Nitric Oxide Alters Renal Function and Guanosine 3',5'-Cyclic Monophosphate

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Endothelium-derived relaxing factor (EDRF) activates soluble guanylate cyclase, resulting in an increase in vascular smooth muscle guanosine 3',5'-cyclic monophosphate (cGMP) levels, which correlates with its relaxing effect. Using a microdialysis technique, we investigated changes in right and left renal interstitial fluid cGMP levels in response to right intrarenal administration of an EDRF inhibitor, N^\text{rg}-monomethyl-L-arginine (L-NMMA). Studies were conducted in anesthetized dogs (n=5) in metabolic balance at a sodium intake of 40 meq/day. Urine was collected directly from the right and left ureters individually. Changes in the right and left urinary cGMP excretion and renal function in response to cumulative doses of L-NMMA were studied. In the right kidney, 20-100 /\mu g/kg/min L-NMMA caused 1) a dose-dependent decrease in renal interstitial fluid and urinary cGMP levels (p<0.0001 and p<0.001, respectively), 2) antinatriuresis (p<0.01), 3) antidiuresis (p<0.01), 4) a decrease in renal blood flow (p<0.01) and glomerular filtration rate (p<0.01), and 5) a decrease in fractional sodium excretion (p<0.01). No changes in left renal interstitial fluid and urinary cGMP levels or excretory and hemodynamic function were observed during right intrarenal administration of L-NMMA at 20 and 60 /\mu g/kg/min. L-NMMA at 100 /\mu g/kg/min produced a significant decrease in left renal interstitial fluid (p<0.01) and urinary (p<0.01) cGMP levels, antidiuresis (p<0.01), antinatriuresis (p<0.01), and decreases in renal blood flow (p<0.0001), glomerular filtration rate (p<0.01), and fractional sodium excretion (p<0.01). These data demonstrate the ability to monitor renal interstitial fluid cGMP levels in anesthetized animals. We conclude that EDRF acts intrarenally to control renal function through the modulation of renal interstitial cGMP. (Hypertension 1992;19:775-779)

Key Words • endothelium-derived relaxing factor • nitric oxide • cyclic GMP • renal function • microdialysis

A great deal of evidence suggests that endothelium-derived relaxing factor (EDRF) is nitric oxide (NO) or a related nitrous compound that originates from the terminal guanidine nitrogen atom of the amino acid l-arginine. The actions of EDRF are local, affecting only the adjacent vascular smooth muscle cells and platelets. There is no downstream or circulating hormonal effect. After production in the endothelial cell, EDRF is transferred to vascular smooth muscle cells where NO activates soluble guanylate cyclase, resulting in an increase in smooth muscle concentration of guanosine 3',5'-cyclic monophosphate (cGMP), which correlates with its relaxation. A variety of agonists has been shown to induce release of NO from endothelium of various vascular beds. The generation of NO by NO synthase can be inhibited by the l-arginine analogue N^\text{rg}-monomethyl-L-arginine (L-NMMA). This inhibitory effect of L-NMMA is reversed or prevented by the simultaneous administration of l-arginine. Although EDRF has been studied extensively in different systems, little is known about its role in the kidney. Previous studies have shown that EDRF decreases renal vascular resistance and causes renal vasodilation and diuresis. Because EDRF is synthesized in the kidney and because of its extreme lability, it is possible that EDRF acts locally as a paracrine substance and alters renal function. The ability to monitor changes in renal interstitial fluid (ISF) cGMP would be of great advantage in clarification of local mechanisms controlling renal function. We conducted the present study to evaluate the role of endogenous intrarenal EDRF in the control of renal function. We hypothesized that EDRF is generated tonically within the kidney and exerts renal effects through modulation of renal ISF cGMP. We used a novel renal ISF microdialysis technique to sample renal ISF cGMP and infused various doses of L-NMMA into the right renal artery while monitoring simultaneously renal ISF and urinary cGMP levels and renal function in both kidneys.

Methods

Renal Microdialysis Technique

For the determination of renal ISF cGMP, a microdialysis probe was constructed. Inflow and outflow hollow polyethylene tube (0.12-mm i.d., 0.65-mm o.d.; Bioanalytical Systems, Inc., West Lafayette, Ind.) was connected to each end of a 5-mm length of hollow fiber dialysis tubing (Clirans TH; 0.3-mm i.d.; molecular mass cutoff, 5,000 d), adjusted such that the distance between...
the ends of the polyethylene tubes was 3 mm (dialysis area), and sealed in place with cyanoacrylic glue. The dead volume of the dialysis membrane and outflow tube was 3.62 µl.

