Effects of long-term vasopressin receptor stimulation on medullary blood flow and arterial pressure

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Cowley, Allen W., Jr., Meredith M. Skelton, and Theresa M. Kurth. Effects of long-term vasopressin receptor stimulation on medullary blood flow and arterial pressure. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1420–R1424, 1998.—Studies were carried out using instrumented unanesthetized rats to determine the long-term effects of arginine vasopressin (AVP) and a specific vasopressin V\textsubscript{1} receptor agonist (V\textsubscript{1}AG; [Phe\textsuperscript{2}, Ile\textsuperscript{3}, Orn\textsuperscript{8}]-vasopressin) on the renal medullary blood flow and arterial blood pressure. It was hypothesized that the hypertension observed with chronic medullary infusion of a V\textsubscript{1} receptor agonist may be associated with a sustained reduction of blood flow, whereas infusion of AVP may fail to produce a sustained reduction of blood flow and thereby be unable to produce hypertension. Uninephrectomized Sprague-Dawley rats were prepared with renal cortical and medullary optical fibers for daily measurements of cortical and medullary blood flow using laser-Doppler flowmetry techniques. An implanted renal medullary interstitial infusion catheter delivered either AVP or a specific V\textsubscript{1}AG at a dose of 2 ng·kg\textsuperscript{-1}·min\textsuperscript{-1} over a period of 5 days. The V\textsubscript{1}AG produced no change of cortical blood flow but a chronic 35% reduction of medullary blood flow (P < 0.05) and mild hypertension (11 ± 4 mmHg, P < 0.05). AVP produced only an initial, nonsignificant 1- to 2-day reduction of medullary blood flow (~13%) and failed to raise arterial pressure significantly. We conclude that a sustained V\textsubscript{1}AG response is necessary to achieve a chronic reduction of medullary blood flow and hypertension. The present data are consistent with the idea that chronic stimulation of V\textsubscript{2} receptors by AVP offsets the vasoconstrictor and hypertension actions of AVP-induced stimulation of medullary V\textsubscript{1} receptors.

vasopressin V\textsubscript{1} receptor agonist; renal blood flow; arterial blood pressure

It is recognized that chronic elevations of plasma arginine vasopressin (AVP) do not produce sustained hypertension (1, 2, 20). This is puzzling given the fact that AVP reduces the rate of fluid excretion, which results in profound volume expansion unless offset by equivalent reductions of fluid intake (2). Furthermore, AVP is one of the most potent circulatory vasoconstrictor peptides that is known (2). Despite the AVP effects on vascular tone and fluid volume, arterial pressure rises only for a period of 7–10 days and then gradually returns to normal levels during chronic administration of excess AVP, even if daily fluid intake is maintained at a constant level (2, 10). Transient volume expansion and sustained hyponatremia with no hypertension are the most prominent features observed with chronic elevations of plasma AVP in dogs (21) and humans (11).

Studies in our laboratory (4) and that of Hall et al. (10) have shown that the “escape” from the antidiuretic actions of AVP is dependent on the rise of renal arterial perfusion pressure brought about by volume expansion.

It is also surprising that AVP does not produce hypertension because the renal medullary circulation is particularly sensitive to the vasoconstrictor effects of the endogenous peptide. Acute studies of anesthetized rats in our laboratory (17) have shown that renal medullary interstitial infusion of AVP can reduce medullary blood flow 20–40%. We have also shown recently that the renal medullary vasculature is as sensitive to small elevations of plasma AVP (6–12 pg/ml) as the epithelial cells of the medullary collecting duct (8). Such physiological elevations of plasma AVP not only reduce renal medullary flow but also substantially blunt the sensitivity of the pressure-natriuresis-diuresis relationship (9). Despite these responses observed in short term (1–4 h), AVP is unable to chronically reset the pressure-natriuresis relationship and produce hypertension as do other vasoconstrictor compounds, such as angiotensin II, norepinephrine, and N\textsuperscript{ω}-nitro-L-arginine methyl ester (2, 22). AVP when infused intravenously (17) or into the renal medullary interstitium of rats for a long period (7–14 days) has failed to produce hypertension (5, 22).

In the present study we explored the hypothesis that AVP could not produce chronic hypertension because it was unable to produce a sustained reduction of renal medullary blood flow. The emphasis on the renal medulla in these studies was motivated not only by the evidence that blood flow to this region can play an important role in long-term pressure regulation (1), but also by observations that hypertension which we have produced with chronic intravenous infusion of a selective vasopressin V\textsubscript{1} receptor agonist (V\textsubscript{1}AG; [Phe\textsuperscript{2}, Ile\textsuperscript{3}, Orn\textsuperscript{8}]-vasopressin; 5) could be prevented if a selective vasopressin V\textsubscript{1} receptor antagonist was administered simultaneously into the medullary interstitial space (22). The renal medulla therefore appears to be a critical site for the vasopressin V\textsubscript{1} receptor-mediated hypertensive effects. Because we have also shown that stimulation of renal medullary vasopressin V\textsubscript{2} receptors with a selective agonist results in an increase of medullary blood flow (17), we speculated that the failure of AVP to produce hypertension may be due to its inability to produce a sustained reduction of blood flow.

