Effect of Arginine Vasopressin on Renal Medullary Blood Flow

A Videomicroscopic Study in the Rat

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Abstract

The role of arginine vasopressin (AVP) in the regulation of renal medullary blood flow is uncertain. To determine if AVP has a direct vasoconstrictive action on vasa recta, the effect of AVP on erythrocyte velocity (V_{RBC}), diameter, and blood flow (Q_{VR}) in descending vasa recta (DVR) and ascending vasa recta (AVR) was studied in the exposed renal papilla of four groups of chronically water diuretic rats using fluorescence videomicroscopy. There were three periods: control (period 1), experimental (period 2), and recovery (period 3). In periods 1 and 3, all groups received hypotonic saline. In period 2, group I rats (AVP) received AVP (45 ng/h per kg body wt); group II (time) received hypotonic saline alone; group III (AVP plus V_1-inhibitor) received AVP plus its vascular antagonist, $d(CH_2)_5Tyr(Me)AVP$; and group IV (V_1-inhibitor) received the vascular antagonist alone. Another group of rats (group V) was employed to demonstrate that the rise in blood pressure induced by a 3- or 10-ng/kg injection of AVP was virtually abolished by the prior infusion of the V_1-inhibitor. The urine of group III as well as group I rats was concentrated (U_{osm} = 721±62 H_2O vs. 670±39 mosM/kg), while urine remained dilute in groups II and IV. In period 2, V_{RBC} and Q_{VR} in DVR and AVR decreased in group I, did not decrease in group III, and increased in groups II and IV. The vascular antagonist thus completely abolished the AVP-induced decrease in Q_{VR} in group III. These findings unequivocally establish that AVP in physiological amounts reduces medullary blood flow, at least in part, by a direct vasoconstrictive action on the medullary microcirculation. They also show that an effect of AVP on medullary blood flow is not necessary for its antidiuretic effect.

Introduction

The actions of arginine vasopressin (AVP)[1] have been related to two distinct intracellular mechanisms (1). First, vasopressor and glycogenolytic activities of AVP are associated with an increase in intracellular calcium concentration and phosphatidyl-inositol metabolism; second, antidiuretic activity is associated with an increase in intracellular cyclic AMP (cAMP) generation. In analogy to adrenergic and histaminergic receptors, Michell et al. (2) proposed that the reactions related to the phosphatidyl-inositol pathway be designated V_1, and those related to cAMP as V_2. Recently potent selective inhibitors to V_1-related actions of AVP have been synthesized (3).

Thurau et al. (4) reported more than two decades ago that vasopressin in physiological amounts decreases inner medullary blood flow without affecting total renal blood or cortical blood flow. It was unclear, however, if the change in medullary flow was a consequence of a direct action of the hormone on vascular receptors (i.e., V_1-receptor–mediated action), or indirectly a consequence of its antidiuretic activity, V_2. This uncertainty was in part a consequence of the technical limitations in investigating medullary blood flow. While unphysiologically high doses of AVP directly reduce blood flow in both cortex and medulla, several investigators have failed to observe an effect on renal blood flow using physiological amounts of AVP (5). We recently adapted videomicroscopy to study blood flow in the renal papilla. This method combined with modern fluorescence techniques (6) allows a continuous and quantitative measurement of blood flow in the exposed renal papilla in the rat (7, 8).

The purposes of the present investigation were to determine, first, if AVP in physiological doses affects vasa recta blood flow (Q_{VR}) and, if so, whether this effect is mediated directly by the V_1-receptor or indirectly by a V_2-receptor.

Methods

Preparation of animals. Young Munich-Wistar rats (n = 37) of both sexes weighing between 93 and 188 g were prepared for videomicroscopy as described previously (9). In brief, the animals received standard rat chow and were kept in a state of water diuresis for 2–4 wk by adding 5% dextrose to the tap water. On the afternoon before the experiment the animals were deprived of food and sustained on tap water containing 5% dextrose and 0.15% NaCl (10). Rats were anesthetized intraperitoneally with Inactin (thiobutabarbital; Byk Gulden, Konstanz, FRG) at 120 mg/kg body weight (BW). The trachea was cannulated and a triple lumen catheter, made of PE 50 tubing that was tapered by pulling the catheter over an alcohol flame, was inserted into the jugular vein. A PE 50 catheter was inserted into the left femoral artery for continuous recording of arterial blood pressure and for withdrawal of blood

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1. *Abbreviations used in this paper:* AVP, arginine vasopressin; AVR, ascending vasa recta; BW, body weight; C_{In}, clearance of insulin; CON, control period; C_{PAH}, clearance of paraaminohippurate; D, diameter; DVR, descending vasa recta; EXP, experimental period; FITC, fluorescein isothiocyanate; HCT, hematocrit; PAH, paraaminohippurate; P_{osm}, plasma osmolality; Q_{VR}, vasa recta blood flow; REC, recovery period; SNGFR, single nephron glomerular filtration rate; UF, urine flow; U_{osm}, urinary osmolality; U/P_{In}, urine-to-plasma inulin; V_{blood}, average blood velocity; V_{RBC}, erythrocyte velocity; VPA, vasopressor antagonist $d(CH_2)_5Tyr(Me)AVP$; VR, vasa recta.

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samples. A PE 50 catheter was inserted into the bladder for collection of urine. The body temperature was kept at 37.5°C with the aid of a feedback-controlled heating table. The left kidney was exposed by a flank incision and carefully isolated from the perirenal tissue. The tissue around the renal pedicle was removed by dissection and the papilla was exposed by excising the ureter (11). The kidney was placed gently in a lucite holder, protected with small cotton balls, and the dorsal aspect of the papilla was displayed. Care was taken not to stretch the renal artery. Any ischemia of the kidney caused the experiment to be discarded. To prevent drying, the kidney was bathed in warm mineral oil and covered with a translucent foil (Saran Wrap) until microscopic observations were performed. During the video recording the foil was removed.

