ORIGINALES

Role of Endogenous Nitric Oxide. Evidence for a Nitric Oxide (NO)-Sensitive Regulation of Tubule Na Transport. Contribution of Atrial Natriuretic Peptide 99-126 [1]

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SUMMARY

Preliminary studies have suggested that nitric oxide (NO) control blood pressure in the basal state and plays a role in the water and sodium handling by the kidneys. Inhibition of NO synthesis with competitive L-arginine analogues leads to increased renal vascular resistance and raised systemic and glomerular blood pressure. We questioned whether the effects of NO synthase inhibition, such as N^G-nitro L-arginine methyl-ester (L-NAME) interferes with the disposal of an acute NaCl load in chronically NO-blocked (N = 8) anesthetized Munich-Wistar rats compared to controls (N = 6). Significant systemic hypertension and a marked renal vasoconstriction was accompanied with a decline in renal plasma flow, without changes in glomerular filtration rate, with filtration fraction thus being increased in the NO-blocked rats. In addition, we observed a marked absolute and fractional excretion of sodium without influences in potassium excretion. These observations could suggest a pressure-natriuresis mechanism plus a reduction in tubular reabsorption of sodium, somewhere in the distal nephron through an NO-sensitive mechanism for regulating tubule Na transport. In an orally L-NAME pretreated rats (N = 6), the effects of ANP 99-126 administration resulted in a transient decrease of RPF (4.22 ± 0.54 mL/min at 60 min) compared with control (N = 5: 6.37 ± 0.77 mL/min at 60 min), with the consequent increase in filtration fraction in the former group. MAP and RVR were maintained without significative changes in each group during the experiment, though L-NAME pretreated rats showed a significant elevation as compared with control. The infusion of ANP 99-126 (0.01 µg/Kg/min) resulted in an 46-fold increase in urinary sodium excretion in the L-NAME pretreated rats, as compared with a 26-fold increase in control rats. However, fractional and absolute potassium excretion was significantly higher in the control rats.

Key words: ***Nitric oxide. Natriuresis. Atrial natriuretic peptide. L-NAME (Nitro-L-arginine methyl ester).***

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PAPEL DEL OXIDO NITRICO ENDOGENO. EVIDENCIA DE UN MECANISMO REGULADOR (NO)-SENSIBLE. CONTRIBUCION DEL PEPTIDO NATRIURETICO ATRIAL 99-126

RESUMEN

Estudios previos han sugerido que el óxido nítrico (NO) controla la presión arterial en situacion basal, al igual que interviene en el manejo del agua y del sodio por parte del riñón.

La inhibición de la síntesis del óxido nítrico con análogos de la L-arginina, en este caso con L-NAME (Nitro-L-arginina metiléster), provoca un incremento de la presión arterial sistémica y glomerular junto a una importante elevación de las resistencias vasculares renales. Para analizar si los efectos de la inhibición crónica de la óxido nítrico sintetasa interfiere con el manejo en la excreción de una sobrecarga de sodio, hemos utilizado ratas Munich-Wistar a las que se les ha administrado L-NAME (100 mg/L) en el agua de bebida diariamente. El experimento fue llevado a cabo en ratas anestesiadas. El grupo tratado con L-NAME (n = 8) mostró frente al grupo de ratas controles (n = 6) una marcada vasoconstricción renal, acompañada de hipertensión arterial sistémica y disminución del flujo plasmático renal, sin cambios en el filtrado glomerular y elevada fracción de filtración. La excreción absoluta y fraccional de sodio se encontró aumentada sin modificaciones en la eliminación de potasio, lo que podría sugerir la existencia de no sólo un mecanismo presión-natriuresis, sino también de una reducción en la reabsorción tubular de sodio en algún lugar de la nefrona distal. Este fenómeno sugeriría un mecanismo óxido nítrico sensible que regulase el transporte tubular de sodio.

En una segunda parte del experimento, un nuevo grupo de ratas pretratadas crónicamente con L-NAME oral (n = 6) fueron expuestas a la administración de factor natriurético auricular de rata (ANP 99-126), frente a un grupo control no pretratado con L-NAME (n = 5). Se encontró una disminución significativa del flujo plasmático renal y elevación de la fracción de filtración en el grupo pretratado con L-NAME. La presión arterial sistémica y las resistencias vasculares renales no se modificaron a lo largo del estudio en cada grupo de animales, aunque estaban significativamente más elevadas en el grupo que recibió L-NAME respecto al grupo control ($p < 0,001$ y $p < 0,05$, respectivamente, a los 180 minutos del experimento). La administración de ANP 99-126 a las dosis utilizadas provocó una excreción absoluta de sodio 46 veces superior a la basal en el grupo tratado previamente con L-NAME. Por el contrario, la excreción absoluta y fraccional de potasio fue significativamente más elevada ($p < 0,05$) en el grupo no tratado previamente con L-NAME.

Estos resultados sugieren una atenuación del efecto kaliurético del ANP en aquellos animales previamente tratados con L-NAME, datos que apoyarían la existencia de mecanismo NO-sensible a nivel tubular.

Palabras clave: ***Oxido nítrico. Natriuresis. Factor natriurético auricular. L-NAME (Nitro-L-arginina metiléster).***

Acethylcholine (ACh)-induced relaxation of arteries is endothelium-dependent and the relaxation is mediated through the release of endothelium-derived relaxing factor(s) (EDRF) [1, 2]. Subsequent studies suggested that at least one EDRF is nitric oxide (NO) [3, 4]. Particularly, the association of ACh-induced vasodilatation and the release of nitric oxide has been demonstrated in the isolated perfused rabbit heart [5]. The synthesis of NO from L-Arginine has been proposed to represent a widely expressed process [6]. NO, much like oxygen is actually a gas with an ultrashort half-life (less than 5 seconds in biological tissues) that is sparingly soluble in aqueous medium and functions biologically as a molecule in solution.