To examine this hypothesis, uninephrectomized Sprague-Dawley rats were prepared with renal cortical and medullary optical fibers for daily measurement of changes of blood flow to these two regions of the kidney while AVP or V\textsubscript{1}AG at equimolar doses were infused over a period of 5 days.
**METHODS**

**Experimental Animals**

Studies were performed on adult male Sprague-Dawley rats (300–350 g) purchased from Harlan Sprague Dawley (Madison, WI). All animals were housed individually in the Animal Resource Center with food and water provided ad libitum. All protocols were approved by the Institutional Animal Care and Use Committee at the Medical College of Wisconsin.

**Surgical Preparation**

All surgical procedures were performed under aseptic conditions. Rats were anesthetized with a mixture of ketamine (100 mg/kg im) and acepromazine (5 mg/kg im) and placed on a heated surgical table to maintain body temperature at 37°C. The right kidney was removed through a right flank incision at least 7 days before catheters were implanted. The right kidneys of these rats were removed because it was not possible to simultaneously infuse the renal medulla of both kidneys in this chronic studies. Because the effects of altering one kidney could be offset by the counterregulatory effects of the contralateral kidney via pressure-diuresis or indirectly via neuroendocrine responses, the right kidney was removed in the present studies.

Rats were given at least 1 wk to recover from this surgery to allow the remaining left kidney to undergo hypertrophy before instrumenting with the medullary catheter and optical fibers. The left kidney was exposed therefore in a second surgery via a flank incision, and the interstitial catheter and optical fibers were constructed and implanted as previously described (7, 13). A polyethylene catheter (heat stretched to a 100-μm diam tip) was implanted in the kidney by inserting it directly into the kidney tissue through a small hole made in the renal capsule with a 25-gauge needle. The catheter was placed at or near the border of the inner and outer medulla, and cyanoacrylate adhesive was used to anchor the catheter to the kidney surface. The renal interstitial catheter was exteriorized with the femoral catheter and optical fibers at the intrascapular region. To maintain patency of the medullary catheter, a continuous infusion of saline or drug was delivered at a rate of 8.3 µl/min (0.5 ml/h) throughout the study.

**Experimental Design**

Group 1. Chronic infusion of AVP into renal medullary interstitial space. After 10 days of recovery from the surgical implantation of the catheters and optical fibers, baseline measurements of MAP and cortical and inner medullary blood flow were begun. Rats were studied in their home cages as previously described. Throughout the recovery period and the days of hemodynamic control measurements, rats received a continuous 24-h infusion of isotonic saline (8.3 µl/min) into the medullary interstitial space. After at least 2 consecutive days of constant pressures and flows, the interstitial infusion was switched from isotonic saline to AVP (Sigma Chemicals, St. Louis, MO) at a dose of 2.0 ng·kg⁻¹·min⁻¹ and continued for 5 days. Saline was infused for 2 postcontrol days after cessation of the AVP infusion. Arterial pressure and regional blood flow were recorded for 2 h daily at a rate of 100 Hz.

After completion of the study, rats were killed with an excess dose of pentobarbital sodium, and the kidneys were removed and placed in 10% Formalin solution for 24 h before examination of the renal morphology and determination of precise placement of the medullary catheter and the optical fibers. Rats that exhibited morphological changes such as necrotic tissue surrounding the track or at the tip of the optical fibers were not included in the analysis of data.

Group 2. Chronic infusion of V₁ AG into renal medullary interstitial space. This study was performed in the same manner as described for group 1 (with an additional control day) except that after control measurements an equimolar amount (2 ng·kg⁻¹·min⁻¹) of the V₁ AG ([Phe⁶, Ile², Orn³]vasopressin; Peninsula Laboratories, Belmont, CA) was infused into the medullary interstitial space for 5 days.

**Hemodynamic Measurements**

Animals were allowed at least 10 days to recover from surgery before any measurements were made. To minimize motion artifacts during recording of cortical and medullary blood flow using the laser Doppler flowmeter, rats were trained to rest quietly for 2 h in a Plexiglas tube placed in the home cage (13). The animals train quickly to this procedure (during the last 3 days of recovery from surgery), and control pressures and flow measurements can then be made. We have demonstrated in previous studies from our laboratory that chronic fiber implantation had no significant effect on mean arterial pressure (MAP), glomerular filtration rate, renal blood flow, or urine concentrating ability (13). The implanted fibers were connected to the external probe of the Laser-Doppler flowmeter (model ALF 21, Advance, Tokyo, Japan) with the light loss minimized between the fiber and probe by introducing fused silica matching liquid (No. 5035-0, Car-gille Laboratories, Cedar Grove, NJ). Arterial pressure was measured with a pressure transducer (Cobe, Arvada, CO), and the signal amplified with an amplifier of our own design. MAP and cortical and medullary blood flows were measured continuously at 100 Hz for 2–3 h daily with an Apollo DN3500 computer system that transformed continuous pulsatile arterial pressure and laser-Doppler flow signals to minute averages.

