Arginine Vasopressin–Mediated Stimulation of Nitric Oxide Within the Rat Renal Medulla

Frank Park, Ai-Ping Zou, Allen W. Cowley, Jr

Abstract—The present study was designed to determine whether arginine vasopressin (AVP) can stimulate nitric oxide (NO) production within the renal medulla and thereby modulate renal medullary blood flow. An in vivo microdialysis/NO trapping technique was used to determine changes in medullary interstitial [NO]. AVP (2 ng/kg per minute) was delivered into the renal medullary interstitium and resulted in a significant increase in renal medullary [NO] of 35%, which was blocked by pretreatment with nitro-L-arginine methyl ester (L-NAME) (1.3 μg/kg per minute) administered into the renal medullary interstitium. The vasopressin V2 receptor agonist 1-desamino-8-D-arginine vasopressin (dDAVP) resulted in a significant increase of 32% in renal medullary interstitial [NO]. No change in renal medullary interstitial [NO] was observed after selective vasopressin V1 receptor stimulation. Laser-Doppler flowmetry with implanted optical fibers was performed to measure cortical and medullary blood flow changes within the kidney. Renal interstitial infusion of dDAVP in rats pretreated with a vasopressin V1 receptor antagonist resulted in a 15% increase (P<0.05) in medullary blood flow, which was completely blocked by pretreatment with L-NAME (1.3 μg/kg per minute). This study demonstrates that AVP increases renal medullary interstitial [NO] through vasopressin V2 receptor stimulation, which in turn elevates blood flow to the renal medulla. (Hypertension. 1998;32:896-901.)

Key Words: nitric oxide ■ renal blood flow ■ L-NAME ■ vasopressin

arginine vasopressin (AVP) is known to be one of the most potent circulating vasoconstrictors in the mammalian organism, and yet physiological elevations in circulating AVP cannot produce sustained hypertension.2,3 This is an interesting phenomenon since recent studies by Franchini et al4–6 have shown that the renal medullary circulation, which is believed to regulate the pressure-natriuresis relationship,7 is highly sensitive to the vasoconstrictor actions of AVP. It was also found in these studies that small physiological increases of plasma AVP could reduce renal medullary blood flow (MBF) in acute anesthetized rats4,5 through the activation of the vasopressin V1 receptor (V,R).

The long-term role of the V,R in the regulation of mean arterial pressure (MAP) has been studied in our laboratory. Chronic intravenous infusions of the V,R agonist [Phe2, Ile3, Orn4]VP to conscious rats resulted in sustained hypertension, which was prevented by infusing a selective V,R antagonist into the renal medullary interstitium.2 Hypertension was also achieved when the V,R agonist was infused directly into the renal medullary interstitium3 at a dose previously shown to reduce blood flow to the renal medulla. These responses appear to be related to the vasoconstriction of the outer medullary descending vasa recta, which we have recently shown to express V,R mRNA.9 Furthermore, it was shown that isolated perfused outer medullary descending vasa recta vasoconstrict in the presence of AVP.10 It appears, therefore, that despite the inability of AVP to produce hypertension, selective activation of the medullary V,R can reduce MBF, which can lead to a sustained elevation in MAP.

A recent study in our laboratory indicates that AVP is unable to maintain a reduction of MBF over prolonged periods.11 This failure of AVP to sustain a reduction of MBF could be related to the stimulation of the vasopressin V2 receptor (V2,R), which may activate an opposing vasodilator response. It has been shown that systemic administration of AVP in the presence of a V2,R antagonist reduces total peripheral resistance and arterial blood pressure.12,13 Moreover, Nakanishi et al14 demonstrated that AVP can increase blood flow within the renal medulla by stimulating the V2,R. With the use of laser-Doppler flowmetry techniques, it was shown that renal medullary interstitial infusion of either AVP or the selective V2,R agonist 1-desamino-8-D-APV (dDAVP) increased MBF in anesthetized Sprague-Dawley rats pretreated with a selective V2,R antagonist. The V2,R-mediated vasodilation of the renal medullary blood vessels does not appear to be through direct vascular effects of AVP since the V2,R mRNA has not been detected within the vasculature of the renal cortex or medulla.9 The present study was therefore designed to determine whether the AVP-mediated medullary vasodilator response could be through the activation of extravascular nitric oxide (NO) production.
A number of studies have demonstrated that intrarenal NO production can importantly influence blood flow to the kidney. It has also been evident that NO production in the renal medulla is especially important in determining MBF since studies in our laboratory have found that NO synthase (NOS) enzyme activity, NOS protein expression, and interstitial NO concentrations were significantly higher in the renal medulla than in the renal cortex. Furthermore, selective inhibition of renal medullary NOS activity by chronic infusion of nitro-L-arginine methyl ester (L-NAME) into the medullary interstitium resulted in sodium retention and hypertension. There is evidence, albeit indirect, that AVP can stimulate NO production and that increased NO counteracts the AVP-induced vasoconstriction. However, regional NO concentrations have not been measured, and the role of NO in buffering the vasoconstrictor effects of AVP specifically within the renal medulla has not been explored.

