Modulation of the Hydro-osmotic Effect of Vasopressin on the Rabbit Cortical Collecting Tubule by Adrenergic Agents

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ABSTRACT The effects of catecholamines on antidiuretic hormone ([Arg^8]-vasopressin [AVP])-induced water absorption were evaluated in cortical collecting tubules isolated from the rabbit kidney and perfused in vitro. In the presence of AVP (100 μU/ml), net fluid volume absorption (J_v, nanoliters per minute per millimeter) was 1.14±0.12 and osmotic water permeability coefficient (P_f, $\times 10^{-4}$ centimeters per second) was 217.3±39.9. The addition of the alpha-adrenergic agonist, phenylephrine (PE), in a concentration of 10^{-6} M resulted in a significant decrease in J_v and P_f to 0.83±0.13 ($P < 0.001$) and 148.8±41.8 ($P < 0.02$), respectively. Increasing the concentration of PE to 10^{-5} M resulted in a further decrease in J_v and P_f to 0.53±0.05 ($P < 0.05$ vs. PE 10^{-6} M) and 88.5±9.0 ($P < 0.05$ vs. PE 10^{-6} M), respectively. In a separate group of tubules, in the presence of AVP (100 μU/ml) and PE (10^{-5} M), J_v and P_f were 0.35±0.07 and 66.0±17.3, respectively. The addition of the alpha-adrenergic antagonist, phentolamine (PH), in a concentration of 10^{-6} M resulted in a significant increase in J_v to 1.07±0.19 ($P < 0.001$) and P_f to 193.3±35.9 ($P < 0.005$). PH (10^{-5} M) alone did not significantly affect J_v and P_f in the presence of AVP (100 μU/ml) nor in the presence of 8-bromo adenosine 3',5' cyclic monophosphate (8-BrcAMP). J_v and P_f were 1.20±0.21 and 174.0±25.8, respectively, in the presence of 8-BrcAMP (10^{-4} M).

We next examined the effect of the beta-adrenergic agonist, isoproterenol (ISO), on J_v and P_f in the presence of AVP. J_v and P_f were 1.04±0.10 and 202.6±17.2, respectively, in the presence of AVP (100 μU/ml) and 1.06±0.18 and 193.4±27.7, respectively, in the presence of AVP (10 μU/ml). However, in the presence of AVP in a concentration of 2.5 μU/ml, J_v was 0.60±0.07 and P_f was 100.7±24.7. ISO (10^{-6} and 10^{-5} M) did not have any significant effect in the presence of the above maximal and submaximal concentrations of AVP. In the absence of AVP, control J_v was 0.01±0.12 and P_f was 4.6±11.0. The addition of ISO at 25 or 37°C did not result in any significant change in J_v or P_f.

These studies indicate that alpha-adrenergic agonists directly inhibit AVP-mediated water absorption at the level of the tubule, an effect that can be blocked by a specific alpha-adrenergic antagonist. This effect appears to be exerted at the level of activation of adenylate cyclase since it is absent in the presence of cAMP. The beta-adrenergic agonists do not directly inhibit or enhance AVP-mediated water absorption at the level of the renal tubule.

INTRODUCTION

There is now considerable evidence to suggest that the adrenergic system is involved in the control of renal water excretion (1–4). A number of studies have shown that the intravenous infusion of adrenal medullary hormones in normal hydropenic man increases solute-free water excretion (C_{H_2O}) in the absence of changes in glomerular filtration rate, renal plasma flow, and osmolar clearance (2, 3). The exact mechanism by which catecholamines modulate water excretion, however, is unclear. Studies both in vivo and in vitro have suggested that alpha-adrenergic stimulation may antagonize the action of vasopressin at the cellular level (4–7). There is also evidence to suggest that beta-ad-

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renergic stimulation enhances vasopressin-induced water movement in both the toad bladder and the distal nephron (6, 8, 9). Alternatively, other studies have suggested that both alpha- and beta-adrenergic stimulation result in changes in water excretion by modulating the rate of release of endogenous vasopressin (10–12). The present studies were performed, therefore, to directly examine the role of catecholamines in water absorption in isolated cortical collecting tubules of the rabbit perfused in vitro.

