ANTIOXIDANTS & REDOX SIGNALING
Volume 4, Number 6, 2002
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Forum Original Research Communication

Renal Dysfunction After Chronic Blockade of Nitric Oxide Synthesis

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ABSTRACT

The effects of the chronic inhibition of nitric oxide (NO) on renal hemodynamics and tubular function were studied in rats treated for 8 weeks with the NO synthesis inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME; 40 mg/kg/day). In addition, the effect of L-NAME administration on vasoactive systems (renin–angiotensin system, aldosterone, catecholamines, endothelin, and thromboxane A_2) was evaluated. Chronic inhibition of NO significantly elevated blood pressure, reduced glomerular filtration rate and renal blood flow, blunted the pressure–diuresis–natriuresis response, and increased protein urine excretion. All these changes were associated with blunted nitrite production in response to acetylcholine in glomeruli. No changes were observed in the plasma levels of either renin activity, aldosterone, or endothelin in L-NAME-treated rats. Similarly, no differences were observed in the urinary excretion of thromboxane B_2 between both group of animals. By contrast, plasma concentrations of both epinephrine and norepinephrine were elevated in rats treated with L-NAME. In summary, the results show that chronic blockade of NO produced not only alterations in renal function, but also renal damage, suggesting an important renoprotective role of NO. An activation of sympathoadrenal system could participate in these renal alterations. *Antioxid. Redox Signal.* 4, 885–891.

INTRODUCTION

NITRIC OXIDE (NO) is an endogenous autacoid that exerts a basal tonic vasodilatory effect on the vascular wall by increasing cyclic GMP levels in smooth muscle cells (18). Thus, acute and chronic inhibition of NO synthesis produced by oral administration of NO synthase inhibitors such as N^G-nitro-L-arginine methyl ester (L-NAME) increased vasoconstrictor tone and consequently blood pressure in a dose–dependent manner (3, 14, 20, 25). NO also controls a variety of biological processes, including neurotransmission, cell growth, apoptosis, inflammation, and renal function (18, 30, 33, 34).

The significance of NO as a mediator of renal function has been extensively studied. Numerous studies have shown that NO is an important mediator of natriuresis, which is not only involved in the control of sodium excretion under physiological conditions, but also exerts an important role in the chronic adaptation to a high sodium intake (14, 34). In addition, it has been shown that NO can modulate the renal excretory response to changes in arterial pressure, the pressure–diuresis–natriuresis mechanism (6–8). NO exerts these effects by controlling not only tubular ion transport, but also renal vascular tone. Indeed, this endothelial factor is an important modulator of renal vasculature because it controls the diameter of several vessels, including afferent and efferent arterioles and vasa recta (5, 10, 12). Thus, NO can play an important role in the regulation of renal hemodynamics.

Numerous studies have shown that NO can also modulate renal function by its ability to antagonize the effects of other vasoconstrictor or antinatriuretic factors that also participate in the regulation of renal function such as angiotensin II, aldosterone, thromboxane A_2 (TxA_2), endothelin, and catecholamines (4, 21, 34). Moreover, NO can affect these factors by negatively controlling their release. As an example, NO inhibits the secretion of aldosterone, catecholamines, and renin

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release (2, 9, 17, 27, 29, 32). Thus, an activation and/or an overexpression of these factors could be the underlying mechanism that causes the renal alterations observed with the chronic inhibition of NO. Therefore, the aim of this study was to evaluate whether the changes in renal function produced by chronic inhibition of NO were associated with an activation of systemic vasoconstrictor factors

MATERIAL AND METHODS

General procedure

Thirty-two male Sprague–Dawley rats (Charles River, Barcelona, Spain) weighing 157 ± 8 g were maintained on standard rat chow (A04; Panlab, Barcelona, Spain) and tap water throughout the study. Half of the animals were treated for 8 weeks with the NO synthesis inhibitor L-NAME (40 mg/kg/day in the drinking water), and the rest, which did not receive any treatment, served as controls. All experiments were performed according to the guidelines for the ethical treatment of animals of the European Union. At the end of the treatment, systolic blood pressure was measured by a tail-cuff plethysmograph (Narco Bio-Systems, Houston, TX, U.S.A.) in trained animals as previously described (20). Likewise, animals were housed in metabolic cages for 24-h urine collection and food and water intake. Afterwards, half of the animals of each group were killed by decapitation for blood sample collection and kidney removal for glomeruli isolation. The other half of the animals were subjected to renal function studies as described below.

