is affected primarily by changing GFR during long-term inhibition of EDNO synthesis. The lack of an effect of L-NAME on \(\text{Fe}_\text{Na}\) in our study may also be attributed to the dose of L-NAME used or to differences in animal species.

L-NAME infusion did not alter PAC despite the large sodium retention. One would have predicted a decrease in PAC when there is a sodium retention of 73.2±14.7 meq sodium. In conscious dogs, Salazar et al\(^{20}\) demonstrated a significant reduction in PAC during a similar sodium retention. Thus, the level of PAC during long-term inhibition of EDNO synthesis appears to be inappropriately high for the level of sodium retention. Further studies are needed to define the long-term effects of EDNO synthesis inhibition on aldosterone secretion.

Infusion of L-Arg alone during 3 consecutive days failed to induce a systemic or renal effect, suggesting that production of EDNO is not limited by the availability of substrate. This confirms the findings of Lahera et al,\(^{22}\) who showed that the intrarenal infusion of L-Arg for 90 minutes (1 mg/kg/min) did not produce changes in renal hemodynamic and excretory function in anesthetized dogs.

In summary, this study provides new evidence that EDNO plays an important role in the long-term regulation of GFR and renal excretory function and that a potential underlying mechanism of the renal effect obtained with the inhibition of EDNO synthesis may be related to an enhancement of intrarenal Ang II levels. Thus, stimuli that alter long-term EDNO synthesis in the kidney can be expected to have profound effects on renal function and subsequently on systemic arterial pressure.

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