Nitric oxide which is generated from L-Arginine by the constitutive type I nitric oxide synthase (NOS), a

Ca2+ /calmodulin-dependent enzyme[7], stimulates increases in cGMP within the isolated aorta and in vascular smooth muscle cells and platelets[8-10]. The increase occurs via stimulation of the soluble, or citosolic guanylate cyclase in an autocrine or paracrine type of action[11].

In light of the evidence indicating that intrarenal infusions or either ACh or bradykinin, endothelium-dependent vasodilators that increase NO synthesis and release, elicit an increase in sodium excretion as well as renal vasodilation[12], it was expected that the inhibition of NO synthesis would result in a decrease in sodium excretion as well as renal vasoconstriction.

To try to examine some of the systemic and intrarenal effects of NO in normal animals, several investigators have given the animals different L-Arginine analogues as specific substrate competitors[12-20]. Systemic blockade of EDRF/NO synthesis with these L-arginine analogue increases both arterial pressure (AP) and renal vascular resistances in anesthetized and conscious animals[21-24]. Baylis et al[24], have demonstrated in conscious rats that the decrease in renal plasma flow (RPF) caused by inhibition of EDRF/NO synthesis was accompanied by a minor decrease in glomerular filtration rate (GFR), resulting in an increase in filtration fraction (FF). The same authors based on these observations describe a new model of systemic hypertension with glomerular capillary hypertension[25]. Variable effects of NO synthesis inhibition on sodium excretion have been reported. Several authors have reported that NO synthase inhibitors *in vivo* induces natriuresis and diuresis[15, 19, 24, 26, 27]. Although some investigators have suggested that the increased excretion of sodium and water during NO inhibition is due to «pressure natriuresis»[16-18, 28], others have hypothesized a proximal direct tubular action[27], or a distal tubular action of the NO synthase inhibition[15, 19, 24]. Navarro et al[29], used increasing concentrations of L-NAME in the drinking water for five weeks, in Sprague-Dawley rats, and only found blood pressure elevation without changes in sodium excretion or diuresis.

In a preliminary study, data from our laboratory[19] in normal and DOCA-salt hypertensive rats L-NAME induced natriuresis with a minimal kaliuretic response, suggesting a terminal nephron site of action of this nitric oxide synthesis inhibitor.

There is a constant controversy about the difficulty in separating direct renal effects of NO synthesis inhibition from systemic hemodynamic effects in the various experimental models.

In view of this apparent discrepancy, the purpose of the present experiment was twofold. Firstly, to investigate the effects on renal hemodynamics and excretory function in anesthetized rats, of chronic NO synthesis inhibition on the response to an acute NaCl load. Since N^G-nitro-L-arginine is not readily soluble in water, we used N^G-nitro-L-arginine methyl ester (L-NAME) an easily dissolved and orally active NO inhibitor[25, 30]. Secondly, we explore the role of ANP 99-126 (atrial natriuretic peptide 99-126) in blood pressure alteration, natriuresis and kaliuresis in L-NAME pretreated rats. Some of the ANP described actions include an elevation of GFR and renal sodium excretion, relaxation of contracted vasculature *in vitro,* and reduction of systemic arterial blood pressure *in vivo*. Therefore, the present study was undertaken to evaluate the effects of ANP on the systemic and renal circulation under NO syntesis inhibition.

Since NO is a very labile substance, direct measurement of NO has proven to be difficult, especially in vivo experiments. We must limited as in other experiences to interpretation of the responses to NO synthase inhibitors.

Materials and methods

Studies were performed on 25 male Munich-Wistar rats (220-300 g body weight) from the Charles River, Wilmington, Mass. All experimental procedures were designed in accordance with 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. Animals were maintained on a 12-hour light/dark cycle and provided normal rat chow and tap water ad libitum. In the first experiment protocol (Study I) eight rats (Group Ia) were placed on oral L-NAME (100 mg/L in drinking water, changed daily) for a continuous 10 to 15 day period. After 5 to 7 days of habituation, systolic blood pressure was recorded every two days in all rats by the awake tail cuff method[31], until hypertensive state was confirmed. A control group of six rats (Group Ib) aged over a similar time period differed only in not receiving L-NAME (figure 1).

The day of the experiment, rats were anesthetized with intraperitoneal thiobarbiturate, Inactin (BYK Gulden, Konstans Fed Rep. Germany) (100 mg/kg body wt.) and placed on a temperature-regulated micropuncture table. Rectal temperature was maintained at 37 ± 1.0 °C. An indwelling polyethylene catheter (PE-50) was placed in the left femoral artery for continuous monitoring of mean arterial pressure (MAP) as well as for collecting blood samples.

MAP was measured utilizing a pressure transducer connected to a direct recorder (Gould Inc. Cleveland, OH. with a thermal writing recorder 8000-S, model 8188-2202). After a baseline blood sample was collected and tracheostomy, both yugular veins were catheterized with PE-50 polyethylene tubing, one for

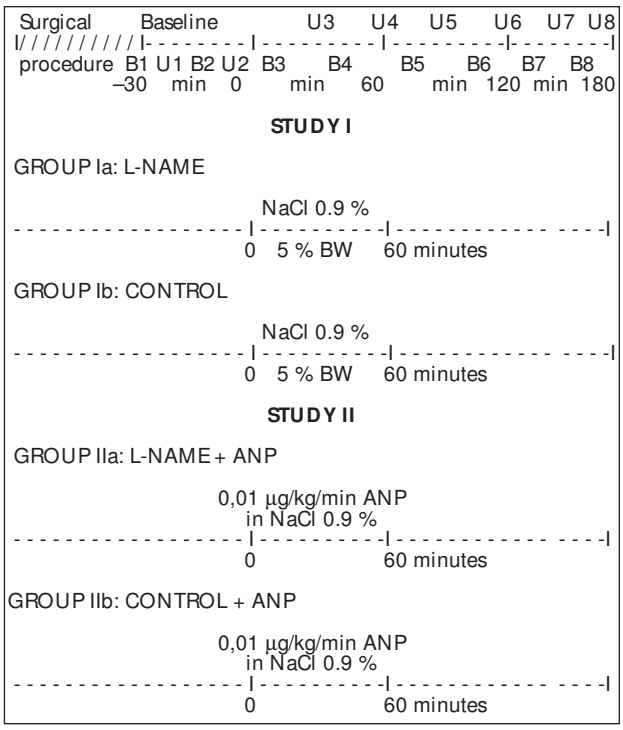

Fig. 1.–Schematic representation of experimental protocols.