METHODS

Segments of cortical collecting tubules (CCT)[1] were isolated and perfused in vitro as described by Burg et al. (13) with few modifications (14). Briefly, experiments were performed on female New Zealand white rabbits weighing 1.5–2.5 kg that had been maintained on tap water and rabbit chow until the time of study. Animals were sacrificed by decapitation and the right kidney was immediately removed, decapsulated, and sliced into transverse sections, 1–2 mm thick. The slices were transferred to a dish containing chilled dissecting solution of the following composition (in millimolars): NaCl, 140; K_2HPO_4, 2.5; $MgSO_4$, 1.2; L-alanine, 6.0; sodium citrate, 1.0; sodium lactate, 4.0; $CaCl_2$, 2.0; and glucose, 5.5. The pH was 7.4 and the osmolality was 290 mosmol/kg H_2O. 5% (vol/vol) of 5 gm/dl defatted bovine albumin (Calbiochem-Behring Corp, San Diego, CA) solution was added to prevent adhesion of tubules to the dish. Cortical collecting tubules (0.8–2.5 mm long) were teased from the slices with fine dissecting forceps. Only the ends of the tubule segments were touched, and these were subsequently trimmed.

The tubules were transferred to a Lucite perfusion chamber mounted on the mechanical stage of an inverted microscope. The chamber contained a bathing solution identical to the dissecting solution. One end of the tubule was aspirated into a constriction pipette and an inner, concentric perfusion pipette containing the perfusion solution was advanced into the lumen of the tubule. Perfusion was initiated by a gravity flow system at a rate of 5–15 $nl \cdot min^{-1}$. The luminal perfusion solution contained (in millimolars): NaCl, 60; K_2HPO_4, 2.5; $MgSO_4$, 1.2; and $CaCl_2$, 2.0. The pH was 7.4 and the osmolality was 125 mosmol/kg H_2O. Exhaustively dialyzed [methoxy-^{3}H]inulin (New England Nuclear, Boston, MA) was added to the perfusate as a volume marker. The other end of the tubule was aspirated into a collecting pipette coated with Sylgard 184 silicone elastomer (Dow Corning Corp., Midland, MI). Mineral oil was layered over the collected fluid to prevent evaporation. The tubules were inspected visually and length and diameter were determined using an eyepiece micrometer. Tubules were discarded if any breaks or denuded areas were visible along the perfused length. Tubules were also discarded whenever there was leakage of [^{3}H]inulin into the bath in excess of 2% of the total perfused counts. The bathing solution was then replaced by another solution of the following composition (in millimolars): NaCl, 115; $NaHCO_3$, 25; K_2HPO_4, 2.5; $MgSO_4$, 1.2; L-alanine, 6.0; sodium citrate, 1.0; sodium lactate, 4.0; $CaCl_2$, 2.0; and glucose, 5.5. 5% (vol/vol) of 5 gm/dl defatted bovine albumin solution was added to the bathing solution. The pH of the bathing solution was 7.4 and the osmolality was 290 mosmol/kg H_2O. The pH was maintained at 7.4 by continuously bubbling the bathing solution with 95% O_2/5% CO_2. The bathing solution was continuously changed at a rate of 0.5 ml/min using a Holter pump (Extracorporeal Medical Specialties, King of Prussia, PA). Unless otherwise specified, the bath temperature was maintained at 25°C.

Perfusion of the tubules was initiated within 30 min from the time of decapitation in most of the experiments. An initial equilibration period of 120–150 min was allowed to elapse to ensure the disappearance of the effects of endogenous vasopressin. Between the experimental periods, a stabilization period of 45–60 min was allowed. Timed samples were collected under oil into constant-volume pipettes that had been advanced into the collecting pipettes. Each sample was placed in 5 ml of Bioflour (New England Nuclear, Boston, MA) and radioactivity was measured in a Tri-Carb liquid scintillation counter (Packard Instrument Co. Inc., Downers Grove, IL).

(Arg^8)-vasopressin (AVP) acetate was obtained from Calbiochem-Behring Corp.; isoproterenol (ISO) HCl from Sterling-Winthrop Research Institute, Rensselaer, NY; L-phenylephrine HCl from Sigma Chemical Co., St. Louis, MO; phentolamine HCl from Ciba Pharmaceutical Co., Summit, NJ; and 8-bromo adenosine 3′,5′-cyclic monophosphate (8-BrcAMP) from Sigma Chemical Co. All solutions were prepared daily and added to the bathing solution only in the desired concentration just prior to the commencement of the experiment.

The following groups of experiments were performed.