Animal Preparation and In Vivo Renal Interstitial Fluid Cyclic GMP Dialysis

Experiments were conducted in five anesthetized female mongrel dogs weighing 15–20 kg. Surgery was performed with dogs under general anesthesia using halothane inhalation. A renal catheter was inserted into the right renal artery. Changes in urine flow and sodium excretion (Uo/V) were monitored by catheterizing each ureter with polyethylene tube (0.04-mm i.d.). The renal capsule was penetrated with an 18-gauge needle that was tunneled in the outer cortex approxi-
mately 3 mm from the outer renal surface for 2 cm before it exited by penetrating the capsule again. One end of the dialysis probe then was pulled through until the dialysis fiber was situated in the renal cortex. The needle then was pulled out. The dialysis probe was inserted into the outer cortex of each kidney. The inflow tube was connected to a gas-tight syringe filled with lactated Ringer’s solution and perfused at 3 µl/min (pump 22, Harvard Apparatus, South Natick, Mass.). A minimum 90-minute recovery period elapsed before the experimental protocol was initiated. The effluent was collected from the outflow tube for 20-minute sample periods in plastic tubes on ice.

Hemodynamic Measurements

Renal blood flow (RBF) of the right and left kidneys was monitored continuously during the study by an ultrasonic flow probe (Transonic System, Ithaca, N.Y.). The RBF for each kidney was calculated as the mean of three recordings, lasting 15 seconds each and taken at intervals of 2 minutes at the middle of each 20-minute period.

Analytical Methods

Plasma and urine sodium levels were measured by an analyzer (NOVA Biomedical, Waltham, Mass.). Urinary and renal ISF cGMP content was measured by radioimmunoassay.

Sodium Balance

Daily sodium intake was maintained at 40 meq by feeding of a low sodium diet (5 meq/day) (Hills H/D, Topeka, Kan.) and intravenous infusion of 35 meq sodium per day (as normal saline) for 5 days before study.

Effects of Intrarenal Endothelium-Derived Relaxing Factor Antagonist

Five dogs were infused intravenously with inulin (Sigma Chemical Co., St. Louis, Mo.). Five percent dextrose solution (Travenol Laboratories, Deerfield, Ill.) was infused intravenously at 0.5 ml/min as a vehicle for L-NMMA (BACHEM Bioscience Inc., Philadelphia, Pa.). Ninety minutes were allowed for adequate equilibration. After the first two consecutive 20-minute periods (pretreatment), cumulative infusion doses of L-NMMA at 20, 60, and 100 µg/kg/min over three consecutive 20-minute periods were given into the right renal artery. The L-NMMA infusion was followed by a 20-minute recovery (posttreatment) period during which 5% dextrose solution was infused at 0.5 ml/min. Renal ISF dialysis effluent was collected for cGMP measurement throughout each period. Urine collections were performed during each period. Blood samples (5 ml) were obtained in the middle of each 20-minute urine collection period. Glomerular filtration rate (GFR) was calculated as the clearance of inulin. Fractional excretion of sodium (FENa) was calculated.

Statistical Analysis of Data

Comparisons among treatments and between the two kidneys were examined by analysis of variance, including a repeated measure term, using the general linear measures models procedures of SAS (Statistical Analysis System). Multiple comparisons of individual pairs of effect means were conducted using least-squares means, pooled variance, and the t test. Comparisons between the two kidneys were examined by paired t test. Data are expressed as mean±SEM. Statistical significance was identified at a value of p<0.05.

Results

In Vivo Renal Interstitial Fluid and Urinary Cyclic GMP in Response to Intrarenal L-Monomethyl-L-Arginine

Renal ISF cGMP was stable during the initial pretreatment period (0–40 minutes) (see Figure 1). A reduction in right renal ISF cGMP content (p<0.001) was observed in response to intrarenal administration of L-NMMA (40–100 minutes). Right renal ISF cGMP levels were reduced significantly at an infusion rate of 20 µg/kg/min (p<0.001). The decrease in right renal ISF cGMP levels in response to L-NMMA was dose dependent, and the largest dose administered (100 µg/kg/min) was associated with a reduction of renal ISF cGMP levels from 0.55±0.01 to 0.22±0.03 pmol/min (p<0.001). The left renal ISF cGMP level was stable and did not show any significant change from pretreatment during right intrarenal infusion of L-NMMA at 20 and 60 µg/kg/min (40–80 minutes). Left renal ISF cGMP content decreased significantly during right intrarenal infusion of L-NMMA at 100 µg/kg/min (p<0.0001). After L-NMMA was discontinued (posttreatment, 100–120 minutes), both right and left renal ISF cGMP levels continued to decrease (p<0.0001).