Glomerular nitrite formation

Glomeruli were isolated from kidneys by successive mechanical sieving in a phosphate-buffered saline solution. Glomerular cell viability (>95%) was tested by using a trypan blue exclusion test, and tubular contamination was <5%. Isolated glomeruli were suspended in well plates (4–5 × 10^3 glomeruli/well) with Dulbecco's modified Eagle's medium (Life Technologies, Barcelona, Spain) supplemented with 10% fetal bovine serum, 10 U/ml penicillin/streptomycin mixture (BioWhitaker), and 0.4 m*M* L-Arg for 24 h at 37°C under 5% CO_2. Nitrite formation in the presence or absence of acetylcholine (10^{-7} *M*) was measured by Griess reaction in cell-free supernatants.

Renal function

Renal hemodynamics and excretory function were measured in response to changes in renal perfusion pressure (RPP) in denervated kidneys of inactin-anesthetized rats (100 mg/kg i.p.; Research Biomedical International, Natick, MA, U.S.A.) maintained at 37°C as previously published (6, 7). All animals received an intravenous infusion of 0.9% NaCl solution containing 1% bovine serum albumin at a rate of 2 ml/100 g/h throughout the experiment. Plasma levels of sodium and water-retaining hormones were maintained at fixed high levels by adding aldosterone (20 ng/kg/min), corticosterone (10 ng/kg/min), vasopressin (0.05 ng/kg/min), and norepinephrine (100 ng/kg/min) to the infusion solution. [^{3}H]Inulin (1 μCi/ml; New England Nuclear, Itisa, Madrid, Spain) was included in the infusion solution to measure glomerular filtration rate (GFR). Renal blood flow (RBF) was measured with an electromagnetic flowmeter (Skalar 1421; Skalar, The Netherlands) placed around the left renal artery. After the stabilization period (45 min), RPP was controlled to about 100, 125, and 150 mm Hg by tightening the clamp above the renal arteries; after 15 min of stabilization for each period, urine and plasma samples were collected. In the hypertensive animals, the RPP levels to which the excretory function was measured were higher (120, 140, and 160) in order to not study the renal function below the different autoregulation range of these animals. Urine samples were collected in all periods into preweighed plastic vials, for 10 min. Blood samples (150 μl) for the determination of hematocrit and plasma inulin were obtained at the midpoint of each clearance period into heparinized microhematocrit tubes. Then the animal was euthanized by thoracotomy, and the left kidney was removed and weighed.

Biochemical measurements

Colorimetric reactions employing commercial kits were used to determine plasma levels of creatinine (Medical Analysis Systems, Camarillo, CA, U.S.A.). An adaptation of the microturbidimetric method using an analyzer (ACA DuPont, Wilmington, DE, U.S.A.) was used to measure urine levels of proteins. Radioimmunologic methods employing commercial kits were used to determine plasma levels of renin activity, aldosterone (Sorin, Biomedica, Saluggia, Italy), and endothelin-1 (Amersham Pharmacia Biotech, Little Chalfont, U.K.). Likewise, radioimmunoassay was used to measure urinary levels of thromboxane B_2 (TxB_2) (Amersham Pharmacia Biotech). Plasma concentrations of norepinephrine and epinephrine were measured by high-performance liquid chromatography with electrochemical detection (Bioanalytical Systems 400, West Lafayette, IN, U.S.A.). Concentrations of sodium were measured by flame photometry (NAK-1, Pacisa, Barcelona, Spain). [^{3}H]Inulin in plasma and urine was measured by counting aliquots of the samples dissolved in scintillation fluid (Ecoscint H, National Diagnostics, Atlanta, GA, U.S.A.) in a β counter (Wallac 1409, EG&G Instruments, Madrid, Spain).

Calculations and statistical methods

Data are presented as means ± SE of eight animals per group. GFR was calculated as the clearance of radioactive inulin (urine to plasma concentration ratio × urine flow) and was normalized per gram kidney weight. Urine flow was determined gravimetrically. The slopes of the relationships between RPP and the excretory parameters were calculated by linear regression. Significant differences of the measured values within or between groups were evaluated using an unpaired Student's *t* test or a repeated measures analysis of variance followed by a post hoc Duncan's test, as appropiate. Differences were considered statistically significant at a p level lower than 0.05.