continuous iv infusion throughout the experiment of isoncotic plasma obtained from normal adult Munich-Wistar rats to maintain an euvolemic condition and for a sustained infusion of p-aminohippurate (400 µl) and inulin (10 ml) solution for the measurement of renal plasma flow (RPF) and glomerular filtration rate (GFR). The left kidney was exposed through a ventral midline incision and its surface was moistened with saline throughout the experiment. The left ureter was cannulated with PE-10 tubing and the bladder vented by a curved 19-gauge needle. Urine was collected in a preweighed plastic vial for gravimetric determination of urine flow rate. After a 60-min equilibration time, when plasma inulin and PAH concentrations had plateaud, a control observation period was begun in which two 15 min urine collections were made and arterial blood samples were taken at the midpoint of each urine.

After completion of control measurements, in Study I the second yugular vein was used with an isotonic infusion of NaCl (5 % BW in 60 min). Six more 30-min urine collections with midpoints bloods were taken.

In Study II, six rats (Group IIa) previously placed on oral L-NAME (100 mg/L in drinking water during a continuous period of 10-15 days) followed a similar surgical protocol to those of Study I with the difference that, during the experimental period 0,01 µg/Kg/min was infused in saline (1.5 µg/mL), of purified synthetic rat atrial natriuretic peptide (ANP 99-126), from Peninsula Laboratories (Belmont CA), during a 60-min period (figure 1). The dose utilized was previously determined to yield effective natriuretic and diuretic effects without a deleterious alteration of rat GFR and minimal changes in blood pressure. Each rat received only one peptide infusion. A control group of five rats (Group IIb) differed only in not receiving L-NAME.

Analytical procedures

Hematocrit was determined by the microcapillary tube method. Urinary and plasma sodium and potassium concentration were measured by standard flame photometry. Protein concentration in femoral arterial blood plasma was determined using the fluorometric method [32]. Inulin concentrations in plasma and urine were measured using a macro-anthrone method [33], and PAH concentrations were measured by the method of Smith et al [34].

Filtration fraction was calculated as FF= GFR/RPF. Renal vascular resistance was estimated by the expression RVR= MAP (1HCT)/RPF, where HCT is arterial hematocrit. Efferent arteriolar protein concentration was estimated by the expression CE=CA/(1-FF), where CA and CE are plasma protein concentrations in afferent (femoral artery determination) and efferent arterioles, respectively.

Results are presented as mean ± 1 SE. Analysis of comparisons between groups was performed by one-way analysis of variance. We considered a P value of less than 0.05 to be statistically significant.

Results

Study I: Rats subjected to a NaCl load

The mean body weight of the rats used in this study was 289 ± 4.6 g and 260.1 ± 11.6 g (NS) for groups Ia and Ib, before placing the former on oral L-NAME.

The effect of NaCl load on mean arterial pressure, renal vascular resistance and urinary sodium and potassium excretion in rats of groups Ia and Ib is shown in figure 2

After 10-15 days rats placed on oral L-NAME were hypertensive. Mean arterial pressure averaged 130.5 ± 2.3 in the L-NAME group, and each value was significantly higher ($P < 0.001$) than the arterial pressure of 97.7 ± 3.9 mmHg for the vehicle-treated control rats (fig. 2A). A marked and significant elevation in systemic MAP was maintained throughout the experiment. The renal vascular resistance (RVR) increased in parallel with MAP and maintained for more than 180 minutes (27.1 ± 1.9 and 22.0 ± 1.1 vs 13.07 ± 1.0 and 12.9 ± 0.7 mmHg/mL/min in groups Ia and