Group I. In the first group of studies, the effect of the alpha-adrenergic agonist, L-phenylephrine (PE), on AVP-mediated water absorption was examined. Following the initial equilibration period, AVP was added to the bathing solution in a concentration of 100 μU/ml. After a 45–60-min stabilization period, three timed samples of collected fluid were obtained. PE (10^{-6} M) was then added to the bathing solution containing AVP and three timed samples of collected fluid were obtained. The effect of PE (10^{-5} M) on AVP-induced water absorption was studied in a third experimental period in a similar fashion. When PE (10^{-4} M) was added to the bathing solution, irreversible cellular damage occurred.

Group II. The effect of the alpha-adrenergic antagonist, phentolamine (PH), on PE-induced changes in water absorption was examined. After obtaining timed samples of collected fluid in the presence of AVP (100 μU/ml), PE (10^{-5} M) was added to the bathing solution containing AVP and samples of collected fluid were obtained. PH was then added to the bathing solution in concentrations of 10^{-6} M and 10^{-5} M and timed samples of collected fluid were obtained.

Group III. In this group of experiments, the effect of PH alone on AVP-induced water absorption was examined. After obtaining collected fluid samples in the presence of AVP (100 μU/ml), PH (10^{-5} M) was added to the bathing solution and collected fluid samples were again obtained.

Group IV. In this group of studies, the effect of PE on cyclic AMP (cAMP)-mediated water absorption was examined. Following the initial equilibration period, 8-BrcAMP was added to the bathing solution in a concentration of 10^{-4} M and samples of collected fluid were obtained following the stabilization period. PE (10^{-5} M) was then added to the bathing solution and a second set of collected fluid samples was obtained.

[1] *Abbreviations used in this paper:* AVP, (Arg^8)-vasopressin; 8-BrcAMP, 8-bromo adenosine 3′,5′ cyclic monophosphate; cAMP, cyclic AMP; CCT, cortical collecting tubule; ISO, isoproterenol; NE, norepinephrine, PE, L-phenylephrine; PH, phentolamine.

Group V. We next examined the effect of the beta-adrenergic agonist, ISO, on AVP-mediated water absorption. First, samples of collected fluid were obtained in the presence of AVP (100 μU/ml) in the bathing solution. Then, ISO in a concentration of 10^{-6} M was added to the bathing solution containing AVP. Following the stabilization period, collected fluid samples were obtained. The effect of ISO in a concentration of 10^{-5} M was studied in a third experimental period in a similar fashion. When ISO was added to the bathing solution in a concentration of 10^{-4} M, irreversible cellular damage occurred. To study the effect of ISO on water absorption when lower concentrations of AVP were present, the above protocol was repeated with AVP in the bathing solution at concentrations of 10 and 2.5 μU/ml.

Group VI. The effect of the beta-adrenergic agonist ISO on water absorption in the absence of vasopressin was studied at both 25 and 37°C. After the initial equilibration period at 25°C, timed samples of collected fluid were obtained. Following these collections, ISO was added to the bathing solution in a concentration of 10^{-5} M and collected fluid samples were obtained after the stabilization period. Then, the temperature of the bathing solution was raised to 37°C and collected fluid samples were obtained in a similar fashion.

Calculations. Net fluid absorption (J_v) was calculated using the formula: J_v (nl/min/mm) = $(V_i - V_o)/L$, where V_i is the perfusion rate in nanoliters per minute, V_o is the collection rate in nanoliters per minute, and L is the length of the tubule in millimeters.

V_i was calculated from the formula: $V_i = V_o \cdot [^3H_o]/[^3H_i]$, where $[^3H_o]$ and $[^3H_i]$ are the inulin counts per nanoliter in the collected and perfused fluids, respectively.

Coefficient of hydraulic conductivity, L_p ($cm \cdot s^{-1} \cdot atm^{-1}$) was calculated from $L_p = V_r/\sigma \cdot A \cdot \Delta\pi$, where V_r is the reabsorbed fluid volume per second (obtained from the difference between the perfusion and collection rates), σ is the reflection coefficient of the solutes determining the osmotic pressure gradient (assumed to be 1.0), A is the tubule luminal area (from the measured length and an arbitrarily assumed diameter of 20 μm), and $\Delta\pi$ is the osmotic pressure difference calculated by the following formula (15): $\Delta\pi = \pi_b - \exp[(\ln\pi_i + \ln\pi_f)/2]$, where π_b is calculated from the measured bath osmolality, π_i from the measured osmolality of the initial perfusion fluid, and π_f from the collected fluid osmolality. Collected fluid osmolality was calculated from the measured perfusate osmolality and the relative increase in the concentration of the volume marker measured in the collected fluid.