After insertion of the arterial catheter, a sample of plasma was taken and an intravenous infusion of normal saline containing 1.5% inulin and 0.15% paraaminohippurate (PAH) was started at a rate of 0.30 ml/min per kg BW. This solution was used during period 2 of groups I, III, IV, and V as a vehicle for the infusion of AVP and/or the pressor antagonist. The protein loss due to surgical manipulation was replaced according to the protocol of Ichikawa et al. (10) modified as follows: bovine serum albumin (Sigma Chemical Co., St. Louis, MO) was dissolved in normal saline to achieve a 5% albumin solution. This solution was infused at a rate of 0.27 ml/min per kg BW for 1 h and then continued at a rate of 0.033 ml/min per kg BW for the remaining time of the experiment.

Videomicroscopy. The exposed papilla was positioned under the objective of an intravital microscope (E. Leitz, Inc., Rockleigh, NJ). At the end of the stabilization period, 0.2 ml of fluorescein isothiocyanate (FITC) gamma globulin was injected intravenously. The preparation of the FITC gamma globulin has been described previously (7). The papilla was illuminated with fluorescent light (excitation wave length < 490 nm, emission wave length > 530 nm), and the image of the microscopic field was transmitted to a high sensitivity television camera (model 4410 SIT; Cohu Inc., San Diego, CA), displayed on a television monitor, and documented on videotape (7, 8). 2–3 different areas near the base of the papilla were recorded, each for 3–5 min. A sketch of the field in view was drawn to insure the recording of the same microscopic field subsequently. At the end of each recording a bolus of 0.03–0.05 ml of FITC gamma globulin was injected to highlight the walls of the vasa recta (VR) to facilitate measuring the vessel diameters for a few seconds. The injection had no effect on blood pressure or VR velocity. The final magnification on the monitor screen with an L 20 (E. Leitz, Inc.) objective, without further ocular magnification, was 850–1,150 ×, depending on the location on the screen (12).

VR diameter (D). The diameter of a VR was measured as the distance between the fluorescent rims of the capillary walls on the monitor corrected for final magnification on the video screen.

Erythrocyte velocity (V_{RBC}). V_{RBC} was measured by a dual-slit technique from the videotape recordings as described previously (7, 8). Photometric signals from two nearby sites on the same capillary were analyzed and the transit time of erythrocytes was obtained by a time series cross-correlation of the two signals using an automated digital cross-correlation system. From transit time and real distance between the two sites the V_{RBC} was calculated.

Experimental protocols. Five groups of rats were studied. The initial measurements in all groups began 2 h after exposure of the papilla. An outline of the experimental protocol for groups I to IV is given in Fig. 1. There were three experimental periods. Periods 1 and 3 were identical in all five groups. The animals were maintained in water diuresis after surgery by infusing a solution containing 0.83% glucose and 0.30% NaCl (glucose-saline infusion) at a rate of 1.2 ml/min per kg BW for 60 min (the infusion was started ~30 min before exposure of the papilla) and continued thereafter at 0.70 ml/min per kg BW for 90 min. Period 1 then began. Urine collection from the right kidney was started and a specimen of plasma was obtained at the midpoint. VR blood flow was determined (period 1 = control period [CON]). The time required for sampling and recording was 20–30 min.

The second and third periods in the different groups were conducted as follows:

Group I (n = 8): AVP-induced antidiuresis. Antidiuresis was induced by infusing AVP intravenously at a rate of 45 ng/h per kg BW (9). The AVP was dissolved in the infusate on the day of the experiment and infused in place of the initial infusion at the same rate (0.30 ml/min per kg BW). After the AVP infusion began, the glucose-saline infusion through a separate line was gradually reduced during the first 20 min to match the falling urine flow (UF), and was maintained at 0.063 ml/min per kg BW thereafter. 60 min after onset of the AVP-infusion, period 2 began, during which the collection of plasma and urine was obtained as in period 1. Videomicroscopic recordings of the same areas studied in period 1 were made. In period 3, the glucose-saline solution was restored to the original rate of 0.70 ml/min per kg BW, and the original infusate without AVP was given. Period 3 began 60 min later and was identical to period 1.

Group II (n = 8): time control. In this group, the original base-line infusion was maintained and the glucose-saline infusion sustained at the rate of 0.70 ml/min per kg BW throughout all three periods. The periods were otherwise identical in design to that of group I.

Group III (n = 8): AVP-induced antidiuresis and inhibition of V_1-effect. The infusion protocol for AVP and glucose-saline infusion was identical to group I. In addition, an antagonist of the vasopressor (V_1-mediated) action of AVP (1-[β-mercapto-β,β-cyclopentamethylene propionic acid],2-[*O*-methyl]tyrosine)AVP (d[CH_2]$_5$Tyr[Me]-AVP) (13) was added to the base-line infusion at the rate of 5 μg/h per kg BW. Concomitantly with the onset of the AVP/antagonist infusion, a precise dose of the antagonist was given 1 μg/h per kg BW in a volume of 2 ml/kg, a protocol similar to that used by Imai et al. (14). After 60 min, sampling and recording of period 2 was started. Period 3 was identical to period 3 in group I.

Group IV (n = 6): V_1-inhibitor alone. The infusion protocol in this group was similar to group II. Glucose-saline infusion was administered at the rate of 0.70 ml/min per kg BW throughout all three periods. The base-line infusion containing the AVP-antagonist used in group III was infused, and the same primary dose as in group III was administered. 60 min later, samples and video recordings were taken. Period 3 was identical to that of group I.

