Role of Nitric Oxide in Mediating Renal Response to Volume Expansion

Antonio Alberola, Jose M. Pinilla, Tomás Quesada, J. Carlos Romero, Miguel G. Salom, and F. Javier Salazar

The objective of the present study was to determine the role of endothelium-derived nitric oxide in mediating the renal response to extracellular volume expansion with isotonic saline (5% body weight). In anesthetized dogs (n=7) and before volume expansion, nitric oxide synthesis was inhibited in the right kidney by continuous intrarenal infusion of N^G-nitro-L-arginine-methyl ester (1 µg/kg/min). Arterial pressure and renal hemodynamics of both kidneys did not change significantly either during nitric oxide synthesis inhibition or during 5% volume expansion. However, in response to extracellular volume expansion, increases in natriuresis, diuresis, and fractional excretion of lithium (an index of proximal sodium reabsorption) were inhibited in the right kidney by 27%, 28%, and 41%, respectively, when compared with the contralateral kidney. Increases of renal interstitial hydrostatic pressure during 5% volume expansion were not statistically different between both kidneys. In another group of dogs (n=4), the administration of L-arginine (0.5 mg/kg/min) into the right renal artery prevented the renal effects induced by the nitric oxide synthesis inhibitor during volume expansion. The findings in this study suggest that nitric oxide production plays an important role in regulating the renal response to extracellular volume expansion. The proximal tubule seems to be involved in the reduced renal excretory response to volume expansion during nitric oxide synthesis inhibition. (Hypertension 1992;19:780-784)

KEY WORDS • natriuresis • diuresis • sodium loading • endothelium-derived relaxing factor • nitric oxide • lithium

Recent studies have provided evidence that the renal response to vasodilators that induce an increase of medullary blood flow seems to be mediated by an increase of endothelium-derived nitric oxide (EDNO).1,2 It also has been suggested that the endothelial cells of the vasa recta are able to produce nitric oxide3 and that increases in flow stimulate endothelial cells of rabbit thoracic aorta to release nitric oxide.4 Finally, it has been shown that EDNO plays an important role in the regulation of sodium excretion.5 These studies suggest that EDNO may play an important role in the regulation of renal function when medullary blood flow increases. One such condition is during extracellular volume expansion (ECVE). However, the role of EDNO in the regulation of the renal excretory response to saline loading has not been assessed.

The hypothesis of the present study is that endogenous EDNO may serve as a mediator in the renal response to volume expansion, because an increase in medullary blood flow seems to play an important role in determining the renal response to ECVE.6-8 To test this hypothesis, we inhibited nitric oxide synthesis in the right kidney by the intrarenal infusion of N^G-nitro-L-arginine-methyl ester (L-NAME) at a dose that does not produce changes in renal function. The natriuretic and diuretic responses of both kidneys to 5% saline loading were compared using the contralateral kidney as a control. It was also determined if the ECVE-induced changes6,8 in renal interstitial hydrostatic pressure (RIHP) and proximal sodium reabsorption (by lithium clearance) are modified by the intrarenal inhibition of nitric oxide synthesis. L-NAME was used to inhibit nitric oxide synthesis because it has been demonstrated to be active in vivo.5,9

Methods

Surgical Preparations

Experiments were performed in mongrel dogs of either sex (14–24 kg) maintained on a standard laboratory diet with free access to water. Lithium was given orally (300 mg) to the dogs the evening before the experiment. On the day of the experiment, food was withheld. Dogs were anesthetized with pentobarbital sodium (30 mg/kg/min i.v.) and were ventilated artificially with a respirator (Harvard Apparatus, Millis, Mass.). Catheters were placed in the femoral artery for measurement of mean arterial pressure and in the femoral vein for infusion of inulin, additional anesthetic (0.04 mg/kg/min), and isotonic saline during volume expansion. Inulin was dissolved in isotonic saline and infused to achieve plasma levels around 30 mg/dl. The

From the Departamento de Fisiologia (A.A., J.M.P., T.Q., M.G.S., F.J.S.), Facultad de Medicina, Murcia, Spain, and the Department of Physiology and Biophysics (J.C.R.), Mayo Clinic, Rochester, Minn.