The osmotic water permeability coefficient, P_f ($cm \cdot s^{-1}$) was calculated from: $P_f = \sigma \cdot L_p \cdot RT/\bar{V}_w$, where R is the gas constant, T is the absolute temperature, and $\bar{V}_w$ is the partial molar volume of water.

All values represent the mean of two or more collections for each experimental period. The data are given as means±SE. Statistical analysis was performed using the Student's t test for paired data (two experimental periods) or by analysis of variance (more than two experimental periods in the same tubule).

RESULTS

Group I. The effect of the alpha-adrenergic agonist, PE, on AVP-mediated water absorption (J_v) and osmotic water permeability coefficient (P_f) is shown in Table I and depicted in Fig. 1. In this group of tubules (n = 7), when AVP was present in the bathing solution in a concentration of 100 μU/ml, J_v averaged 1.14±0.12 $nl \cdot min^{-1} \cdot mm^{-1}$, and P_f averaged 217.3±39.9 × 10^{-4} $cm \cdot s^{-1}$. The addition of PE (10^{-6} M) resulted in a significant decrease of J_v and P_f to 0.83±0.13 $nl \cdot min^{-1} \cdot mm^{-1}$ ($P < 0.001$ vs. AVP alone) and 148.8±41.8

TABLE I
Effect of PE on AVP-mediated J_v and P_f in the CCT of Rabbit

	AVP 100 μU/ml			AVP 100 μU/ml + PE 10^{-6} M			AVP 100 μU/ml + PE 10^{-5} M		
	J_v	P_f	V_i	J_v	P_f	V_i	J_v	P_f	V_i
	0.73	118.3	11.2	0.41	65.5	11.2	0.31	49.2	11.5
	0.98	254.2	6.7	0.74	121.5	7.9	0.54	116.2	8.9
	1.63	434.4	10.8	1.47	393.4	9.5	0.45	114.7	8.8
	1.12	140.8	11.5	0.77	100.5	6.5	0.69	86.1	8.7
	1.41	195.3	8.5	1.05	129.3	7.1	0.72	86.5	7.0
	0.93	205.6	7.6	0.63	135.4	8.7	0.45	96.9	8.1
	1.21	172.5	9.5	0.76	95.8	10.0	0.56	70.2	9.8
Mean	1.14	217.3	9.4	0.83‡	148.8‖	8.7*	0.53‡§	88.5‡§	9.0*
±SE	0.12	39.9	0.7	0.13	41.8	0.6	0.05	9.0	0.5

Tubules were studied at 25°C with a 165 mosmol/kg H_2O transepithelial osmotic gradient. J_v is fluid volume reabsorption in nanoliters per minute per millimeter. P_f is the osmotic water permeability coefficient in × 10^{-4} centimeters per second. V_i is the perfusion rate in nanoliters per minute. Mean length of the tubules is 1.7 mm.

* P = not significant vs. AVP alone.

‡ $P < 0.001$ vs. AVP alone.

§ $P < 0.05$ vs. AVP + PE 10^{-6} M.

‖ $P < 0.02$ vs. AVP alone.

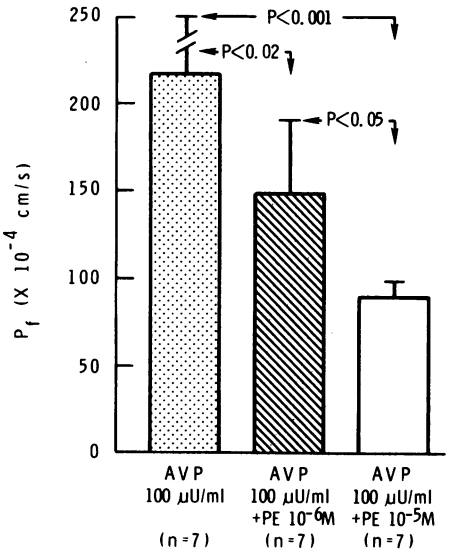

FIGURE 1 Effect of PE on AVP-mediated water absorption in the CCT of rabbit. P_f is the osmotic water permeability coefficient. n Is the number of tubules studied. Tubules were studied at 25°C with a 165-mosmol/kg H_2O transepithelial osmotic gradient. All values are expressed as means±SE.