Group V(a) (n = 4) and V(b) (n = 3): effectiveness of pressor antagonism. Animals were prepared as described before except that the left kidney was not dissected. The animals received the same base-line infusion as groups I–IV. Glucose-saline was infused at 0.70 ml/min per kg BW. 2 h after completion of surgery a bolus of saline (2 ml/kg) was given and the rise in blood pressure noted. 5 min later, AVP was given in a dose of 10 ng/kg (group V[a], n = 4) or 3 ng/kg (group V[b], n = 3) in a volume of 2 ml/kg. The peak of the change in blood pressure immediately after injection was recorded. After the pressure had returned to base line (30 min later), the pressor antagonist was infused as in period 2 of groups III and IV: a bolus of 1 μg/kg d(CH_2)$_5$Tyr(Me)AVP followed by an infusion of 5 μg/h per kg added to the base-line infusion. 60 min after onset of the antagonist infusion, another bolus of AVP was given in the same two doses and in the same manner as before and the pressure change recorded.

Analyses. Blood pressure was recorded using a Statham strain gauge and recorded on a strip chart recorder (model 110; Gould Inc., Cleveland, OH). Hematocrit (HCT) was determined after centrifugation in a hematocrit centrifuge for 5 min. Plasma and urine osmolality (U_{osm}) were determined by freezing point depression using a Ramsay Brown osmometer (11). Inulin was determined with the anthrone method (15). PAH was detected chemically according to Smith et al. (16). During the period of the experiments we experienced problems with the detection of PAH, and in three experiments (one in group I and two in group IV) used radioactive ^{3}H-PAH (New England Nuclear, Boston, MA) infused in the base-line infusion at a rate of 1.8 μCi/h per kg BW. UF was determined gravimetrically.

Calculations. Blood in individual VR was calculated from cross-sectional area and average blood velocity according to the following equation:

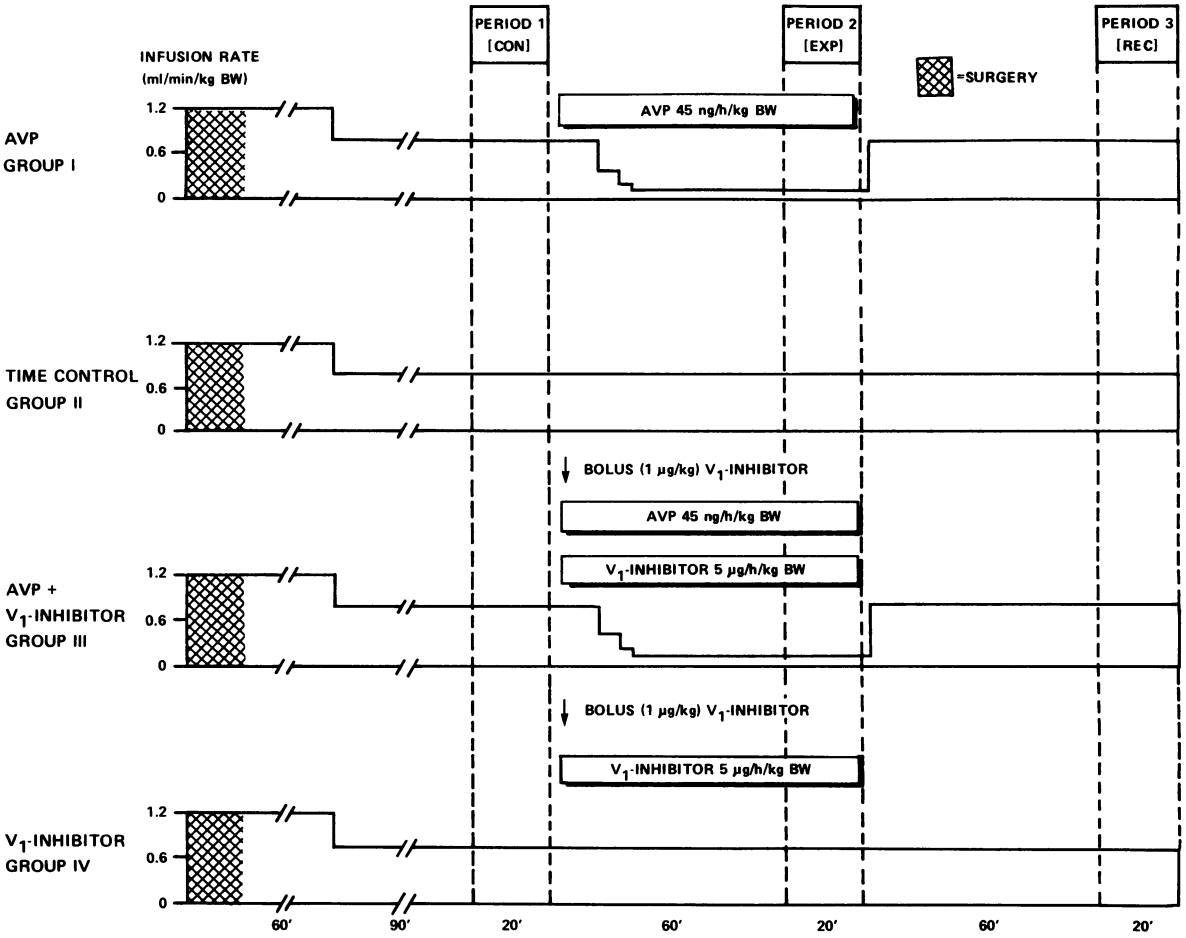

Figure 1. Experimental protocol. Videomicroscopic recordings and clearance studies were performed during periods 1–3, CON, EXP, and REC, respectively. At the time indicated by the vertical arrow, a bolus of 2 ml/kg BW saline containing the V_1-inhibitor of AVP $d(CH_2)_5Tyr(Me)AVP$ was injected intravenously in groups III and IV. The height of the ordinate represents the infusion rate of hypotonic glucose-saline solution in millimeters per minute per kilogram BW, which was adjusted according to urinary flow. In addition, a base-line infusion of 30 µl/min per 100 g BW normal saline containing 5% albumin was given. The horizontal axis represents the time scale in minutes.

$$Q_{VR} = V_{blood} \cdot \pi \cdot D^2/4, \quad (1)$$

where Q_{VR} is the mean blood flow in a vas rectum, V_{blood} is the mean blood velocity, and D is the diameter of a vas rectum.