RESULTS

As shown in Fig. 1A, prolonged oral administration of the NO synthesis inhibitor induced an increase in systolic blood pressure. These higher pressure levels in L-NAME-treated rats were accompanied by no changes in sodium excretion (Fig. 1B).

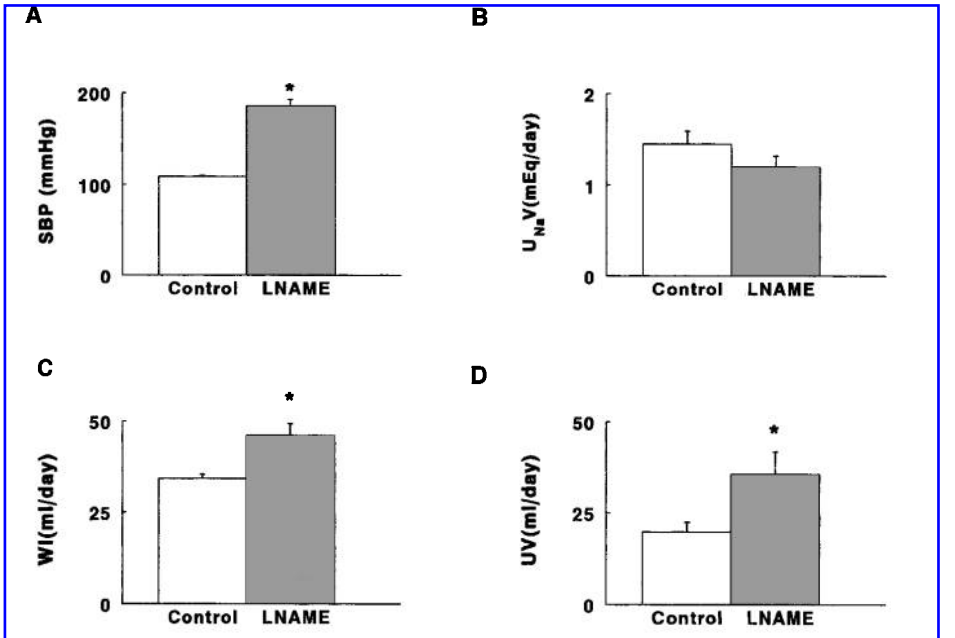

FIG. 1. Effects of oral administration of the NO synthesis inhibitor L-NAME (40 mg/kg/day) for 8 weeks on systolic blood pressure (SBP) (A), on natriuresis ($U_{Na}V$) (B), water intake (WI) (C), and diuresis (UV) (D). Values are means ± SEM of 8 rats. *$p < 0.05$ compared with control group.

By contrast, L-NAME animals showed an increase in both water intake and urine excretion (Fig. 1C and D, respectively). No differences were observed in body weight (data not shown).

As compared with the control group, L-NAME-treated animals presented higher plasma creatinine levels and urine protein excretion (Fig. 2A and B, respectively), suggesting an impairment of renal function. The ratio acetylcholine/basal nitrite production in isolated glomeruli was lower from L-NAME-treated animals than from control ones (Fig. 2C).

The natriuretic and diuretic responses to changes in RPP were blunted in the L-NAME group in comparison with those observed in control animals (Fig. 3A and C, respectively), so the slopes of these relationships were significantly reduced (0.18 ± 0.03 vs. 0.61 ± 0.07; 1.44 ± 0.26 vs. 5.11 ± 0.6, natriuretic and diuretic response, respectively). In addition, GFR and RBF were reduced in those animals. However, changes in perfusion pressure did not modify those hemodynamic parameters in any group (Fig. 3B and D, respectively). No significant changes in hematocrit values were observed in any group throughout the experiment.

Regarding vasoactive factors, Table 1 shows that L-NAME-treated animals presented a decrease in plasma renin activity and aldosterone plasma levels in comparison with control animals. However, they had both higher epinephrine and norepinephrine plasma concentration. As compared with control animals, no changes were found in either endothelin plasma levels (16.1 ± 0.8 vs. 15.2 ± 0.9 fmol/ml) or urine excretion of TxB_2 (17.8 ± 1.3 vs. 15.1 ± 1.5 ng/24 h) in L-NAME-treated animals.