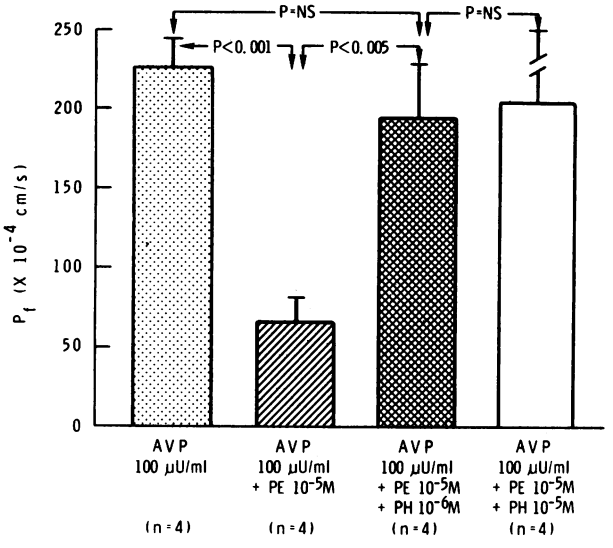

FIGURE 2 Effect of PE and PH on water absorption in the CCT of rabbit. P_f is the osmotic water permeability coefficient. n Is the number of tubules. Mean length of the tubules is 1.2 mm. There is no difference in the perfusion rate between the different experimental periods. Tubules were studied at 25°C with a 165 mosmol/kg H_2O transepithelial osmotic gradient. All values are expressed as means±SE.

$\times 10^{-4}$ cm·s^{-1} ($P < 0.02$ vs. AVP alone), respectively. Increasing the concentration of PE to 10^{-5} M resulted in a further decrease in J_v and P_f to average values of 0.53±0.05 nl·min^{-1}·mm^{-1} ($P < 0.05$ vs. PE 10^{-6} M) and 88.5±9.0 $\times 10^{-4}$ cm·s^{-1} ($P < 0.05$ vs. PE 10^{-6} M), respectively.

Group II. To determine whether the effect of PE was specific, the effect of the alpha-adrenergic antagonist, PH, on water absorption was examined (Fig. 2) in this group of tubules (n = 4). Again, AVP (100 μU/ml) alone in the bathing solution resulted in a J_v of 1.16±0.07 nl·min^{-1}·mm^{-1} and a P_f of 225.8±19.6 $\times 10^{-4}$ cm·s^{-1}. The addition of PE (10^{-5} M) resulted in a significant decrease in J_v and P_f to 0.35±0.07 nl·min^{-1}·mm^{-1} ($P < 0.001$ vs. AVP alone) and 66.0±17.3 $\times 10^{-4}$ cm·s^{-1} ($P < 0.001$ vs. AVP alone), respectively. The addition of PH (10^{-6} M) to the bathing solution containing AVP and PE resulted in a significant increase in J_v to 1.07±0.19 nl·min^{-1}·mm^{-1} ($P < 0.001$ vs. AVP + PE, P = NS vs. AVP alone) and P_f increased to 193.3±35.9 $\times 10^{-4}$ cm·s^{-1} ($P < 0.005$ vs. AVP + PE, P = NS vs. AVP alone). When the concentration of PH was increased to 10^{-5} M, J_v was 1.17±0.18 nl·min^{-1}·mm^{-1} (P = NS vs. PH 10^{-6} M) and P_f was 223.8±43.5 $\times 10^{-4}$ cm·s^{-1} (P = NS vs. PH 10^{-6} M).

Group III. In this group of tubules (n = 3), shown in Table II, in the presence of AVP (100 μU/ml), J_v and P_f were 1.38±0.03 nl·min^{-1}·mm^{-1} and 208.3±34 $\times 10^{-4}$ cm·s^{-1}, respectively. The addition of PH (10^{-5} M) in the presence of AVP did not result in any significant change in J_v and P_f, which were 1.31±0.09 nl·min^{-1}·mm^{-1} and 199.7±42.9 $\times 10^{-4}$ cm·s^{-1}, respectively.