To convert V_{RBC} determined with this technique into V_{blood}, an equation was used that was derived from studies of the relationship between V_{RBC} and V_{blood} employing in vitro perfusion of quartz capillary tubes, which were reported elsewhere (12). In capillary tubes, 12–26 µm in diameter, and for HCT ranging from 10 to 37%, V_{blood} and V_{RBC} are related according to the equation (12):

$$V_{blood} = 0.88\ V_{RBC} - 0.11 \quad \text{for} \quad 0.2 < V_{RBC} < 2 \text{ mm/s}. \quad (2)$$

Total papillary blood inflow was calculated as the product of average value of Q_{DVR} multiplied by the total number of functioning DVR of 906; and total papillary outflow was calculated from Q_{AVR} and number of functioning AVR of 2,038. The number of DVR and AVR were obtained from the total number of VR of 2,944 (7) at the base of the exposed papilla, and the functional ratio of AVR to DVR of 2.25 (8) was obtained at the same location. The volume of fluid reabsorbed in the exposed papilla and removed by VR was taken as the difference between inflow and outflow of blood (7, 8). As carefully delineated by Holliger and his colleagues (7), these calculations incorporate mean values for Q_{DVR} and Q_{AVR}, and number of DVR and AVR in the papilla. Each of these four mean values has confidence limits such that the upper and lower bounds to the calculated water uptake are relatively far apart (7). Thus, the calculated values for fluid uptake are meant to infer only the direction of changes.

Statistics. Data are given as mean±SE. Periods 1–3 were compared using paired determinations or nonpaired determinations as appropriate using t test (17). Statistically significant difference was assumed if the P values were <0.05 (two-tailed t test).

Results

Groups I–IV

General parameters. Mean arterial pressure, systemic HCT, and plasma osmolality (P_{osm}) remained unchanged except for a slight fall (3 mmHg) in pressure in the third period in groups I–IV and a slight decline (5 mosM/kg H_2O) in plasma osmolality in the second period in group II. The BW in groups I–IV was 125±10 g (mean±SEM), 116±9, 124±5, and 135±8, respectively.

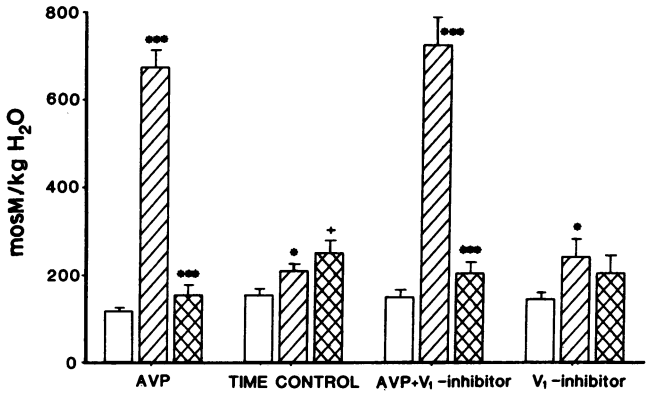

Figure 2. Urinary osmolality. The mean values (±SEM) for osmolality of urine collected during periods 1–3 from the right kidney in groups I–IV are illustrated. A significant difference from the value in the preceding period is indicated by *, $P < 0.05$; **, $P < 0.001$; ***, $P < 0.005$, and a significant difference in values between periods 1 and 3 in the same group is indicated by +, $P < 0.05$, and ++, $P < 0.01$. □, CON; ■, EXP; ■, REC.

Renal function. Figs. 2 and 3 illustrate the data for U_{osm} and urinary flow (V), respectively, obtained during the control (CON) (period 1), experimental (EXP) (period 2), and recovery (REC) (period 3) periods. In period 2 of group I (AVP), U_{osm} increased significantly and UF decreased. Infusion of the pressor antagonist d(CH_2)$_5$Tyr(Me)AVP (group III) did not alter this response (period 2, group III). The changes in U_{osm} and flow in groups II or III were modest by comparison.

The mean urine-to-plasma inulin (U/P_{In}) increased from 9±1 to 60±8 in the experimental period ($P < 0.001$) and returned to 11±1 in the recovery period in group I. Corresponding values in group III were similar: 9±1 to 63±1 ($P < 0.005$) to 13±3, respectively. In contrast, the mean U/P_{In} remained essentially unchanged throughout all three periods in groups II and IV.

In Fig. 4 the clearance of inulin is depicted. No significant changes occurred between periods among any of the groups. The clearance of PAH (C_{PAH}) was determined in most rats in each group—in group I ($n = 7$), group II ($n = 6$), group III ($n = 6$), and group IV ($n = 4$). As shown in Fig. 5, C_{PAH} did not differ significantly between period 1 and 2 in any of the four groups, but showed a tendency to decrease with time, reaching significance in period 3 in group II.