The laser-Doppler flowmeter was calibrated each day using an internal electrical standard (0–1 V). Readings in a motility standard solution (PF-100, Perimed, 5% diluted) showed only small daily fluctuations of the laser-Doppler signal (2.59 ± 0.05 V). We have previously demonstrated that changes in blood flow measured with this flowmeter correlate with changes measured by in vivo videomicroscopy (17). Preliminary in vitro experiments also showed that the laser-Doppler flowmeter ALF 21RD is linear within the physiological flow range and generates a signal that is similar to the signal of the Perimed laser-Doppler flowmeter (model PFI, Perimed, Stockholm, Sweden), which had been used in prior studies (12–17).

**Neuroendocrine Control**

The experimental design was as follows:

- **Group 1**: Infusion of AVP into renal medullary interstitial space (days 11–16 of the study).
- **Group 2**: Infusion of V₁ AG into renal medullary interstitial space (days 11–16 of the study).

**Control Measurements**

Baseline measurements of MAP and cortical and inner medullary blood flow were begun. Rats were studied in their home cages as previously described. Throughout the recovery period and the days of hemodynamic control measurements, rats received a continuous 24-h infusion of isotonic saline (8.3 µl/min) into the medullary interstitial space. After at least 2 consecutive days of constant pressures and flows, the interstitial infusion was switched from isotonic saline to AVP (Sigma Chemicals, St. Louis, MO) at a dose of 2.0 ng·kg⁻¹·min⁻¹ and continued for 5 days. Saline was infused for 2 postcontrol days after cessation of the AVP infusion. Arterial pressure and regional blood flow were recorded for 2 h daily at a rate of 100 Hz.

After completion of the study, rats were killed with an excess dose of pentobarbital sodium, and the kidneys were removed and placed in 10% Formalin solution for 24 h before examination of the renal morphology and determination of precise placement of the medullary catheter and the optical fibers. Rats that exhibited morphological changes such as necrotic tissue surrounding the track or at the tip of the optical fibers were not included in the analysis of data.
RESULTS

Group 1. Chronic infusion of AVP into renal medullary interstitial space.

Figure 1 summarizes the daily arterial pressure and blood flow responses of the renal inner medulla and cortex during 5 days of renal medullary interstitial infusion of AVP. Although there was a tendency for inner medullary blood flow to decrease and MAP to rise during the first several days of AVP infusion, these changes were not statistically significant. Cortical blood flow remained unchanged throughout the study. We have shown previously that medullary interstitial infusion of isotonic saline at the same rate resulted in no change of medullary or cortical blood flow over 11 days of daily recording (13).

Group 2. Chronic infusion of V1 AG into renal medullary interstitial space.

Figure 2 summarizes the responses to medullary interstitial infusion of the selective V1 AG of MAP and cortical and medullary blood flow. In contrast to equimolar amounts of AVP, the infusion of V1 AG resulted in a sustained reduction of blood flow to the inner medulla which averaged 35–40% less than that measured during the control period. Cortical blood flow remained unchanged from control throughout the period of V1 AG infusion. Absolute cortical blood flow signals tended to be lower in this group of rats perhaps because of the deeper placement of the optical fibers to reduce the incidence of pulling out which occurs more frequently with the more superficially placed fibers. The reduction of medullary blood flow was associated with a modest, but sustained elevation of MAP, which rose from an average control of 110 ± 6 mmHg. Both MAP and medullary blood flow returned toward control levels on the day after V1 AG infusion, but on the second postcontrol day medullary flow averaged a value less than control.

DISCUSSION

It is well recognized that prolonged elevations of AVP do not produce sustained hypertension (2). In contrast, chronic administration of a V1 AG delivered either systemically or into the renal medullary space of rats does result in a mild and sustained form of hypertension (22). It has been shown in anesthetized rats that both AVP and V1 AG effectively reduce renal medullary blood flow (17). If such effects were sustained, one would anticipate that both compounds could produce a sustained elevation of arterial pressure because chronic reductions of blood flow to the medulla result in hypertension (1, 12, 15). We hypothesized therefore that the failure of AVP to produce hypertension could be related to an inability of this endogenous peptide to produce a sustained reduction of medullary blood flow. The re-
the present study support this hypothesis and show that chronic reduction of blood flow to the inner medulla was not achieved with continuous administration of AVP but did occur with infusion of the V₃AG in addition to a sustained hypertension.