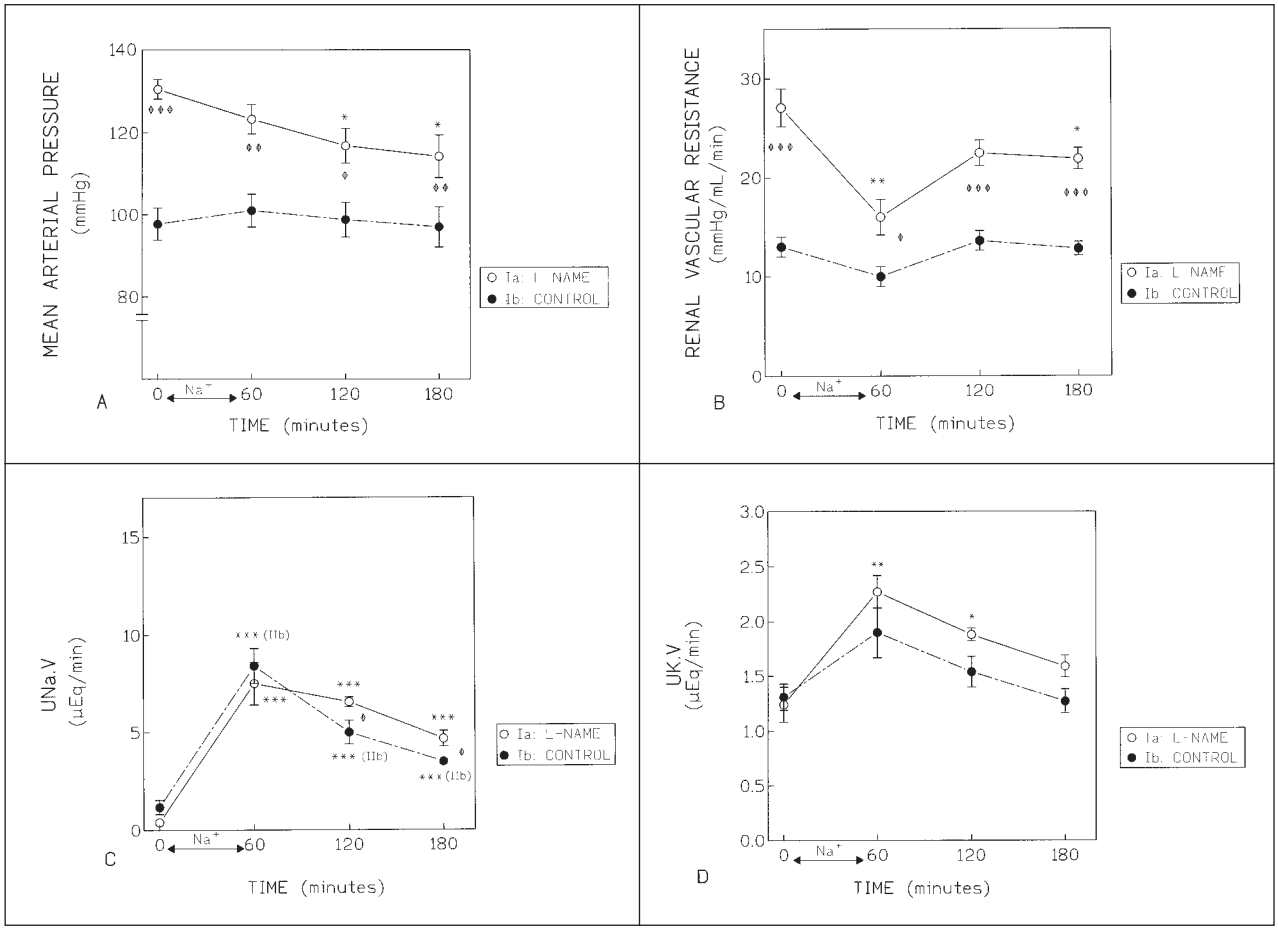

*Fig. 2.–Effect of NaCl load in rats of study I in the baseline and during the experimental protocol. Mean arterial pressure (A), renal vascular resistance (B), urinary excretion rates of sodium (C), and potassium (D). * P < 0.05, ** P < 0.01, *** P < 0.001 vs baseline. ♦ P < 0.05, ♦♦ P < 0.01, ♦♦♦ P < 0.001 group Ia vs group Ib.*

Ib respectively). A slight decrease in RVR was observed in groups Ia and group Ib during NaCl infusion although it was more pronounced in the L-NAME treated rats (fig. 2B). Since the glomerular filtration rate was unchanged in L-NAME and in the control group of rats by NaCl infusion, RPF was significantly reduced (P< 0.01) in group Ia. These hemodynamic changes yielded at 120 and at 180 min with a significant increase in FF. No significant differences in urine output was seen between L-NAME and control group. The magnitude of the slight decrease in arterial hematocrit was similar in groups Ia and Ib. Associated with the pressor responses elicited by L-NAME were a striking increase in urinary sodium excretion during NaCl load (fig. 2C) also evident at 180 min (4.67 ± 0.4 µeq/min) and significantly higher (P< 0.05) than the 180 min sodium excretion rate measured in the vehicle-treated animals (3.5 ± 0.23 µeq/min). Both groups of rats excreted with minor differences (NS) the same sodium load throughout the experiment. On the other hand, urinary potassium excretion did not differ in Ia and Ib groups respectively (fig. 2D). In group Ia mean arterial pressure and RVR had decreased somewhat by the end of the protocol (basal vs final 130.5 ± 2.36 vs 114.2 ± 5.2 mmHg, P< 0.05, and 27.1 ± 1.9 vs 21.9 ± 1.1 mmHg/mL/min, P < 0.05 respectively), however RPF (basal vs final 2.67 ± 0.22 vs 3.1 ± 0.11 mL/min, P = NS) were unchanged over the course of the experiment. In both groups Ia and Ib, a significant increase (P< 0.001) in urine flow rate and UNa.V was observed over the 180 minutes experimental period.

Study II: Rats subjected to atrial natriuretic peptide (ANP 99-126) infusion

Arterial hcts were similar between experimental (IIa) and control (IIb) groups. There were no signifi-

cant differences in mean body weight for both group of rats 280 ± 2.2 g (group IIb) and 280.8 ± 7.5 g (group IIa).

Figure 3 shows the data for mean arterial pressure, glomerular filtration rate and urinary sodium and potassium excretion measured before and 60, 120 and 180 minutes after infusion of ANP in L-NAME and vehicle-control groups. MAP slightly decreased (Fig. 3A) after ANP 99-126 infusion in L-NAME group, but still remained significantly higher than the vehicle group (116.2 ± 2.39 vs 93.5 ± 2.2 mmHg respectively at 180 min after ANP). In control rats and in those pretreated with L-NAME coincident with the ANP infusion, glomerular filtration rate increased but, return to basal levels straightaway (Fig. 3B), without any difference between IIa and IIb groups. The filtration fraction was significantly higher ($P < 0.01$ and $P < 0.05$ at 120 and 180 min respectively) in the group IIa associated with a decreased RPF at baseline in this group of rats ($p < 0.05$).