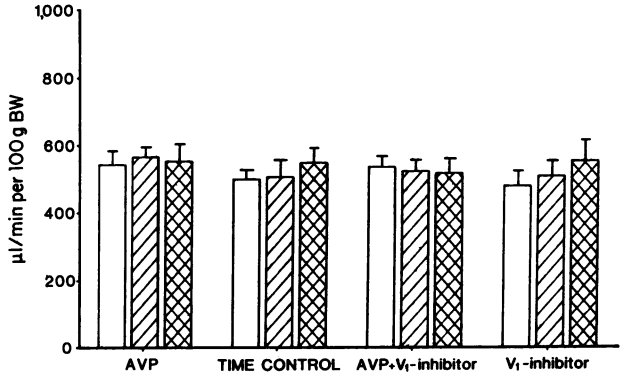

Figure 4. Insulin clearance. C_{In} corrected for 100 g BW in periods 1–3 from the right kidney, determined for groups I–IV, are illustrated. For further details, see the legend to Fig. 2. □, CON; ■, EXP; ■, REC.

The filtration fraction, calculated as the ratio of clearance of inulin (C_{In}) to C_{PAH}, tended to increase with time; however, this was significant only in period 2 of group I (from 0.31±0.03 to 0.32±0.04, $P < 0.001$) and period 3 in group II (from 0.32±0.04 to 0.37±0.03, $P < 0.025$).

Q_{VR}, V_{RBC}, D, and Q_{blood} (Table I and Fig. 6)

Group I (n = 44 VR): AVP-induced antidiuresis. Mean V_{RBC} in DVR and AVR fell significantly from period 1 to 2 after AVP and then rose again during the return from antidiuresis to diuresis. The average VR diameter did not change. Accordingly, as illustrated in Fig. 6 *A*, mean blood flow fell in both DVR and AVR after AVP administration and increased after AVP was discontinued.

Group II (n = 35 VR): time control. Mean V_{RBC} and blood flow increased steadily from period 1 to 2, and from period 2 to 3 in both DVR and AVR. Capillary D did not change.

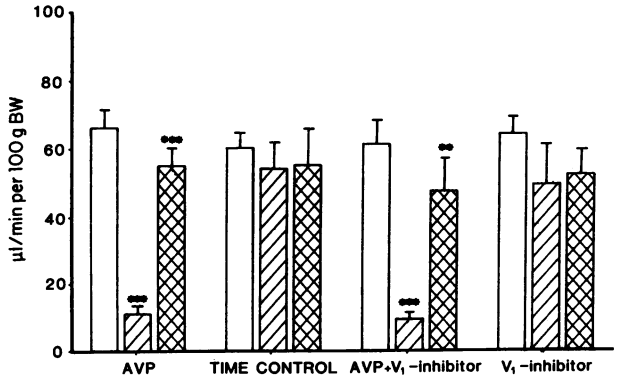

Figure 3. Urine flow. The mean values (±SEM) for urine flow (corrected for 100 g BW) collected during periods 1–3 from the right kidney in groups I–IV are illustrated. For further details, see the legend to Fig. 2. □, CON; ■, EXP; ■, REC.

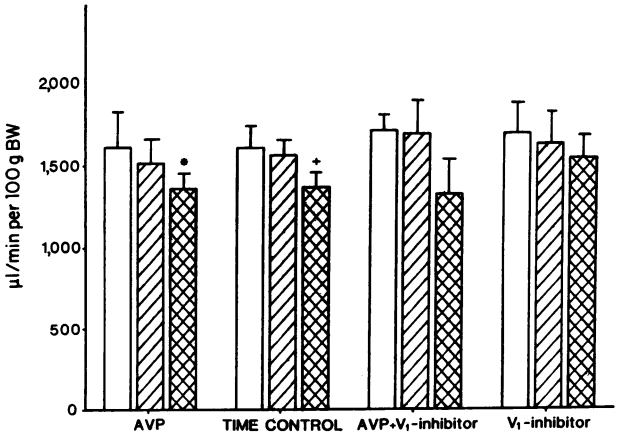

Figure 5. PAH clearance. C_{PAH} corrected for 100 g BW in periods 1–3 from the right kidney for groups I–IV are illustrated. For further details, see the legend to Fig. 2. □, CON; ■, EXP; ■, REC.

Table I. V_{RBC} and VR Diameter in DVR and AVR in Groups I–IV

	V_{RBC} in DVR				V_{RBC} in AVR			
	Group*				Group*			
Period‡	I	II	III	IV	I	II	III	IV
	mm/s	*mm/s*	*mm/s*	*mm/s*	*mm/s*	*mm/s*	*mm/s*	*mm/s*
CON	1.25±0.09	1.37±0.16	1.24±0.19	1.27±0.18	0.62±0.05	0.57±0.05	0.51±0.05	0.54±0.09
P§ EXP → CON	<0.05	<0.01	NS	<0.005	<0.005	NS	NS	NS
EXP	1.13±0.09	1.50±0.15	1.27±0.21	1.51±0.21	0.55±0.05	0.61±0.06	0.56±0.07	0.59±0.08
P§ REC → EXP	<0.025	NS	<0.05	NS	<0.025	NS	0.005	<0.005
REC	1.39±0.12	1.64±0.17	1.50±0.24	1.56±0.21	0.69±0.06	0.68±0.05	0.76±0.10	0.71±0.12
P§ REC → CON	NS	<0.25	<0.01	NS	NS	<0.005	<0.005	<0.005
	Diameter of DVR				Diameter of AVR			
	μm	*μm*	*μm*	*μm*	*μm*	*μm*	*μm*	*μm*
CON	15.1±0.7	15.5±0.5	15.2±0.2	15.4±0.5	19.6±0.5	20.1±0.8	20.8±1.1	20.0±0.7
EXP	15.3±0.5	15.3±0.5	15.2±0.3	15.2±0.5	19.6±0.4	19.8±0.7	20.7±1.1	20.1±0.8
REC	15.2±0.8	15.7±0.7	15.3±0.3	15.1±0.5	19.9±0.5	20.2±0.7	20.9±1.1	20.2±0.8

None of the differences between periods is statistically significant. * Group I, AVP-induced antidiuresis; group II, time control; group III, AVP-induced antidiuresis plus V_1-inhibitor; group IV, V_L-inhibitor alone. ‡ Period 1, CON; period 2, EXP; period 3, REC. § *P*, Significance of difference between (→) periods.