In the present study we hypothesized that AVP stimulates the regional production of renal medullary NO production, which would play a role in the modulation of AVP-induced changes in MBF. To address this hypothesis, we used an in vivo microdialysis/oxyhemoglobin-NO trapping technique developed in our laboratory for direct measurement of renal interstitial NO concentrations. The effect of medullary interstitial AVP administration and the receptor type(s) responsible for the AVP-induced increase of interstitial [NO] were determined. Laser-Doppler flowmetry with optical fibers implanted in the renal cortex and medulla was, in turn, used to determine whether NO was involved in the AVP-mediated increase in MBF.

Methods

Experimental Animals
Male Sprague-Dawley rats weighing 280 to 360 g obtained from Harlan Inc (Madison, Wis) were used for these studies. All animals were given free access to tap water and fed a standard pellet diet (Purina Mills). All protocols were approved by the Institutional Animal Care Committee. On the day of the experiment, the rats were anesthetized with thiobutabarbital (100 mg/kg IP) and ketamine (50 mg/kg IM) and placed on a heated surgical table to maintain body temperature at 37°C. Cannulas were placed in the femoral arteries for measurement of MAP and blood sampling and in femoral veins for infusion of solutions. Surgical fluid losses were replaced by continuous intravenous infusion of 2% bovine serum albumin (fraction V, Sigma Chemical Co) in 0.9% sodium chloride solution at 1 mL/h per 100 g body wt throughout the experiment. The left kidney was exposed through a midline incision, isolated, and placed in a holder.

In Vivo Microdialysis/Hemoglobin Trapping Technique
In vivo microdialysis studies were performed as we have described previously. In brief, a small channel was made with a 23-gauge needle in the exposed left kidney through the renal capsule and the cortex to allow the microdialysis probe (Bioanalytic Systems) to be inserted into the renal medulla (5.5 mm in depth). The dialysis probe, which had a 0.5-mm-diameter tip and a 20-kDa transmembrane diffusion cutoff, was perfused at a rate of 2 µL/min with a solution containing (in mmol/L): NaHPO₄ 40.5, Na₂HPO₄ 9.5, NaCl 205, and oxyhemoglobin 0.003, with the pH adjusted to 7.4. The microdialysis probe was then perfused for 2 hours to enable the tissue to recover from the insertion of the probe and for equilibration to be established. During this time, the animal received an intravenous infusion of 2% bovine serum albumin in 0.9% NaCl, at a rate of 1 mL/h per 100 g body wt. After equilibration, dialysate fluid was collected (60 µL in volume) over two 30-minute intervals for a 1-hour control measurement period. Dialysate samples were analyzed as previously described. Briefly, 50-µL samples were added to a quartz cuvette and analyzed to record changes in absorbance using a wavelength scanning mode of a DU-640 Beckman spectrophotometer (Beckman Instruments, Inc). Methemoglobin (ie, NO concentrations) were calculated according to the following equation: c = A/eb, where c is methemoglobin, A is the increase of absorbance at 401 nm (absorbance difference between 401 and 411 nm), e is the extinction coefficient of methemoglobin, and b is the light path in centimeters.