The peptide caused a significant increase in urine flow in group IIa ($P < 0.05$) and IIb ($P < 0.01$) at 120 minutes respect to baseline as well as an increase in urine sodium excretion ($P < 0.05$) and ($P < 0.01$) for both groups respectively. The infusion of ANP 99-126 in group IIa animals resulted in a 46-fold increase in urinary sodium excretion averaged 13.84 ± 2.1 and 9.67 ± 1.0 at 60 ($P < 0.01$) and 120 ($P < 0.05$) min vs 5.32 ± 1.36 and 5.56 ± 0.93 µeq/min in group IIb (a 26-fold increase in UNa.V), and each value was significantly higher than the basal excretion rate in both groups 0.3 ± 0.05 (group IIa) ($P < 0.05$) and 0.2 ± 0.09 (group IIb) µeq/min ($P < 0.01$) at 120 min (Fig. 3C). Fractional excretion of potassium as well as absolute urinary potassium excretion (Fig. 3D) were significantly higher in group IIb as compared with L-NAME-ANP rats (IIa) at 120 min ($P < 0.05$) and at 180 min however, at that latest period this difference was not statistically significant for the fractional excretion rate ($P < 0.1$). Thus, the «potassium sparing»

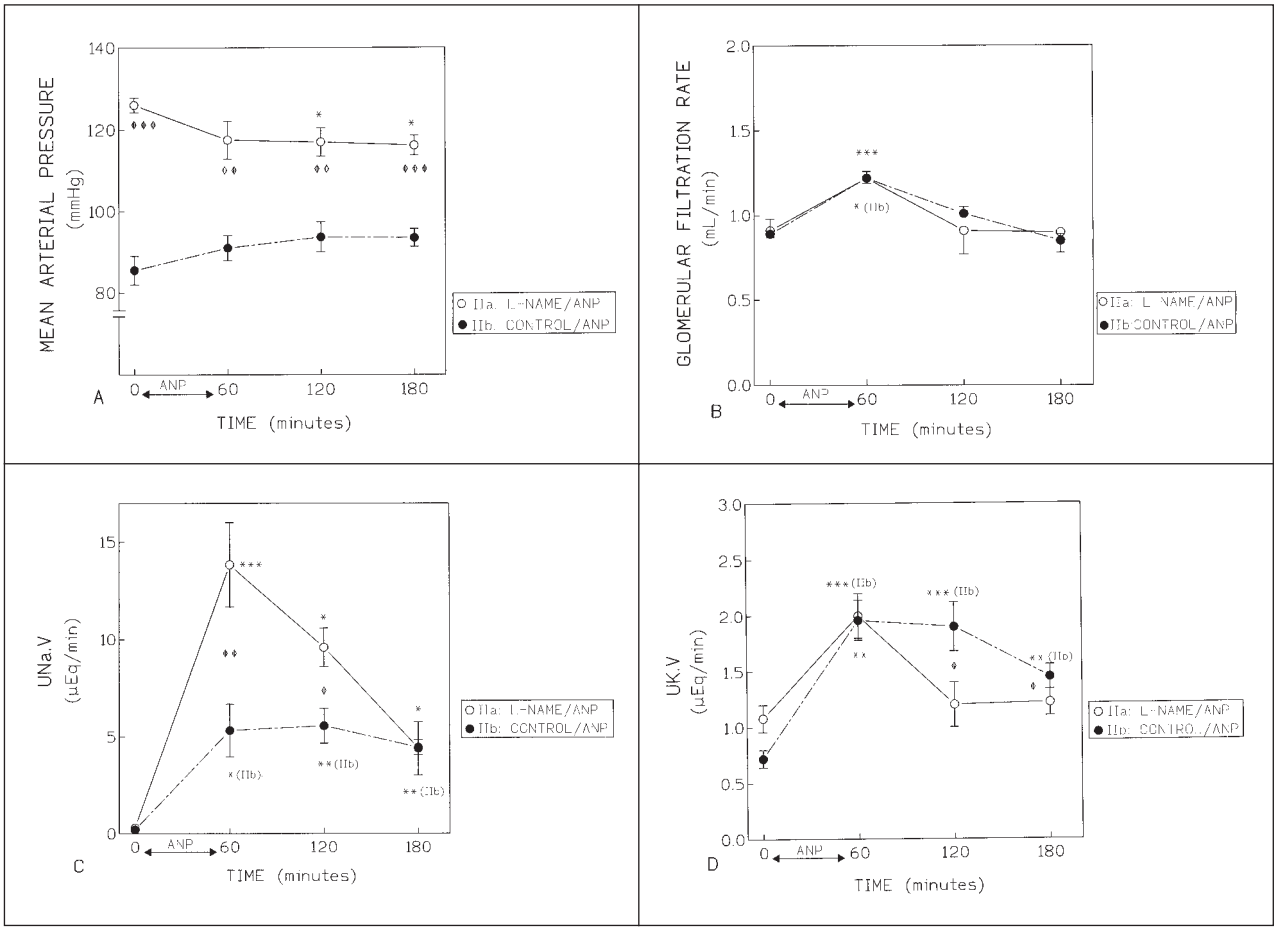

*Fig. 3.–Effect of ANP (99-126) administration in control and L-NAME rats (Study II) in baseline and over the course of the experiment. Mean arterial pressure (A), gomerular filtration rate (B), urinary excretion rates of sodium (C), and potassium (D). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs baseline. ♦ $P < 0.05$, ♦♦ $P < 0.01$, ♦♦♦ $P < 0.001$ group IIa vs group IIb.*

Table I. Systemic and renal variables in anesthetized male Munich-Wistar rats studied in the basal conditions and after chronic EDRF-Blockade with L-Name. Groups Ia (A) and Ib (B) were loaded with NaCl 5 % BW, and groups IIa (C) and IIb (D) with ANP 99-126 (0.01 µg/kg/min) during 60 min after basal measures[a]