Supported by a grant (PM88-94) from the Dirección General de Investigación Científica y Tecnica of Spain. J.C.R. was supported by grant HL-16496 from the National Institutes of Health. A.A. was a postdoctoral fellow from the Department of Physiology, School of Medicine, University of Valencia, Spain.

Address for reprints: F. Javier Salazar, PhD, Departamento de Fisiologia, Facultad de Medicina, 30100 Murcia, Spain.
left and right kidneys were exposed through flank incisions, and the ureters were cannulated to allow for comparison of renal function of both kidneys. The dogs were placed in a metal frame that mimicked their usual standing position, and both renal arteries were fitted with noncannulating electromagnetic flow probes connected to flowmeters (Carolina Medical Electronics, Inc., King, N.C.). A curved 23-gauge needle was inserted into the right renal artery and connected to a peristaltic pump (Watson-Marlow 204, Falmouth, UK).

The RIHP was measured in both kidneys using the acutely implanted capsule technique. The capsule was inserted into a hole in the renal cortex that created a fluid-filled space in free communication with the interstitium, and pressure was recorded using a Hewlett-Packard 1280 transducer connected to a Hewlett-Packard 8805C amplifier and a four-channel polygraph (Hewlett-Packard Co., Palo Alto, Calif.). Finally, a 45-minute stabilization period was allowed before experiments began.

**Experimental Groups**

**Group 1** (n=7). After two 15-minute control clearances, L-NAME (Sigma Chemical Co., London) was infused into the right renal artery (1 μg/kg/min) for the duration of the experiment. Twenty minutes after initiation of the L-NAME infusion, two 15-minute clearances were obtained. An ECVE (5% body weight) with isotonic saline during 45 minutes was then started, with two clearances performed during the last 10 minutes of saline infusion and 10 minutes after cessation of volume expansion. Finally, 30 minutes after the end of volume expansion, two more 15-minute clearances were obtained. Renal clearances were taken during each period to determine glomerular filtration rate; fractional sodium, lithium, and potassium excretion; and urine flow. Blood samples for plasma inulin, lithium, sodium, and potassium concentrations also were obtained during each experimental period.

**Group 2** (n=4). A similar protocol to that of group 1 was performed, with the difference being that after the control period, L-arginine (L-Arg) (Sigma) was infused into the right renal artery (0.5 mg/kg/min) for the duration of the experiment. Fifteen minutes after initiation of L-Arg infusion, two 15-minute clearances were obtained. L-NAME infusion started after these clearance periods, with the protocol being similar to that of group 1.

**Group 3** (n=5). Isotonic saline, instead of L-NAME or L-Arg, was infused through the needle inserted into the right renal artery. Saline loading (5% body weight) was performed after a control period of 30 minutes. Renal clearances from both kidneys were obtained during the control period, at the end of volume expansion, and 30 minutes after cessation of ECVE.

**Analytical Methods**

Glomerular filtration rate was measured by the clearance of inulin. Inulin concentrations were analyzed by the Anthrone method. Concentrations of sodium and potassium were measured by flame photometry (Corning 435, Corning Ltd., Halstead, UK). Proximal tubular reabsorption of sodium was estimated by the lithium clearance technique. Lithium concentrations were measured by flame emission spectrophotometry (model 5500, The Perkin-Elmer Corp., Norworth, Calif.). That lithium is a marker for changes in proximal tubular sodium reabsorption is suggested by previous studies showing that fractional excretion of lithium (FeLi) increased during administration of proximal but not distal acting diuretics. Furthermore, micropuncture studies in rats have further supported the use of lithium as a means of assessing proximal tubular sodium reabsorption, although small amounts of lithium may be reabsorbed in the distal tubular segments when fractional excretion of sodium (FeNa) is less than 0.4%. In humans, there is indirect evidence showing that distal lithium reabsorption is insignificant even at very low sodium excretion rates.

**Statistical Analysis**

Data are expressed as mean±SEM. The data for the two clearance periods for each condition were averaged for statistical comparisons. Significance of differences in values for each period in the same group and kidney was evaluated using a one-way analysis of variance and the Duncan multiple-range test. The significant difference between the same period of different kidneys and groups was calculated with a three-way analysis of variance and the Duncan test.