Experimental Protocols for Microdialysis Studies
MAP was continuously recorded throughout each of the experimental protocols with the use of a computer-operated data acquisition software (AT-CODAS; DATAQ Instruments) program.

Group 1: Effects of AVP on Medullary Interstitial [NO] To determine whether AVP can increase renal medullary interstitial [NO], a subpressor dose of AVP (2 ng/kg per minute) was infused into the renal medullary interstitium at a rate of 8.3 µL/min for 100 minutes. Dialysis fluid was collected in two 30-minute intervals during the final 60 minutes of drug infusion for measurement of [NO].

Group 2: Effects of AVP on Medullary Interstitial [NO] After Partial Inhibition of NOS Activity L-NAME (1.3 µg/kg per minute) was infused into the renal medullary interstitium at a rate of 8.3 µL/min throughout the experiment. After the initial 60-minute equilibration period, dialysate was collected for two 30-minute periods. After the second dialysate collection, AVP (2 ng/kg per minute) was added to the medullary interstitium with L-NAME for 100 minutes. Dialysate was collected for two 30-minute periods in the final 60 minutes of AVP infusion.

Group 3: Effect of V₂R Stimulation on Medullary Interstitial [NO] To determine whether the increase in [NO] was mediated by the stimulation of the V₂R, a selective V₂R agonist, [Phe², Ile³, Orn⁴]VP, was infused at 2 ng/kg per minute into the renal medullary interstitium for 100 minutes. Dialysis fluid was collected in two 30-minute intervals during the final 60 minutes of agonist infusion.

Group 4: Effect of V₁R Stimulation on Medullary Interstitial [NO] A highly selective V₁R antagonist, d(CH₂)₅[Tyr(Me)₂, Ala⁻NH₂]AVP, was administered intravenously at 5 ng/kg per minute throughout the protocol to block any endogenous effects of the V₁R. After an equilibration period of 20 minutes, dialysate fluid was collected during two 30-minute periods for [NO] determinations. Subsequently, a selective V₂R agonist, dDAVP, was infused at 4 ng/kg per minute directly into the renal medullary interstitium at a rate of 8.3 µL/min for 100 minutes, where two 30-minute collections were obtained during the final 60 minutes of dDAVP infusion.

Experimental Protocols for Laser-Doppler Flowmetry
To determine the role of NOS in the V₁R-mediated increase in renal medullary blood flow, optical fibers were implanted into the renal cortex (1 mm deep) and the medulla (5.5 mm deep) of the left kidney to simultaneously measure changes in blood flow to these regions. A renal medullary interstitial catheter was also inserted from the lateral border of the kidney through the cortex into the renal medullary interstitium, with its tip (~100 µm in diameter) placed at the border of the inner and outer medulla. Isotonic saline (or
Group 5: Effect of V₁R Stimulation on MBF
After the equilibration period, a selective V₁R antagonist, d(CH₂)₅[Tyr(Me)₂, Ala-NH₂]AVP, was intravenously infused at 5 ng/kg per minute throughout the protocol. After the V₁R antagonist was infused for 30 minutes, MBF was recorded for 20 minutes. The V₁R agonist dDAVP (4 ng/kg per minute) was infused at a rate of 8.3 µL/min into the renal medullary interstitium for 40 minutes while the infusion of the V₁R antagonist was continued. A 20-minute recording of MBF was made in the final 20 minutes of dDAVP infusion.

Group 6: Effect of L-NAME on the V₂R Response of MBF
After the equilibration period, a selective V₂R antagonist, d(CH₂)₅[Tyr(Me)₂, Ala-NH₂]AVP, was intravenously infused at 5 ng/kg per minute along with a renal medullary interstitial infusion of L-NAME (1.3 µg/kg per minute) throughout the protocol. After 60 minutes of V₂R antagonist and L-NAME infusion, MBF recordings were made for 20 minutes. The V₂R agonist dDAVP (4 ng/kg per minute) was then added to the renal medullary infusate with L-NAME and then infused at a rate of 8.3 µL/min for 40 minutes. MBF recordings were made in the final 20 minutes of the dDAVP infusion in the presence of L-NAME and the V₂R antagonist.