	Basal	60'	120'	180'
L-NAME group (Ia) (A)				
HCT (%)	46.5 ± 0.63	40.9 ± 0.65**, ♦	40.6 ± 0.89**, ♦	41 ± 0.85**, ♦
MAP (mmHg)	130.5 ± 2.36 ♦♦♦	123.3 ± 3.58 ♦♦	116.8 ± 4.23*, ♦	114.2 ± 5.2*. ♦♦
GFR (mL/min)	0.88 ± 0.05	1.28 ± 0.09**	1.02 ± 0.04	0.96 ± 0.4
RPF (mL/min)	2.67 ± 0.22 ♦♦	4.9 ± 0.48**	3.12 ± 0.15 ♦♦	3.1 ± 0.11 ♦♦♦
FF	0.33 ± 0.01 ♦♦♦	0.26 ± 0.01**	0.33 ± 0.01 ♦♦	0 31 ± 0 01 ♦
RVR (mmHg/mL/min)	27.1 ± 1.9 ♦♦♦	16.0 ± 1.8**, ♦	22.5 ± 1.3 ♦♦♦	21.9 ± 1.1*. ♦♦♦
UV (mL/min)	0.006 ± 0.0008	0.039 ± 0.005***	0.033 ± 0.001***	0.027 ± 0.001***
UNa.V (µEq/min)	0.39 ± 0.12	7.5 ± 1.09***	6.55 ± 0.25***, ♦	4.67 ± 0.4***. ♦
FENa (%)	0.29 ± 0.09	4.4 ± 0.78***	4.55 ± 0.24***, ♦	3.4 ± 0.27***. ♦
UK.V (µEq/min)	1.24 ± 0.16	2.27 ± 0.15**_	1.88 ± 0 06*	1 59 ± 0.1
FE K (%)	31.1 ± 2.87	41.8 ± 2.3*	43.9 ± 2.3**-	39.3 ± 2.0*
CA (g/dl)	5.0 ± 0.03	4.0 ± 0.08**	4.3 ± 0.1**	4.4 ± 0.1*
CE (g/dl)	7.5 ± 0.1 ♦♦	5.6 ± 0.2**	6.5 ± 0.28*	6.4 ± 0.3*
Control group (Ib) (B)				
HCT (%)	46.7 ± 0.64	43 ± 0.5*	43.5 ± 0.81*	43.6 ± 0.81*
MAP (mmHg)	97.7 ± 3.91	101 ± 4.0	98.8 ± 4.17	97 ± 4.89
GFR (mL./min)	1.01 ± 0.08	1.58 ± 0.19	1.10 ± 0.09	1.08 ± 0.06
RPF (mL/min)	4.09 ± 0.31	5.91 ± 0.49*	4.18 ± 0.2	4.26 ± 0.19
FF	0.24 ± 0.01	0.27 ± 0.04	0.26 ± 0.01	0.25 ± 0.01
RVR (mmHg/mL/min)	13.0 ± 1.0	10.0 ± 1.0	13.6 ± 1.0	12.8 ± 0.7
UV (mL/min)	0.0082 ± 0.0016	0.04.3 ± 0.005**	0.032 ± 0.002***	0.024 ± 0.002***
UNa.V (µEq/min)	1.17 ± 0.36	8.4 ± 0.9***	5.0 ± 0.6***	3.5 ±i 0.23***
FENa (%)	0.8 ± 0.2	4.2 ± 0.9*	3.4 ± 0.7*	2.3 ± 0.3**
UK.V (µEq/min)	1.31 ± 0.12	1.90 ± 0.23	1.54 ± 0.14	1.27 ± 0.11
FE K (%)	33.7 ± 4.0	37.3 ± 8.7	40.8 ± 7.2	32.7 ± 4.1
CA (g/dl)	5.0 ± 0.1	4.3 ± 0.07**	4.5 ± 0.14*	4.5 ± 0.12*
CE (g/dl)	6.7 ± 0.16	6.0 ± 0.34	6.2 ± 0.21	6.1 ± 0.17*
L-NAME-ANP group (IIa) (C)				
HCT (%)	46.7 ± 0.36	42.6 ± 1.79	41.3 ± 0.59**-	42.0 ± 0.4**
MAP (mmHR)	126.0 ± 1.81 ♦♦♦♦	117.5 ± 4.63 ♦♦	117.0 ± 3.48*, ♦♦	116.2 ± 2.39*. ♦♦♦
GFR (mL/min)	0.91 ± 0.04	1.22 ± 0.03 ***	0.91 ± 0.14	0.90 ± 0.12
RPF (mL/min)	2.99 ± 0.18 ♦	4.22 ± 0.54 ♦	3.52 ± 0.57	3.39 ± 0.37
FF	0.31 ± 0.015 ♦♦	0.31 ± 0.04 ♦	0.26 ± 0.006*, ♦♦	0.26 ± 0.007*. ♦
RVR (mmHg/mL/min)	22.9 ± 1.82 ♦♦♦	17.0 ± 2.0 ♦♦	20.6 ± 2.6 ♦	20.0 ± 2.0 ♦
UV (mL/min	0.0054 ± 0.00047	0.065 ± 0.0092***, ♦♦	0.026 ± 0.0046*	0.024 ± 0.0048*
UNa.V (µEq/min)	0.30 ± 0.05	13.84 ± 2.16***, ♦♦	9.6 ± 1*, ♦	4.39 ± 1.37*
FENa (%)	0.22 ± 0.04	8.07 ± 1.19***, ♦♦	6.59 ± 0.62**, ♦♦	3.52 ± 1.04*
UK.V (µEq/min)	1.08 ± 0.12	2.0 ± 0.2**-	1.2 ± 0.20 ♦	1.22 ± 0.12 ♦
FE K (%)	27.2 ± 1.6	44.8 ± 4.9*	34.3 ± 2.7*, ♦	37.3 ± 3.72*
CA (g/dl)	5.18 ± 0.06	4.56 ± 0.16*, ♦	4.62 ± 0.094*, ♦	4.65 ± 0.095*. ♦
CE (g/dl)	7.51 ± 0.11♦♦♦	6.87 ± 0.63 ♦	6.24 ± 0.12**, ♦♦	6.38 ± 0.14 *. ♦♦
Control-ANP group (IIb) (D)				
HCT (%)	45.9 ± 0.46	40.7 ± 0.46**-	41.2 ± 0.47**	41.1 ± 0.51**
MAP (mmHg)	85.5 ± 3.49	91 ± 3.06	93.7 ± 3.72	93.5 ± 2.21
GFR (mL/min)	0.89 ± 0.09	1.22 ± 0.04*	1.01 ± 0.04	0.85 ± 0.04
RPF (mL/min)	4.19 ± 0.47	6.37 ± 0.77*	5.03 ± 0.34	3.87 ± 0.61
FF	0.21 ± 0.02	0.20 ± 0.01	0.20 ± 0.01	0.23 ± 0.02
RVR (mmHg /mL/min)	11.4 ± 1.09	8.95 ± 1.09	11.03 ± 0.55	15.27 ± 2.28
UV (mL/min)	0.0038 ± 0.00028	0.027 ± 0.0054**	0.029 ± 0.0041**	0.024 ± 0.0020**
UNa.V (µEq/min)	0.20 ± 0.09	5.32 ± 1.36*	5.56 ± 0.9**	4.44 ± 0.38**
FENa (%)	0.17 ± 0.07	2.95 ± 0.77*	3.81 ± 0.70**	3.76 ± 0.39***
UK.V (µEq/min)	0.72 ± 0.08	1.96 ± 0.18***	1.90 ± 0.22***	1.45 ± 0.11**
FE K (%)	22.0 ± 4.04	40.1 ± 3.89*	49.7 ± 4.71**	47.3 ± 2.01**
CA (g/dl)	5.03 ± 0.033	4.08 ± 0.037**	4.3 ± 0.07**	4.3 ± 0.07**
CF: (g/dl)	6.45 ± 0.18	5.1 ± 0.09***	5.4 ± 0.07**	5.6 ± 0.2*