Group III (n = 39 VR): AVP plus inhibitor VPA. V_{RBC} increased slightly but not significantly from period 1 to 2 after administration of AVP and the V_1-inhibitor (VPA) in both DVR and AVR. The return to water diuresis, period 3, was accompanied by a further increase in V_{RBC} in DVR and AVR that was significant. Diameters remained unchanged throughout the experiment. Thus, as shown in Fig. 6 *C*, blood flow changed little in DVR but tended to increase in AVR from period 1 to 2. During the return from antidiuresis (AVP and V_1-inhibitor) to water diuresis (period 3), blood flow increased significantly in both DVR and AVR.

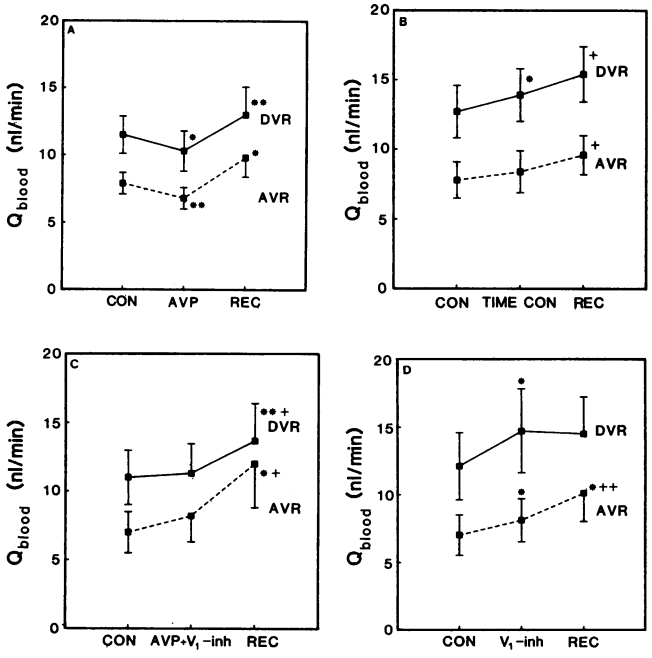

Figure 6. Q_{blood} (= Q_{VR}) in DVR and AVR of the exposed papilla during the three periods in group I (*A*), AVP (0.75 ng/min per kg BW); group II (*B*), time control; group III (*C*), AVP plus V_1-inhibitor; and group IV (*D*); V_1-inhibitor. For further details, see legend to Fig. 2.

Group IV (n = 28 VR): inhibitor VPA alone. During infusion of VPA in period 2, V_{RBC} increased in DVR and AVR (the latter change was not statistically significant). After discontinuation of VPA in period 3, V_{RBC} increased further in DVR (NS) and in AVR ($P < 0.025$). The diameters remained unchanged. Accordingly, as illustrated in Fig. 6 *D*, blood flow increased from period 1 to 2 in DVR and AVR. From period 2 to period 3, it leveled off in DVR, but continued to increase in AVR.

Group V: verification that AVP pressor effect was inhibited. The degree of inhibition of the vasopressor effect of AVP was studied in group V(a). First a bolus of AVP, 10 ng/kg BW, was administered. Mean arterial pressure increased by 27±3 mmHg. The infusion of the same volume of vehicle (normal saline) alone increased the blood pressure by 1.7±0.6 mmHg. Next the vascular inhibitor VPA was infused for 60 min, after which the pressure response to the same amount of AVP was nearly completely abolished, 4.0±1.6 mmHg, which was significantly different from the first AVP infusion ($P < 0.001$). In group V(b), the same protocol was followed, except that a lower dose of AVP, 3 ng/kg BW, was injected. The corresponding changes in blood pressure were 12±2 mmHg after AVP, 0.3±0.3 mmHg after the vehicle, and 0.7±0.7 mmHg after the administration of both AVP and the inhibitor VPA, a significantly lower response than that from AVP alone ($P < 0.001$).

Discussion

Renal function. The effect on urinary flow and osmolality by AVP (group I) within 1 h is striking, compared with changes with time (group II) (Figs. 2 and 3), and occurred without detectable changes in blood pressure, C_{In}, or PAH clearance (Figs. 4, 5, and 7), thus resembling precisely the physiological

transition from water diuresis to antidiuresis. These changes were completely reversible, or at least nearly so. Exactly the same sequence of events occurred after the combined administration of AVP and its V_1 (vascular) inhibitor, VPA (group III), as happened after AVP alone, which indicated that the vascular mediated (V_1-receptor) effects of AVP are not important for its antidiuretic effects. The inhibitor given alone had no effects of its own (group IV), but it effectively blocked the pressor response to a large dose of AVP (group V).

Q_{VR}. After AVP, Q_{VR} fell significantly (Figs. 6 and 7), and then increased after AVP was discontinued (group I). The fall in blood flow between period 1 and 2 was abolished if the vascular inhibitor (VPA) was administered concomitantly with AVP (group III). That this was the result of inhibition of the AVP effect and not caused by the inhibitor alone is shown in group IV (VPA given alone in period 2), in which the blood flow rose with time exactly as it did in the time control rats (group II).