Group 7: L-NAME Time Control
The protocol was the same as in group 6 except that dDAVP was not infused in the final infusion period. L-NAME infusion into the renal medullary interstitium was continued along with the intravenous infusion of the V₂R antagonist. MBF recordings were made at the same time periods as in group 6.

Drugs
L-NAME (Sigma), AVP (Sigma), [Phe², Ile³, Orn⁴]VP (Bachem), d(CH₂)₅[Tyr(Me)₂, Ala-NH₂]AVP (Manning compound; Bachem), and dDAVP (Bachem) were used.

Statistical Analysis
Data are presented as mean±SEM. An ANOVA for repeated measures followed by Duncan’s multiple range test was used to determine within-group differences (control versus drug periods). All statistical analyses were performed on the raw data. P<0.05 was considered significant.

Results
Group 1: Effect of AVP on Renal Medullary Interstitial [NO]
To determine whether subpressor doses of AVP can stimulate an increase in the [NO] within the renal medulla, we infused AVP into the renal medullary interstitium at a concentration of 2 ng/kg per minute. Figure 1 (top panel) demonstrates that AVP stimulation resulted in a significant increase of 35% (n=10; P<0.01) in renal medullary interstitial [NO] (151±11 nmol/L) compared with the control (saline-infused) period (112±6 nmol/L). Figure 1 (bottom panel) shows that no significant change in MBF occurred during the control (109±3 mm Hg) and AVP infusion (114±3 mm Hg) periods.

Figure 1. Top, Response of renal medullary interstitial [NO] collected by microdialysis to saline (control; white bars) or AVP (black bars; 2 ng/kg per minute). Bottom, MAP responses during the saline and AVP interstitial infusion (i.i.) periods. *Significant difference (P<0.05) from control.

Figure 2. Effects of medullary interstitial infusion of AVP on renal medullary interstitial [NO] after NOS inhibition with L-NAME. Top, NO measurements in dialyzed renal medullary interstitial fluid during interstitial infusion (i.i.) of saline (control; white bars) and L-NAME (1.3 µg/kg per minute; gray bars) and L-NAME+AVP (black bars; 2 ng/kg per minute). Bottom, MAP values during the 1-hour saline control, L-NAME, and L-NAME+AVP interstitial infusion periods. *Significant difference (P<0.05) from control.
between the control (104±2 mm Hg), L-NAME alone (107±3 mm Hg), and L-NAME+AVP (111±3 mm Hg) time periods (Figure 2, bottom panel).

**Group 3: Effect of V1R Stimulation on Renal Medullary Interstitial [NO]**

To determine whether selective V1R stimulation could mediate the increase in renal medullary interstitial [NO], a specific V1R agonist, [Phe2, Ile3, Orn8]VP, was infused into the renal medullary interstitium. Figure 3 (top panel) shows that stimulation of the V1R with the V1R agonist did not alter renal medullary interstitial [NO] (121±6 versus 115±5 nmol/L; n=8). Figure 3 (bottom panel) demonstrates that renal interstitial infusion of [Phe2, Ile3, Orn8]VP did not change MAP (108±4 mm Hg) compared with the control period (112±4 mm Hg).

**Group 4: Effect of V2R Stimulation on Renal Medullary Interstitial [NO]**

To determine whether selective V2R stimulation could mediate the increase in renal medullary interstitial [NO], a specific V2R agonist, dDAVP, was infused into the renal medullary interstitium in the presence of a V1R antagonist. Figure 4 (top panel) shows that intravenous infusion of the V1R antagonist d(CH2)5[Tyr(Me)2, Ala-NH2]AVP did not significantly alter renal medullary interstitial [NO] compared with the control period (122±6 versus 115±5 nmol/L; n=8). Figure 4 (bottom panel) demonstrates that renal interstitial infusion of [Phe2, Ile3, Orn8]VP did not change MAP (108±4 mm Hg) compared with the control period (112±4 mm Hg).

**Figure 3.** Response of renal medullary interstitial [NO] to interstitial infusion of V1R agonist (V1agnet). Top, NO measurements in dialyzed renal medullary interstitial fluid in the presence (black bars) and absence (white bars; saline control) of the selective V1R agonist [Phe2, Ile3, Orn8]VP infusion into the renal medullary interstitium at 2 ng/kg per minute. Bottom, MAP values during the 1-hour control and V1R agonist infusion periods.