**Results**

**Group 1**

Table 1 shows that arterial pressure and renal hemodynamics of both kidneys did not change during 5% ECVE. The intrarenal infusion of L-NAME into the right renal artery produced no significant changes either in basal renal hemodynamics or in basal excretory function, but it partially inhibited the renal excretory response to the volume expansion. The 5% ECVE induced significant increases (p<0.05) in urinary sodium and potassium excretion in both kidneys (Table 1). However, the increment of sodium excretion in the contralateral kidney (375±54 μeq/min) was greater (p<0.05) than that in the ipsilateral kidney (274±53 μeq/min). With respect to diuresis, it can be seen that the increase induced by volume expansion was also greater (p<0.05) in the contralateral (3.43±0.44 ml/min) than in the L-NAME-infused (2.46±0.47 ml/min) kidney. Natriuresis and diuresis decreased significantly during the postexpansion period, but both were still greater (p<0.05) in the contralateral than in the ipsilateral kidney (Table 1). Although not shown in Table 1, it should be noted that cumulative sodium excretion from initiation of saline loading through the end of the experiment, was significantly higher (p<0.05) in the contralateral (25.6±2.9 meq of sodium) than in the L-NAME-infused (18.9±2.3 meq of sodium) kidney. The increase in RIHP during saline loading was slightly but not significantly higher in the contralateral (7.2±2.1 mm Hg) than in the ipsilateral (4.7±1.5 mm Hg) kidney (Table 1). During the postexpansion period, RIHP decreased in both kidneys to levels similar to those found in the control period.

Figure 1 shows increments of FeNa and FeLi in both kidneys during 5% ECVE. It can be seen that the increments in FeNa and FeLi were greater (p<0.05) in the contralateral than in the ipsilateral kidney. During
volume expansion, FeNa increased \((p<0.05)\) from 0.8±0.2% to 5.7±0.8% in the ipsilateral kidney and from 0.8±0.2% to 7.3±1.0% in the contralateral kidney. FeLi increased \((p<0.05)\) from 33.5±3.7% to 60.3±5.2% in the contralateral kidney. From this, it can be seen that both parameters increased \((p<0.05)\) to similar levels during 5% ECVE when L-Arg was simultaneously infused with the nitric oxide synthesis inhibitor.

**Group 3**

As expected, during infusion of saline into the right renal artery, 5% ECVE did not induce significant changes in arterial pressure or renal hemodynamics but did induce similar increments \((p<0.05)\) in natriuresis and diuresis in the ipsilateral (236.6±8.5 \(\mu\)eq/min and 2.3±0.3 ml/min) and contralateral (226.4±14.5 \(\mu\)eq/min and 2.2±0.2 ml/min) kidneys. Increases \((p<0.05)\) in RIHP and FeLi were also similar in both kidneys during saline loading.

**Discussion**

The present study demonstrates that natriuresis and diuresis induced by a 5% ECVE are reduced during the intrarenal infusion of a low dose of L-NAME, suggesting that EDNO plays an important physiological role in modulating the renal excretory response to ECVE. The increase in FeLi during volume expansion was also reduced in the kidney in which nitric oxide synthesis was inhibited. These results suggest that the proximal tubule may be involved in the reduced natriuretic and diuretic responses to a 5% ECVE during nitric oxide synthesis inhibition.