[a] Values are means ± SE. HCT, hematocrit; MAP, mean arterial pressure; GFR, glomerular filtration rate; RPF, renal plasma flow; FF, filtration fraction; RVR, renal vascular resistance; UV, urinary volume; UNa. V, sodium excretion rate; FENa, fractional excretion of sodium; UK.V, potassium excretion rate; FE K, fractional excretion of potassium; CA and CE, afferent and efferent arteriolar protein concentration.
Significant differences between subsequent time points and baseline within each group: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Significant differences between groups Ia and Ib and IIa IIb: ♦ $p < 0.05$; ♦♦ $p < 0.01$; ♦♦♦ $p < 0.001$.

effect of ANP 99-126 was only maintained in the control-vehicle group.

The increased renal vascular resistances in group IIa dropped slightly as well as in group IIb after the initiation of ANP 99-126 infusion. As a consequence of the duration of the experiment RVR in group IIb exhibits a tendency to be higher than basal levels but without any statistical significance.

Table I illustrates the variations in the systemic and renal parameters throughout the experiment.

Discussion

Control of vascular function by the endothelium is quite complex and involves a balanced synthesis and release of both vasodilator and vasoconstrictor substances[35].

The important modulatory effects of nitric oxide on regional hemodynamics and renal vascular tone have recently been well demonstrated. To examine the systemic and renal hemodynamic effects of NO in normal animals, most investigators have given the animals NO synthesis inhibitors acutely [12-15, 19, 24] directly into the renal artery, by venous infusion or chronically in the drinking water [25, 26, 29, 36]. Some differences were observed in conscious versus anesthetized rats [37-39]. These differing responses were apparently due to different animal models or to varying circulating levels of angiotensin II [38].

Meanwhile acetylcholine infusion to the animals resulted in systemic hypotension and renal vasodilation with diuretic and natriuretic effect [40], the administration of NO synthesis inhibitors to animals promotes a marked increase in mean arterial pressure and renal vascular resistance, with a decrease in renal plasma flow and variable (null or minor) effects on glomerular filtration rates with the concommitant elevation in filtration fraction. Baylis and coworkers [25], using 50 mg/L of L-NAME in the drinking water for a two months period, reported a mean arterial pressure of 136 ± 4 mmHg with 30.1 ± 5.6 mmHg/mL/min of renal vascular resistance. Meanwhile, Ribeiro et al [36] using 10 fold higher dose over 4-6 weeks of continual NO blockade with L-NAME reported a significantly greater hypertension. In the study presented here, the results are compatible with those of recent reports [25, 41-43], confirming a previous suggestion, where there is a dose-dependency to the magnitude of the systemic hypertension achieved with chronic NO blockade. This increase in renal vascular resistance is specifically related to the EDRF-NO synthesis inhibition. The renal vasoconstriction observed in this study is not due to an autoregulatory phenomena elicited by the concurrent rise in systemic arterial pressure (using subpressor doses of L-NAME in the drinking water still increases the calculated RVR; unpublished observations), but rather to a greater sensibility of the vascular renal bed to L-arginine analogues or to an angiotensin II mediated renal vasoconstriction [38]. The latter would be in accordance with the reversal decrease in renal plasma flow after the administration of an angiotensin II receptor antagonist [37]. In the absence of high circulating levels of angiotensin II another possibility could be the underlying myogenic mechanism, as suggested by Ito et al [44]. However this vasoconstriction was found to be confined to the afferent arteriole, in controversy with this study where vasoconstriction affect predominantly postglomerular renal vasculature, as indicated by a significant decrease in RPF with no alterations in GFR.