A dose-dependent decrease in right kidney urinary cGMP (p<0.0001) occurred during intrarenal L-NMMA administration. L-NMMA at 20 and 60 µg/kg/min did not change left urinary cGMP content. In contrast, L-NMMA at 100 µg/kg/min significantly decreased right and left urinary cGMP levels from 0.81±0.03 to 0.25±0.04 pmol/min (p<0.0001) and from 0.78±0.02 to 0.32±0.11 pmol/min (p<0.0001), respectively. After L-NMMA was discontinued, urinary cGMP continued to decrease from both right and left kidneys (p<0.0001).

Renal Excretory Changes in Response to Intrarenal Nω-Monomethyl-L-Arginine

Urinary flow rate was stable during the initial pretreatment period (0–40 minutes) (see Figure 2). Right intrarenal L-NMMA administration caused a significant
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Figure 1. Line plots show renal interstitial fluid cyclic GMP (cGMP) (top panel) and urinary cGMP levels (bottom panel) of anesthetized dogs (n=5) in metabolic balance at 40 meq/day sodium intake in response to right intrarenal infusion of N\textsuperscript{G}-monomethyl-l-arginine (L-NMMA). •, Right kidney; ○, left kidney. *p<0.0001 compared with pretreatment; +p<0.0001 compared with corresponding left kidney.

The right kidney pretreatment values for UN\textsubscript{V} and FE\textsubscript{Na} were not significantly different from the left kidney values. Intrarenal administration of L-NMMA resulted in a dose-dependent decrease in right kidney UN\textsubscript{V} from 20±1.5 μeq/min to a nadir of 4±1.2 μeq/min at 100 μg/kg/min (p<0.01). Left kidney UN\textsubscript{V} increased slightly during right intrarenal L-NMMA administration at 20 μg/kg/min. Left kidney UN\textsubscript{V} continued to increase with the increase in L-NMMA dose to 60 μg/kg/min (p<0.01 from pretreatment; p<0.01 from corresponding right kidney). L-NMMA at 100 μg/kg/min significantly decreased UN\textsubscript{V} from both kidneys (p<0.01 from pretreatment). After right intrarenal L-NMMA administration was discontinued, UN\textsubscript{V} continued to decrease in both kidneys (p<0.01). Right intrarenal inhibition of EDRF generation with L-NMMA resulted in overall reduction in right kidney UN\textsubscript{V} (p<0.01, analysis of variance). A similar pattern was observed for FE\textsubscript{Na} as for UN\textsubscript{V} in response to right intrarenal L-NMMA. From an initial control value of 0.38±0.03%, intrarenal administration of L-NMMA produced a dose-dependent decrease in right renal FE\textsubscript{Na} to 0.11±0.03% (p<0.0001) at 100 μg/kg/min. Left renal FE\textsubscript{Na} did not show any significant changes during right intrarenal L-NMMA administration at 20–60 μg/kg/min. L-NMMA at 100 μg/kg/min significantly decreased FE\textsubscript{Na} in the left kidney (p<0.01). Intrarenal L-NMMA caused an overall reduction in right renal FE\textsubscript{Na} (p<0.01, analysis of variance).
used the putative synthesis inhibitor L-NMMA to evaluate the role of intrarenal EDRF and cGMP in the maintenance of renal function.

Urinary excretion of cGMP has been identified as a biological marker of EDRF activity in vivo. Intrarenal EDRF/NO inhibition with L-NMMA at 20 and 60 \( \mu \text{g/kg/min} \) caused a highly significant dose-dependent decrease in right renal ISF and urinary cGMP levels, whereas left renal ISF and urinary cGMP levels were not affected. This suggests that L-NMMA at these doses was confined mainly to the right kidney and that very little, if any, L-NMMA traversed the right kidney to enter the systemic circulation during intrarenal administration. In contrast, L-NMMA at 100 \( \mu \text{g/kg/min} \) caused a significant decrease in renal ISF and urinary cGMP levels from both kidneys, suggesting that L-NMMA had traversed the right kidney, entered the systemic circulation in significant quantities, and affected the left kidney. After cessation of L-NMMA infusion, renal ISF and urinary cGMP levels continued to be suppressed, probably because of the well-documented, long-lasting effect of this agent. Because L-NMMA decreased basal levels of renal ISF and urinary cGMP, it is apparent that there is a basal rate of NO synthesis by kidney cells that may be important in the modulation of renal function.