We have shown previously in anesthetized rats that blood flow to the renal medulla is reduced immediately on medullary infusion of V₃AG. This would indicate that the rise of arterial pressure with V₃AG was a consequence of the reduction of medullary blood flow rather than a result of the hypertension (17). It was also found that hypertension which resulted from chronic intravenous infusion of V₃AG could be prevented by infusion of a V₁ receptor antagonist into the renal medulla (22). Taken together these studies demonstrated that the renal medulla is a critical site for vasopressin V₁-mediated hypertensive effects.

It is interesting that even though AVP has been shown to be a potent constrictor of the medullary circulation acutely, it is unable to chronically sustain a reduction of medullary blood flow. The inability of AVP to maintain a reduced flow to this region could be related to the simultaneous actions of the endogenous peptide on both vasopressin V₁ and V₂ receptors. We have shown that V₂ receptor stimulation in the presence of a V₁ receptor antagonist increased blood flow to this region (17, 19). It was also observed acutely that medullary interstitial infusion of V₃AG resulted in nearly twice the reduction of blood flow to the inner medulla as did equimolar amounts of AVP (17). AVP therefore appears to be a less effective vasoconstrictor in the renal medulla of rats than compounds that preferentially stimulate V₁ receptors. These opposing effects of vasopressin V₁ and V₂ receptor stimulation are consistent with the responses observed in the present chronic studies as shown in Figs. 1 and 2.

It is difficult to carry out a study that would directly define the chronic effects of selective V₂ receptor stimulation on medullary blood flow. Administration of a V₂ receptor antagonist would be expected to produce a diabetes insipidus state with changes in the electrolyte and volume status of the animals with many secondary neural and hormonal responses influencing medullary blood flow and arterial pressure. Alternately, chronic administration of a V₂ agonist into the renal medulla to determine whether this would result in an elevation of medullary blood flow and reduce arterial pressure is also problematic. Such prolonged stimulation of V₂ receptors with AVP or 1-(3-mercaptopropanoic acid)-8-D-arginine vasopressin (DDAVP) would result in fluid retention, volume expansion, and a rise of arterial pressure. This in turn would initiate a pressure-diuresis response and drive an escape from the antidiuretic and hypertensive effects of the V₂ receptor agonist (4, 10). Nevertheless, the results of the present study are consistent with the idea that chronic stimulation of V₂ receptors by AVP offsets the vasoconstrictr and hypertensive actions of the AVP-induced stimulation of medullary V₁ receptors.

The long-term chronic effects of AVP stimulation are undoubtedly complex. The present results imply, but do not prove, that AVP not only stimulates V₁ receptors to reduce medullary blood flow but also stimulates vasodilator pathways that can offset these constrictor effects. This could occur either through stimulation of the classic V₂ receptor cyclic AMP-mediated pathways or other yet unknown receptors and pathways. We have recently reported an absence of mRNA for vasopressin V₂ receptors in microdissected vessels from both the cortex and medulla, including vasa recta (18). It appears that V₂ receptors residing on medullary collecting duct epithelial cells and/or interstitial cells are responsible for the vasodilatory responses observed with AVP in the presence of V₁ receptor blockade. Studies in our laboratory have shown that the concentrations of AVP used in the present study result in an increase of medullary nitric oxide concentrations and these responses are mediated via the V₂ receptor (19). This could explain at least in part why an increase of medullary flow occurs with infusion of AVP in the presence of a selective V₁ receptor antagonist or with administration of a V₂ receptor agonist (DDAVP). The chronic events that appear to evolve over time may be a consequence of enhanced production of nitric oxide or other vasodilator substances that require 24–48 h to reach significant concentrations in the medulla. Clearly, there are many unanswered questions at this time regarding the chronic effects of AVP on the regulation of blood flow to the renal medulla that need to be explored.

Finally, it should also be recognized that the inability of AVP to produce a sustained reduction of medullary blood flow may depend on the state of hydration of the animal. Under conditions in which AVP is normally elevated, such as dehydration, this endogenous peptide is capable of effectively reducing medullary blood flow for at least 48 h. Specifically, a rise of plasma AVP from 3.4 to 20.5 pg/ml was observed during 2 days of water restriction in rats which was associated with a sustained reduction of medullary blood flow of nearly 34% (7). Although other factors, such as the stimulation of the sympathetic nervous system, could have contributed to this response, at least one-half of this response could be accounted for by V₁ receptor stimulation, as determined in rats that were administered a selective V₁ receptor antagonist throughout the study. In summary, the results of the present study indicate that the reason AVP is unable to produce sustained elevations of arterial pressure is because this endogenous vasoconstrictor peptide cannot chronically reduce blood flow to the renal medulla.

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