In the present study, the intrarenal inhibition of nitric oxide synthesis did not significantly affect baseline renal hemodynamics and excretory function but partly inhibited the increases in natriuresis and diuresis induced by saline loading. These results could be interpreted as indicating that the low dose of L-NAME (1 \(\mu\)g/kg/min) produced only a partial intrarenal inhibition of nitric oxide synthesis, because others groups of investigators have found that larger doses of this inhibitor induce a fall in renal blood flow and glomerular filtration rate. The actual percentage of nitric oxide synthesis inhibition obtained in this study is unknown, because there are no techniques that allow accurate measurements of nitric oxide production from in vivo animal preparations. However, the purpose of the present study was to inhibit nitric oxide synthesis in the right kidney with a dose of L-NAME that does not...
The intrarenal infusion of L-NAME significantly blunted the natriuretic and diuretic responses to relatively large saline loading without affecting renal hemodynamics, suggesting that EDNO plays an important role in the regulation of tubular reabsorption of sodium and water during saline loading. There are several possible mechanisms whereby EDNO could contribute to the ECVE-induced natriuresis and diuresis. Previous studies have provided evidence that the endothelial cells of the vasa recta are able to produce nitric oxide and that an increase of flow stimulates endothelial cells of thoracic rabbit aorta to release nitric oxide. These results prompt us to suggest that the increase in medullary blood flow that occurs during saline loading induces the release of nitric oxide that then produces a further increase in medullary blood flow and a decrease in sodium reabsorption. The initial increase in medullary blood flow during saline loading could be secondary to the decrease in intrarenal angiotensin II levels. The difference in FeLi between both kidneys during saline loading suggests that the inhibition of nitric oxide synthesis has an effect on the proximal tubule reabsorption of sodium. It is not known whether the different increments of FeLi in both kidneys are secondary to a direct or indirect effect of EDNO synthesis inhibition. However, it could be speculated that the difference in FeLi between both kidneys during saline loading could be due to greater intrarenal angiotensin II levels in the L-NAME-infused kidney than in the contralateral kidney. This is supported by previous studies showing that inhibition of nitric oxide synthesis induces an increase in renin release and that changes in intrarenal angiotensin II levels are important in modulating both the renal response to saline loading and proximal sodium reabsorption. Furthermore, it has been demonstrated that pretreatment with an angiotensin II receptor antagonist blunted the renal effects induced by the acute infusion of a nitric oxide synthesis inhibitor. Further studies are needed to define the mechanism by which the inhibition of nitric oxide synthesis induces an increase in proximal sodium reabsorption during volume expansion. Besides the proximal tubule, it is also possible that the inner medullary collecting ducts also could be involved in the lesser natriuresis and diuresis found in the L-NAME-infused kidney during saline loading. This hypothesis is supported by studies showing that EDNO increases the release of cyclic GMP and that cyclic GMP decreases sodium reabsorption in the inner medullary collecting ducts.

Increases in RIHP have been suggested to contribute to the changes in natriuresis and diuresis induced by volume expansion. In this study, the infusion of L-NAME was expected to partially inhibit the increase in RIHP during saline loading, because the inhibition of nitric oxide synthesis may affect the increased medullary blood flow during saline loading and changes in medullary blood flow are important in determining the increment of RIHP. The change of RIHP in the cortex during saline loading could be secondary to the increase in interstitial pressure in the medulla that occurs as a consequence of the elevation in medullary blood flow. However, in our present study, the increase in RIHP in the contralateral kidney was slightly but not significantly higher than in the L-NAME-infused kidney. Our results suggest that changes in RIHP are not involved in the reduced natriuretic and diuretic response to saline loading when nitric oxide synthesis is inhibited by a low dose of L-NAME.

Infusion of L-Arg alone failed to induce an effect on renal hemodynamics or excretory function, suggesting that nitric oxide production is not limited by the availability of substrate. This confirms the findings of Salom et al., who showed that the intrarenal infusion of L-Arg (1 mg/kg/min) did not induce changes in renal hemodynamics and excretory function even during the intrarenal infusion of acetylcholine. Our results also demonstrated that the intrarenal infusion of L-Arg prevented the blunted response of the L-NAME-infused kidney on the ECVE-induced increases in natriuresis and diuresis. These results confirm previous studies in vivo supporting the hypothesis that L-NAME inhibits the formation of nitric oxide by acting competitively with endogenous L-Arg.

In summary, the findings of this study are consistent with the hypothesis that EDNO contributes to the renal excretory response to saline loading. The reduced natriuretic and diuretic responses to volume expansion during nitric oxide synthesis inhibition seem to be, at
least partly, mediated by an increase in proximal sodium reabsorption. It is suggested that a deficiency in the synthesis of EDNO may constitute an important factor in the development of systemic hypertension, because such a deficiency interferes with the ability of the kidney to excrete sodium and water.

Acknowledgments
We thank Mark Strong and Maria J. Salazar for typing this manuscript.

References
Role of nitric oxide in mediating renal response to volume expansion.
A Alberola, J M Pinilla, T Quesada, J C Romero, M G Salom and F J Salazar

Hypertension. 1992;19:780-784
doi: 10.1161/01.HYP.19.6.780

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/19/6_Pt_2/780

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/