**Figure 4.** Effects of medullary infusion of V2R agonist dDAVP on renal medullary interstitial [NO]. Top, NO values in dialyzed renal medullary interstitial fluid during saline-control (white bars) and intravenous infusion of V1R antagonist (V1 ANTA; 5 ng/kg per minute) in the presence (black bars) and absence (gray bars) of interstitially infused dDAVP (4 ng/kg per minute). Bottom, MAP values during 1-hour saline control, V1R antagonist, and V1R antagonist+dDAVP infusion periods. *Significant difference (P<0.05) from V1R agonist; #significant difference (P<0.05) from control.

**Groups 5 and 6: Effect of V2R Stimulation on MBF in the Presence and Absence of L-NAME**

Figure 5 summarizes that renal medullary infusion of dDAVP in the presence of the V1R antagonist resulted in a significant 15±2% increase of MBF (n=7; P<0.05). No change in renal cortical blood flow or MAP was observed during these periods.

To determine whether NOS stimulation was responsible for the V2R-mediated vasodilation after V1R blockade in the renal medulla, L-NAME was administered into the renal medullary interstitium at 1.3 mg/kg per minute before the renal medullary infusion of dDAVP. Figure 5 demonstrates that L-NAME pretreatment completely blocked the V2R-mediated increase in MBF (n=7), as it had prevented the rise in medullary interstitial [NO] shown in Figure 2 (top panel). No changes in renal cortical blood flow (n=6) and MAP were observed (n=7).

**Group 7: L-NAME Time Control**

A time control experiment for groups 2, 3, 5, and 6 was performed with medullary interstitial infusion of L-NAME (1.3 µg/kg per minute) and an intravenous infusion of the V1R antagonist (5 ng/kg per minute) to determine whether MBF was stable throughout the period of the study. Figure 5 demonstrates the constancy of renal cortical blood flow, MBF, and MAP throughout the experimental protocol.

**Discussion**

The present study addressed 3 issues: first, whether AVP at a dose that only moderately reduces MBF could increase renal medullary interstitial [NO]; second, which AVP receptor type mediated the observed changes in renal medullary interstitial [NO]; and third, whether AVP stimulation of NO was responsible for V1R-mediated increases of blood flow to the renal medulla.
The Role of AVP in the Regulation of MBF

Previous studies in our laboratory by Nakanishi et al.\textsuperscript{14} using anesthetized rats found that renal medullary interstitial infusion of the selective V\textsubscript{2}R agonist [Phe\textsuperscript{2}, Ile\textsuperscript{3}, Orn\textsuperscript{8}]VP caused a greater reduction in inner MBF than an equimolar dose of AVP. It was also found that renal medullary stimulation of the V\textsubscript{2}R resulted in an increase in inner MBF in the presence of a selective V\textsubscript{1}R antagonist.\textsuperscript{14} The present study confirmed that selective V\textsubscript{1}R stimulation with dDAVP increased MBF and demonstrated that this response was associated with a significant increase of medullary interstitial [NO]. Medullary interstitial infusion of L-NAME, a nonselective NOS inhibitor, at a dose of 1.3 μg/kg per minute was sufficient to eliminate the AVP-induced elevations of medullary interstitial [NO] and prevented the V\textsubscript{2}R-mediated increase in MBF. It is important to note that the dose of L-NAME used in these studies resulted in no significant alteration in basal levels of MBF, which is consistent with our findings in a previous study.\textsuperscript{24} In contrast, we have shown that higher doses of L-NAME (>6 μg/kg per minute) into the renal medullary interstitium significantly reduced MBF nearly 30% and produced sustained hypertension.\textsuperscript{39} These results suggest that an excess pool of NO is normally present in the renal medulla and that interstitial [NO] must be lowered below some threshold level (>≈30%) before basal tone to medullary vessels is influenced. This was the intention of the dose for this study since it was our goal to maintain MBF relatively unchanged by L-NAME while partially reducing the activity of the NOS enzymes.