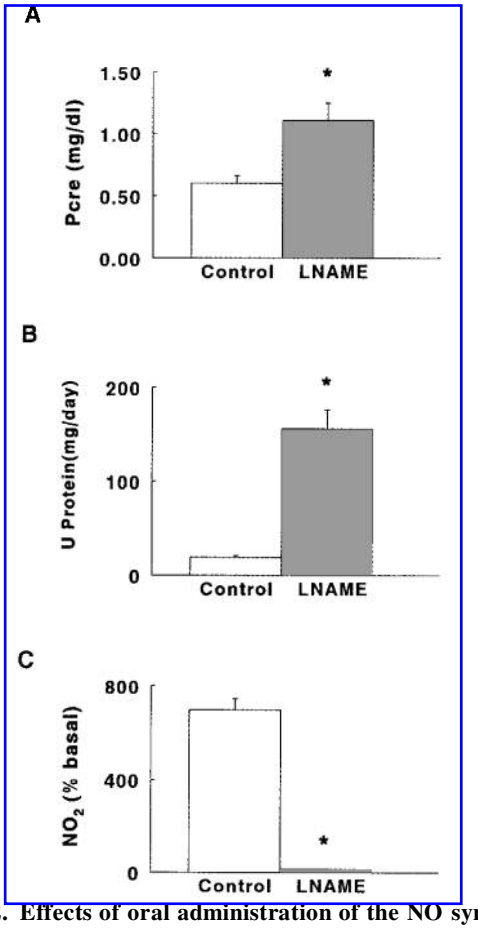

FIG. 2. Effects of oral administration of the NO synthesis inhibitor L-NAME (40 mg/kg/day) for 8 weeks on plasma creatinine (Pcre) (A), urine protein excretion (U protein) (B), and ratio acetylcholine/basal glomerular nitrite formation (NO_2) (C). Values are means ± SEM of eight rats. *$p <$ 0.05 compared with control group.

DISCUSSION

The present study shows that chronic inhibition of NO produces significant alterations in renal function because it