In the present study, EDRF inhibition and reduction of ISF cGMP levels were associated with significant decreases in renal excretory and hemodynamic functions. Renal epithelial cells are able to produce cGMP, a response that is inhibited by L-NMMA. \(^{16,17}\) Whether EDRF-mediated increases in tubular epithelial cell cGMP content modulate transcellular Na\(^+\) flux remains to be determined. The observed decrease in RBF is in agreement with previous reports that L-NMMA increases renal vascular resistance. \(^{8,13,16}\) Recently, it has been suggested that EDRF may play an important role in the local regulation of renal cortical blood flow. \(^{16,18}\) The renal vasculature and glomeruli have the capacity to respond to agonist-induced release of EDRF. \(^{16,19-21}\) The decrease in RBF, GFR, and FE\(\text{NO}\), suggests that the observed changes during EDRF inhibition are mediated conjointly through renal hemodynamic and tubular mechanisms.

In the present study, we were able to monitor basal changes in renal ISF cGMP levels in response to EDRF inhibition. The exact source of renal cGMP has not been elucidated. The microvasculature of the glomerular tuft is able to release and respond to EDRF \(^{21,22}\) by augmenting mesangial cell cGMP, a response markedly blunted by hemoglobin and methylene blue. This suggests a direct effect of EDRF on glomerular function (e.g., GFR).

**Discussion**

Little is known about the role of EDRF in the control of renal function. In the rat kidney, a role for EDRF in the control of arteriolar resistance has been proposed. \(^{8,14}\) In the conscious rat, EDRF inhibition increased systemic blood pressure and reduced total RBF. \(^{15}\)

In the present study, we tested the hypothesis that EDRF is a paracrine substance in the control of renal function through modulation of renal cGMP levels. We used the following rationale. If EDRF produced intrarenally acts locally within the kidney through generation of cGMP to regulate renal function, intrarenal inhibition of EDRF/NO generation should decrease renal ISF cGMP formation and engender measurable physiological changes. Thus, in the present study, we used the putative synthesis inhibitor L-NMMA to evaluate the role of intrarenal EDRF and cGMP in the maintenance of renal function.

FIGURE 3. Line plots show renal blood flow (RBF) (top panel) and glomerular filtration rate (GFR) (bottom panel) of anesthetized dogs (n=5) in metabolic balance at 40 meq/day sodium intake in response to right intrarenal infusion of \( \text{N}^{6}\)-monomethyl-L-arginine (L-NMMA). •, Right kidney; ○, left kidney. *\( p<0.01 \) compared with pretreatment; +\( p<0.01 \) compared with corresponding left kidney.

Renal Hemodynamic Changes Induced in Response to Intrarenal \( \text{N}^{6}\)-Monomethyl-L-Arginine

There was a significant dose-dependent decrease in right kidney RBF and GFR in response to intrarenal L-NMMA (see Figure 3). In contrast, left kidney RBF and GFR did not change with L-NMMA at 20 and 60 \( \mu \text{g/kg/min} \). L-NMMA at 100 \( \mu \text{g/kg/min} \) significantly decreased RBF (\( p<0.01 \)) and GFR (\( p<0.01 \)) in both kidneys, and RBF and GFR continued to decrease after L-NMMA administration was discontinued. Intrarenal L-NMMA caused an overall decrease in RBF (\( p<0.01 \)) and GFR (\( p<0.01 \), analysis of variance).
not be expected. It is interesting that left renal function increased mildly, which could constitute a compensatory mechanism for the decrease in right renal function.

In conclusion, we have provided new evidence that EDRF/NO generated intrarenally affects renal excretory and hemodynamic functions. Intrarenal administration of an EDRF inhibitor produced decreases in renal cGMP levels and excretory and hemodynamic functions. Because the antidiuresis and antinatriuresis was accompanied by a significant decrease in GFR and FENa, the results provide evidence that the action of intrarenal EDRF/NO is mediated both at the glomerulus and probably at the renal tubule.

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References
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