Direct Evidence for AVP Stimulation of Renal Medullary Interstitial NO Concentration

The present study determined whether AVP could directly mediate an increase in interstitial [NO] within the renal medulla. Previous studies have suggested that AVP could stimulate NO production on the basis of pharmacological inhibition of NOS activity and increased cGMP formation as an index of NOS activity.\textsuperscript{20–23} Specifically, Rudichenko and Beierwaltes\textsuperscript{20} observed that suppresor doses of intravenously administrated AVP to anesthetized rats resulted in a reduction in renal vascular resistance, which was blocked by pretreatment with L-NAME. It was also found that urinary cGMP levels were increased by AVP, although it was unclear whether the kidney was the source of these elevations. Ikenaga et al.\textsuperscript{23} demonstrated an augmented vasoconstrictor response to AVP in the presence of a NOS inhibitor using the in vitro blood-perfused juxtamedullary nephron preparation. Consistent with these studies, intravenous infusion of L-NAME was found to attenuate the vasodilator response of the V\textsubscript{2}R in anesthetized dogs.\textsuperscript{21,22} Similar results have been observed in humans in studies of AVP-mediated forearm vasodilation.\textsuperscript{26,27}

In the present study we were able to directly determine the effects of elevated AVP levels within the renal medulla on interstitial [NO] using the in vivo microdialysis/oxyhemoglobin-NO trapping technique. Suppresor doses of AVP delivered into the renal medullary interstitium significantly elevated medullary interstitial [NO], and this response was blocked by reducing medullary NO activity. This experiment demonstrates that AVP stimulates NO production within the renal medulla.

Direct Evidence of V\textsubscript{2}R-Mediated NO Release

The next goal of our study was to define which AVP receptor was involved in mediating the increase in medullary interstitial [NO]. The results demonstrate that the stimulation of medullary interstitial [NO] was mediated by stimulation of the V\textsubscript{2}R, and that selective stimulation of the V\textsubscript{1}R did not alter medullary interstitial [NO]. These observations are in contrast to a recent study by Hirata et al.,\textsuperscript{28} who concluded from studies using an ex vivo isolated perfused rat kidney preparation that AVP stimulated an endothelial V\textsubscript{1}R to increase NO production. In contrast, Barthelmebs et al.\textsuperscript{29} using the isolated perfused rat kidney preparation, found that intrarenal infusion of low doses of AVP did not result in vascular dilation but instead showed vasoconstriction. The latter study is more consistent with the in vivo and ex vivo functional studies which have demonstrated that AVP constricts the medullary circulation through V\textsubscript{1}R stimulation.\textsuperscript{4,5,14} We concluded from the present study and the preponderance of published data that AVP stimulation of extravascular V\textsubscript{2}R increases medullary interstitial [NO], which can reduce medullary vascular tone.

The specific site(s) where AVP mediates the increase in [NO] within the renal medulla remains to be determined.
Recent studies in our laboratory using reverse transcription–polymerase chain reaction on microdissected cortical and medullary blood vessels, including the outer medullary descending vasa recta, could not detect the presence of the V₃R mRNA. Since extravascular sites within the kidney, in particular the medullary thick ascending limbs of Henle and collecting ducts, contain not only the V₂R but also the 3 NOS isoforms, the V₂R-mediated release of NO may occur in these site(s). In this way, NO may act as a paracrine hormone to dilate the renal medullary vasculature. However, the mechanism by which the V₂R stimulates NO remains to be elucidated. The V₂R is known to activate adenyl cyclase, yet the constitutive isoforms for NOS are known to be calcium dependent. Recently, a number of investigators have suggested that there may be a novel V₂-like receptor that stimulates a transient elevation in intracellular Ca²⁺, and therefore it is possible that this V₂-like receptor is responsible for the AVP-mediated stimulation in NO.

In summary, the present study demonstrated that acute subpressor doses of AVP increased the production of renal medullary interstitial NO, which in turn participated in the renal medullary vasodilator mechanism. Thus, the V₂R-mediated vasodilator mechanism appears to modulate reductions in MFB through the activation of NOS. Further studies are needed to determine whether this mechanism is a reason why chronic elevations in AVP cannot produce sustained hypertension.

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