In concordance with a Shultz and Tolins suggestion [28], concerning the difficulty to evaluate the different results of the effects of NO synthase inhibitors on renal hemodynamic and excretory function, our findings are consistent with previous reports [15, 19, 24, 27]. Meanwhile Zats and De Nucci [15], and Baylis et al [24], have suggested a possible inhibitory effect associated with NO inhibition at the level of the distal or collecting tubule, De Nicola et al [27], evidenced a possible inhibitory effect on proximal tubular reabsorption. Our results demonstrate that chronic inhibition of basal EDRF/NO synthesis in anesthetized rats produces a substantial pressor response associated with a marked natriuresis. Thus, the reduction in tubular reabsorption of sodium (fractional excretion of sodium clearly elevated in group Ia without significant modification in the glomerular filtration rate) without any modification in potassium secretion, must suggest the existence of an NO-sensitive mechanism for regulating distal tubule Na transport, more than a mere pressure natriuresis effect. These findings are compatible with a recent report and indicate that, in the kidney-rat the distribution of NO synthase examined by three different approaches (immunocytochemistry, enzymatic activity and mRNA expression) revealed the strongest signals for NO synthase in the macula densa cells of the juxtaglomerular apparatus [45]. In this regard, Neuringer et al [19], reported a significant reduction in fractional reabsorption of sodium in normal and DOCA-salt hypertensive rats after infusion of L-NAME; furthermore, Radermacher et al [46], in isolated perfused rat kidney believe that reduction in fractional reabsorption of sodium after NO inhibition is partially due to a specific tubular effect of NO. Previously in a recent report Green and coworkers [47], have shown that 8-bromo-cyclic GMP can also stimulate the Na^+-H^+ antiporter in renal brush border membranes and thus increase sodium uptake.

Our observations of the effects of NO inhibition on urine flow and urinary sodium excretion are different

from the results of some experiments in rats in which systemic administration of NO synthesis inhibitors induced an antidiuretic and antinatriuretic response [16-18, 28]. In view of this apparent discrepancy we cannot provide any satisfactory explanation. However, this may be due to the influence of species and strain differences, distinct methodological approach with the use of not always the same L-Arginine analogues, or the influence of some other activated mechanism like the release of ANP during NO-inhibitors administration [48]. These findings could suggest a dissociation of the sodium excretory responses from the hemodynamic changes during NO synthesis inhibition.

A transient increase in GFR and renal plasma flow was observed in group IIb after ANP 99-126 administration in accordance with other studies using different ANP peptides [49, 50], a similar increase was observed in the NO synthesis blocked group. In contrast, DePriest, Zimmermann and Baylis [51], using different doses of a 28 aminoacid α rat ANP for a 60 min period to conscious unstressed rats failed to produce statistically significant changes in GFR with a slight reduction in renal plasma flow and increased renal vascular resistance although these effects were not statistically significant. These and other studies have provided significant evidence that GFR-enhancing effect of ANP may result from the use of the surgically anesthetized preparation. Although in the present study we do not conduct any micropuncture studies, the increased GFR could result from the suggested afferent arteriolar vasodilatation and concurrent efferent arteriolar vasoconstriction [50]. In the present study, this was in accordance with a stable filtration fraction and renal vascular resistance in both (IIa and IIb) groups without significant modifications in MAP, probably in relation with the low doses or particular properties of the peptide reached. The high renal vascular resistance obtained in the group IIa after pretreatment with L-NAME, declined slightly coinciding with an increase in renal plasma flow after infusion of ANP 99-126 but without statistical significance, in contrast with previous works where ANP infusion can vasodilate renal blood vessels under conditions of high vasoconstrictor tone or where renal vasculature is precontracted with norepinephrine and angiotensin II [50, 52, 53]. This apparent discrepancy could be associated with the different ANP molecule or doses used, as well as duration of the experience.

Some investigators have proposed that the increase in GFR alone can account for the natriuresis and diuresis induced by ANP [53,54], whereas others supported that ANP also directly alters tubule Na^+ and water reabsorption [55-57]. As in our study, ANP 99-126 has been capable of stimulate natriuresis and diuresis without producing detectable and maintained alterations of GFR [56, 58], suggesting that this may be due to the lower doses used. At higher dosis, an increase in GFR is marked [56, 59]. Maack [60], has argued that even during brisk natriuresis, undetectable changes in GFR could contribute to the proper natriuresis observed with ANP administration. Nevertheless, several observations like in toadfish [61], a species that lacks glomeruli, ANP induces an accentuated natriuresis, indicating that changes in GFR alone do not account for the important natriuresis and diuresis observed in response to ANP infusion.

As was indicated in Table I, potassium excretion was much more variable and much less pronounced than sodium excretion rate. The absolute and fractional potassium excretion increased in groups Ia and Ib, but without statistical significance between the vehicle-control group and L-NAME treated group. In contrast, the L-NAME prevented the increased rate of potassium excretion reached with the use of ANP 99-126 alone in the study II. Such findings suggest that L-NAME blunted the action of ANP 99-126 at distal portions of the nephron, however, there is also little evidence of an action of ANP in the distal or collecting duct. Presently, the in vivo studies do not assess whether the hormone acts directly on the epithelial cells or alters the transepithelial driving forces [62, 63], and whether these segments are devoid or not of receptors for ANP [64].

The current study therefore suggests, by the use of the NO synthase inhibitor L-NAME, that NO is an important modulatory agent in both the renal vascular and tubular function, and probably independent of changes in the renal perfusion pressure.

In conclusion, the present investigation indicates that EDRF/NO exerts a substantive role in maintaining the normally low renal vascular tone, the renal vasoconstriction observed in this study is not due to an autoregulatory phenomena elicited by the concurrent rise in systemic arterial pressure. The use of L-NAME an orally active NO-synthase inhibitor was followed in our model with a marked natriuretic response without any modification in the potassium excretion rate, suggesting an NO-sensitive mechanism for regulating distal tubule Na-transport. With the use of the peptide ANP 99-126 a potent and maintained diuresis and natriuresis was observed even in the L-NAME pretreated group with minimal and excretion response the L-NAME treated transient elevations in GFR. The potassium reached with this peptide was attenuated in animals. Further studies are needed to clarify the exact mechanism responsible for NO induced changes in tubular function, and the physiological role of this particular ANP 99-126 peptide in the regulation of salt, water and potassium homeostasis.

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