The results clearly demonstrate that during antidiuresis induced by AVP, papillary blood flow is diminished. Moreover, the study demonstrates for the first time that AVP induces a fall in both papillary blood inflow and outflow (see below). Thurau and co-workers (4), who measured medullary blood inflow indirectly by a dye dilution curve, observed that it fell after AVP. A decrease in medullary flow has also been reported by several investigators using indirect techniques (18–21). Conversely, either no change or an increase in medullary flow after AVP was found by Aukland (22), Persson et al. (23), Kurtzman et al. (24), Johnson et al. (25), and by our own laboratory, Gussis et al. (9). Persson et al. (23) observed an increased passage time of radiolabeled erythrocytes (i.e., a decrease in erythrocyte velocity) that was qualitatively similar to the findings by Thurau et al. (4) and in the present study. They (23) calculated, however, that erythrocyte flow during antidiuresis was unchanged as a consequence of higher erythrocyte concentrations in the tissue, determined concomitantly. Each of the foregoing investigations is open to criticism. Aukland (22) used the H_2-clearance, a technique that is affected by urine flow and therefore not accurate enough to estimate medullary blood flow. Persson et al. (23) could not convert their values for erythrocyte flow to absolute blood flow. Johnson et al. (25) employed the distribution of microspheres within the renal cortex as an index of medullary blood flow. It is generally agreed that this method does not reliably indicate medullary blood flow (26). Kurtzman et al. (24) used pharmacological doses of AVP, such that a vasoconstrictive effect would be expected. Compared with our initial study of the effect of AVP on papillary blood flow (9), several substantive improvements in the videomicroscopy technique were introduced in the present study, as described recently by Holliger and co-workers (7). These methodological improvements enabled us to detect real differences between V_{RBC} in AVR and DVR (7, 8), whereas in our first study no difference in V_{RBC} between AVR and DVR was demonstrable. Thus it is not surprising that an effect of AVP not demonstrable in our initial study is now clearly detectable in the present experiments.

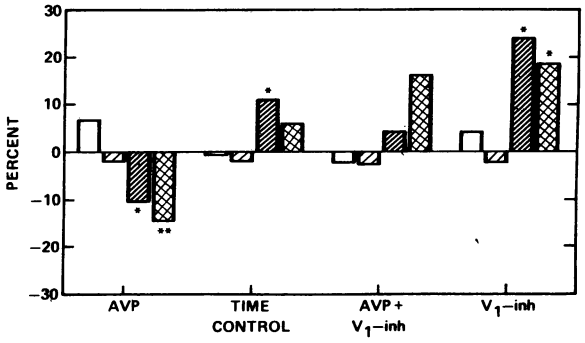

Figure 7. Relative changes (%) in VR blood flow (EXP vs. CON) between periods 1 and 2 for C_{In}, C_{PAH}, Q_{DVR}, and Q_{AVR} in groups I (AVP), II (time control), III (AVP + V_1-inhibitor), and IV (time control with V_1-inhibitor [V_1-inh] infusion). For further details, see the legend to Fig. 2. □, C_{In}; □, C_{PAH}; ■, Q_{DVR}; ■, Q_{AVR}.

The concentration of AVP in plasma was not determined in this study, but from the amount infused it may be estimated by assuming a biological half-life of ~2.5 min in rats and a volume of distribution equal to the extracellular space (27) to be 11 pg/ml. Gellai et al. (28) measured a plasma concentration of AVP of 8 pg/ml with a slightly higher infusion rate of AVP of 100 pg/min per kg BW in Brattleboro rats. Both estimates are well within the range of AVP values found under physiological conditions (28, 29).

Medullary blood flow increased with time in the control experiments (groups II and IV). This is in agreement with earlier observations that severing the ureter is associated with an increase in medullary plasma flow (9, 30). The reason for this increase is not clear; a suggestion that release of prostaglandins might play a role (30) has been rejected by another group as an explanation (31).

The reduction of papillary blood flow found in the present study is qualitatively similar to previous observations (4, 21), but proportionally much less (Fig. 7). From the study of Thurau et al. (4) it can be calculated (32) that the twofold increase in transit time in antidiuresis corresponds to a decrease in papillary plasma flow of ~50%. It should be noted, however, that the infusion rate of vasopressin was higher in their study than in the present one. Bayle et al. (21) also reported a reduction in papillary plasma flow of almost 50%. However, in their study the comparison between antidiuresis and water diuresis was made between different strains of rats. In addition, they used the accumulation of albumin as an index of papillary plasma inflow (33), a technique of questionable accuracy (5, 33).

Mechanism of the effect of AVP on Q_{VR}. The decrease in papillary flow induced by AVP could be the consequence of a direct vasoconstrictive effect of vasopressin mediated by V_1-receptors, or the indirect result of the antidiuretic action (V_2-receptor mediated) of AVP. During AVP-induced antidiuresis, reabsorption of water is markedly increased in the collecting ducts in the renal cortex, sharply diminishing flow entering the collecting duct in the medulla. Consequently, a smaller volume of fluid is reabsorbed from the inner medullary collecting ducts in antidiuresis than in water diuresis, despite the presence of AVP (34). There are two major consequences for the renal medulla which could indirectly lower Q_{VR}. First, there is less fluid to be removed and therefore less uptake of water by the AVR, which would reduce AVR. Fluid uptake by VR, calculated as the difference between papillary blood inflow and outflow (ΔQ), is summarized in Table II (keep in mind the cautionary note about these calculations stated in

Table II. Estimate of Total Papillary Blood Inflow, Blood Outflow, and Difference in Groups I–IV

Period‡		Group* I	II	III	IV
		μl/min	*μl/min*	*μl/min*	*μl/min*
CON	IN	10.4	11.5	10.0	11.0
	OUT	16.1	15.9	14.3	14.3
	ΔQ	5.7	4.4	4.3	3.3
EXP	IN	9.3	12.6	10.2	13.3
	OUT	13.9	17.1	16.7	16.5
	ΔQ	4.6	4.5	6.5	3.2
REC	IN	11.8	14.0	12.4	13.1
	OUT	20.0	19.6	24.5	20.6
	ΔQ	8.2	5.6	12.1	7.5

IN, total papillary blood inflow; OUT, total papillary blood outflow; ΔQ, difference.