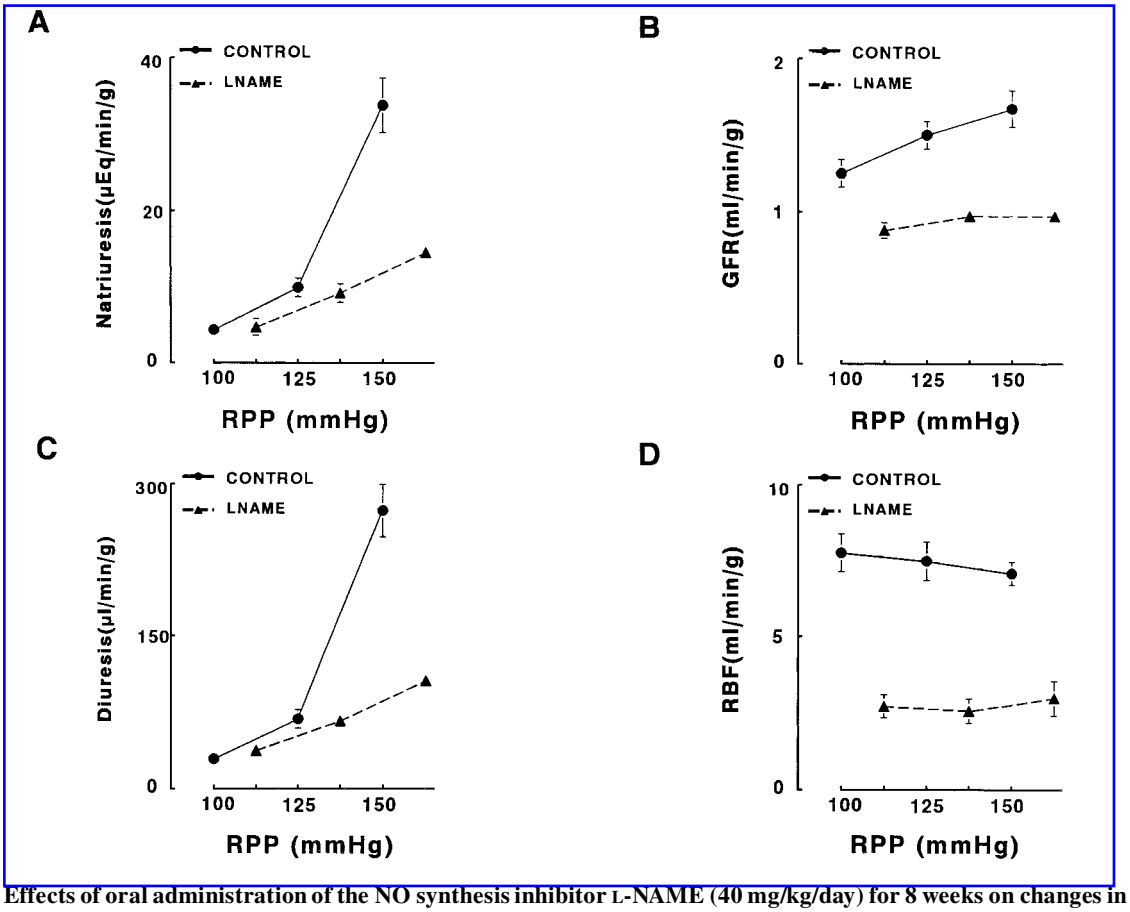

FIG. 3. Effects of oral administration of the NO synthesis inhibitor L-NAME (40 mg/kg/day) for 8 weeks on changes in natriuresis (A), GFR (B), diuresis (C), and RBF (D) in response to changes in RPP. Values are means ± SEM of eight rats.

blunted the pressure–natriuresis–diuresis response and reduced GFR and RBF. In addition, L-NAME administration was associated with renal damage as suggested by the high increase in urine protein excretion. This suggests not only a mediatory role of NO in renal function, but also a renoprotective effect. These observed renal alterations were associated with an overexpression of the systemic catecholamines, but not of either the circulating renin–angiotensin–aldosterone system, endothelin, or renal TxA_2.

As previously reported (14, 19, 25), chronic inhibition of NO produces an increase in blood pressure, confirming the tonic vasodilatory effect exerted by NO in the control of vascular tone (18). It has been shown that the renal vasculature is more sensitive than other vascular beds because the acute inhibition of NO synthesis reduced renal plasma flow even before it produced changes in systemic blood pressure (14). In this regard, the present data showed that renal NO inhibition, as suggested by blunted glomerular NO_2 production, was associated with a reduction in RBF and GFR, indicating that NO exerts a major role in the regulation of renal hemodynamics. This effect could involve modulation of vascular tone not only of afferent arterioles, but also of efferent arterioles because the reduction in RBF was bigger than in GFR (65% vs. 30%). However, although the administration of L-NAME produced an important reduction in renal hemodynamics, renal autoregulation was well preserved, suggesting that NO controls the total level of renal perfusion, but not its pressure dependency.

Acute studies have shown that NO is an important natriuretic factor that plays an important role in the control of so-

TABLE 1. EFFECT OF ORAL ADMINISTRATION OF THE NO SYNTHESIS INHIBITOR L-NAME (40 MG/KG/DAY) FOR 8 WEEKS ON PLASMA LEVELS OF RENIN ACTIVITY (PRA), ALDOSTERONE (PA), NOREPINEPHRINE (NE), AND EPINEPHRINE (E)

Group	*PRA (ng of ANGI/ml/h)*	*PA (pg/ml)*	*NE (pg/ml)*	*E (pg/ml)*
Control	3.8 ± 0.3	479 ± 52	390 ± 65	289 ± 36
L-NAME	1.9 ± 0.2*	189 ± 15*	1,056 ± 142*	923 ± 87*

Values are means ± SEM of eight rats. ANGI, angiotensin I.
*$p < 0.05$ compared with control group.

dium excretion (14, 34). However, the present study shows no differences in natriuresis between control and L-NAME-treated rats, confirming previous observations that indicated that chronic inhibition of NO seems to have a minor impact on sodium excretion (16, 20, 34). This lack of effect on natriuresis could be explained by the fact that the blood pressure increase produced by L-NAME administration can be an important compensatory mechanism that overcomes the initial antinatriuretic effect of NO synthesis inhibition, bringing sodium excretion back to normal. In agreement with previous data, the present study showed that inhibition of NO not only displaced to the right the natriuretic and diuretic response to changes in RPP, but also blunted this response because the slope of the relationship was significantly lower than in control animals. As the pressure–diuresis–natriuresis mechanism participates in the long-term control of blood pressure, the reduced pressure–diuresis–natriuresis response may contribute to the elevation and/or maintenance of hypertension in these animals. The prolonged inhibition of NO also produces an increase in urine excretion. This increase was accompanied by a higher water intake, probably to maintain water balance. This higher urine excretion could be due to changes in antidiuretic hormone, either its release or its action, because it was associated with no changes in natriuresis. In this regard, it has been reported that NO stimulates the basal release of antidiuretic hormone (13, 24).