* Group I, AVP-induced antidiuresis; group II, time control; group III, AVP-induced antidiuresis plus V_1-inhibitor; group IV, V_1-inhibitor alone.

‡ Period 1, CON; period 2, EXP; period 3, REC. The calculation of papillary blood inflow and outflow is described in the last part of Methods. The difference, ΔQ, equals OUT minus IN and is equivalent to water uptake by the capillaries.

Methods). Uptake was lower by 1.2 μl/min during AVP infusion in group I—5.7 μl/min during control (period 1), 4.5 μl/min during antidiuresis. In group II, water uptake remained practically unchanged with time—4.4 μl/min in period 1 and 4.5 μl/min in period 2.

The second consequence that might reduce Q_{VR} is that the medullary interstitial concentration of NaCl and urea will likely increase. We have previously found that net fluid removal—not uptake—occurs in DVR, and suggested at the time that the removal is due to a net outward osmotic driving force created by the transcapillary difference in concentration of nonprotein solutes, NaCl and urea (35). A rise in the medullary interstitial concentration of these solutes is likely to enhance the gradient for water removal from DVR, and thus reduce blood flow.

With the changes in groups I and II as a reference, the findings in group III are critical. The infusion of VPA along with AVP abolished the fall in DVR blood flow (Fig. 6 *C*), although the fall in UF and rise in U_{osm} (and, by inference, the rise in medullary interstitial osmolality) were exactly the same in these animals as in those given AVP alone. If the fall in DVR blood flow were solely an indirect effect of less water reabsorbed from the collecting duct, DVR blood flow should have fallen to the same extent in group III as in group I, but it did not. Note in Table II that infusion of VPA with AVP abolished the fall in water uptake by AVR (ΔQ) in period 2 (4.3 μl/min during control, 6.5 μl/min during antidiuresis), raising the possibility that fluid uptake in the AVR may be plasma-flow dependent.

The foregoing results suggest strongly that the decrease in papillary blood flow induced by AVP is caused, at least in part, by a direct vasoconstrictor (V_1) action of AVP on the renal microvasculature rather than by an indirect antidiuretic effect on the renal tubule. The findings do not completely exclude an indirect effect, however, because the V_1 inhibitor given alone (group IV) caused a rise in DVR blood flow (Fig. 6 *D*) that seemed to be slightly greater than that which occurred with time alone (Fig. 6 *B*), so it might be argued that VPA had an unexpected direct vasodilator effect to increase DVR blood flow that obscured the indirect effect of AVP to decrease DVR blood flow. Furthermore, the difference in water uptake between period 1 and period 2 in group IV (inhibitor alone) (3.3 μl/min in period 1 and 3.2 μl/min in period 2) was smaller than in group III (inhibitor plus AVP). Finally, these vasoactive effects of AVP were demonstrable in rats during a change from water diuresis to moderate rather than maximal antidiuresis. It is possible that the indirect antidiuretic effect of AVP to lower Q_{VR} predominates over the direct effect of AVP in states of greater antidiuresis. Nonetheless, these studies strongly suggest a vasoconstrictive effect of AVP in physiological amounts on the medullary microcirculation.

Site of vasoconstrictive effect of AVP. Vasoconstriction of renal vessels can occur at different sites within the kidney. First, it can occur in the renal artery, thus decreasing total renal blood flow. That the decrease in medullary blood flow was not a consequence of a decreased renal plasma flow is shown by the lack of fall of C_{PAH}, or glomerular filtration rate, in period 2 (Figs. 4 and 5). Changes in renal plasma flow were similar in all four groups. These findings are in accordance with earlier observations (28). Second, vasoconstriction can occur by action of AVP directly on VR. We found no significant difference in the diameters of papillary VR, however, which confirms earlier unpublished experiments by Kramer and associates (cited in reference 36) and is consistent with the morphology of the endothelia of DVR in the renal papilla, which lack smooth muscle cells, and of AVR, which are thin and identical to those of systemic capillaries (37).

The exact site of AVP action is not clear. Afferent and efferent arterioles in the renal cortex clearly are sites of vasoconstriction. Furthermore, the initial segments of DVR in the outer stripe of the outer medulla have cells resembling smooth muscle in their walls (37). Since total plasma flow remained unchanged, it is unlikely that AVP caused vasoconstriction of all arterioles (afferent or efferent). It is possible, however, that juxtamedullary arterioles are more sensitive to AVP than are superficial arterioles. Both afferent and efferent arterioles of juxtamedullary glomeruli differ in appearance from those of superficial glomeruli. They are larger in diameter and packed with more smooth muscle cells (37). It seems possible therefore that vasopressin might cause preferential vasoconstriction of juxtamedullary arterioles, although direct evidence is lacking. Alternatively, AVP might cause constriction of the proximal segments of DVR. A preferential action of AVP at sites distal to the juxtamedullary glomerulus—efferent arterioles or proximal VR—was suggested by Davis and Schnermann (20), who reported that single nephron glomerular filtration rate (SNGFR) increases during antidiuresis, which suggested an increase in efferent arteriolar resistance and effective filtration pressure. However, we could not detect a significant change in juxtamedullary SNGFR (38), nor could Trinh-Trang-Tan (39), although in the latter study a mean increase of 25% was not significant because of scatter. These apparent differences in experimental findings can be reconciled

in the following way. The increase in juxtamedullary glomerular transcapillary hydraulic pressure caused by AVP-induced vasoconstriction of the efferent arteriole or proximal DVR, which would increase juxtamedullary SNGFR, could have been offset by the AVP-induced decrease in the ultrafiltration coefficient (mediated by the V_2-receptor) (40), which would decrease juxtamedullary SNGFR.

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