As previously reported (20, 23, 25), the present data show that the chronic administration of L-NAME also causes a massive proteinuria, supporting the concept of a renoprotective role of NO. This renal damage seems to be a consequence of functional rather than structural disruption of the glomerular wall, because these alterations can be reversed by cessation of NO administration. Moreover, it seems to be not only a consequence of the increase in blood pressure, because it can be prevented by the administration of indomethacin even without a reduction in mean arterial pressure, supporting the role of local factors such as TxA_2 in these alterations. These functional changes could involve modifications in the glomerular permeability of proteins as a consequence of changes in both glomerular size and charge selectivity due to the depletion of anionic charges in glomerular basement membrane (1, 11).

The consequences of the chronic NO inhibition on circulating vasoactive systems have yielded various results. In this regard, the present data show that the administration of L-NAME for 8 weeks did not activate the circulating renin–angiotensin–aldosterone system because plasma renin activity and aldosterone were lower in L-NAME-treated animals than in control ones. In fact, no changes, reduction or even an increase, in plasma renin activity have been reported (16, 19, 22, 23). These contradictory results could be due to the timing because it has been shown that plasma renin activity varied widely during the inhibition time (19, 23). These changes could be the result of the balance of different mechanisms, including the negative effect of NO on renin release and the high blood pressure levels. It is important to mention that although the present data show a lower than normal level of the renin–angiotensin–aldosterone system, it is likely that in the absence of NO these levels may be harmful, because it has been shown that treatment with drugs that interact with this system can partially reduce the changes in renal hemodynamics and proteinuria (7, 20, 21).

The present study also shows that renal impairment is not associated with an increase in urine excretion of TxB_2, which is an index of renal TxA_2 production. This suggests that an activation of TxA_2 synthesis seems not to be responsible for the functional and renal damage observed in these animals. However, the role of this vasoconstrictor factor in the renal damage produced by chronic inhibition of NO cannot be totally eliminated because the coadministration of indomethacin prevents the proteinuria without modifying the effects of L-NAME on renal function or blood pressure (15). A similar situation could be the case for endothelin because we have found no increase in endothelin plasma levels. However, several data have shown that the administration of endothelin antagonist attenuates the deleterious consequences of NO inhibition (21, 28). Therefore, these data support that, in the absence of NO, both TxA_2 and endothelin actions would be overexpressed in the kidney.

In agreement with previous studies, we have observed that NO inhibition is associated with an increase in circulating epinephrine and norepinephrine levels, suggesting an activation of the sympathoadrenal system (14, 26, 31). This activation might be dependent on the degree of inhibition of NO synthase because plasma concentrations of epinephrine and norepinephrine were not modified with low doses of L-NAME (14). It is known that catecholamines can modulate renal function in a direct and indirect manner by affecting both tubular function and renal hemodynamics. Therefore, it is possible to suggest the participation of the sympathetic nervous system in the renal alterations induced by chronic inhibition of NO. Indeed, its participation in the hypertension induced by NO synthesis inhibition has been proposed, among other mechanisms, through an increase in renal nerve activity (17). This increase in catecholamine levels observed in L-NAME animals could be a consequence of enhanced sympathoadrenal outflow due to NO inhibition because it has been shown that NO exerts a negative modulatory effect on catecholamine release in both central nervous system and adrenal medulla (2, 17).

In summary, the present data show that the kidney is an important target of chronic inhibition of NO because L-NAME-treated rats present important alterations in renal function, as well as renal damage. The chronic blockade of NO synthesis only produced the activation of the catecholamines, but not of other vasoconstrictor factors such as the renin–angiotensin system, endothelin, or TxA_2. These effects observed with NO synthesis inhibitors on the kidney are produced by a decreased availability of NO, which leaves unbalanced the activity of pressor systems such as the sympathetic nervous system.

ACKNOWLEDGMENTS

The authors' work was made possible by grants from the Ministry of Science of Spain SAF 2000-0176 and Fondo de Investigaciones Sanitarias of Spain FIS 98/0003-02.

ABBREVIATIONS

GFR, glomerular filtration rate; L-NAME, N^G-nitro-L-arginine methyl ester; NO, nitric oxide; RBF, renal blood

flow; RPP, renal perfusion pressure; TxA_2, thromboxane A_2; TxB_2, thromboxane B_2.

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Received for publication January 9, 2002; accepted July 3, 2002.

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