Group IV. In this group of tubules (n = 3), 8-BrcAMP (10^{-4} M) in the bathing solution resulted in a J_v of 1.20±0.21 nl·min^{-1}·mm^{-1} and P_f of 174.0±25.8 $\times 10^{-4}$ cm·s^{-1}. The addition of PE (10^{-5} M) to the bathing solution containing 8-BrcAMP resulted in a

TABLE II
Effect of PH Alone on AVP-mediated J_v and P_f in the CCT of Rabbit

	AVP 100 μU/ml			AVP 100 μU/ml + PH 10^{-6} M		
	J_v	P_f	V_t	J_v	P_f	V_t
	1.34	151.1	9.8	1.23	132.4	10.1
	1.36	205.1	9.3	1.22	187.4	8.0
	1.45	268.6	7.5	1.48	279.3	7.5
Mean	1.38	208.3	8.9	1.31*	199.7*	8.5*
±SE	0.03	34.0	0.7	0.09	42.9	0.8

Tubules were studied at 25°C with a 165-mosmol/kg H_2O transepithelial osmotic gradient. J_v is fluid volume reabsorption in nanoliters per minute per millimeter. P_f is the osmotic water permeability coefficient in $\times 10^{-4}$ centimeters per second. V_t is the perfusion rate in nanoliters per minute. Mean length of the tubules is 1.8 mm.

* P = not significant.

TABLE III

Effect of PE on 8-BrcAMP-mediated J_v and P_f in the CCT of Rabbit

	8-BrcAMP 10^{-4} M			8-BrcAMP 10^{-4} M + PE 10^{-5} M		
	J_v	P_f	V_t	J_v	P_f	V_t
	0.97	133.8	13.8	0.94	126.0	11.9
	1.25	166.1	9.6	1.23	158.0	11.0
	1.38	222.0	9.1	1.46	231.6	10.3
Mean	1.20	174.0	10.8	1.21*	171.9*	11.1*
±SE	0.21	25.8	1.5	0.26	31.3	0.5

Tubules were studied at 25°C with a 165-mosmol/kg H_2O transepithelial osmotic gradient. J_v is fluid volume reabsorption in nanoliters per minute per millimeter. P_f is the osmotic water permeability coefficient in $\times 10^{-4}$ centimeters per second. V_t is the perfusion rate in nanoliters per minute. Mean length of the tubules is 2.0 mm.

* P = not significant.

J_v of 1.21±0.26 nl · min^{-1} · mm^{-1} and a P_f of 171.9±31.3 $\times 10^{-4}$ cm · s^{-1} (Table III). Neither of these values was significantly different from those obtained with 8-BrcAMP alone.

Group V. The results of studies examining the effect of the beta-adrenergic agonist, ISO, on water absorption in the CCT are given in Table IV. When AVP was present in the bathing solution in a concentration of 100 μU/ml, J_v averaged 1.04±0.1 nl · min^{-1} · mm^{-1} and P_f was 202.6±17.2 $\times 10^{-4}$ cm · s^{-1}. The addition of ISO in concentrations of 10^{-6} and 10^{-5} M resulted in no significant change in either J_v or P_f. Similarly, when AVP was present in the bathing solution in the concentration of 10 μU/ml, J_v averaged 1.06±0.18 nl · min^{-1} · mm^{-1} and P_f 193.4±27.7 $\times 10^{-4}$ $\times$ cm · s^{-1}. Again, addition of ISO did not have any significant effect on J_v or P_f. However, in the presence of AVP (2.5 μU/ml), J_v was 0.60±0.07 nl · min^{-1} · mm^{-1} and P_f was 100.7±24.7 $\times 10^{-4}$ cm · s^{-1}. Even in the presence of this submaximal concentration of AVP, ISO did not have any significant effect on J_v or P_f.

Group VI. The effect of ISO alone on J_v and P_f was examined (Table V). Control J_v averaged 0.01±0.12 nl · min^{-1} · mm^{-1} whereas P_f was 4.6±11.0 $\times 10^{-4}$ cm · s^{-1}. The addition of ISO (10^{-5} M) at 25°C resulted in a J_v of −0.08±0.05 nl · min^{-1} · mm^{-1} and P_f of −9.3±4.5 $\times 10^{-4}$ $\times$ cm · s^{-1}. Neither value is significantly different from the control values. Warming the bathing solution to 37°C in the presence of ISO (10^{-5} M) had no significant effect on J_v or P_f.

DISCUSSION

A number of nonosmolar factors have been shown to affect the renal handling of water (16). Investigations both in man (2, 3, 17) and in experimental animals (4, 18, 19) have shown that the infusion of catecholamines profoundly alters the renal water excretion. The intravenous infusion of norepinephrine (NE) has been known to be associated with solute-free water diuresis in the absence of changes in the glomerular filtration rate, the renal plasma flow, or the osmolar clearance (2, 3, 17). This effect has been ascribed to alpha-adrenergic stimulation, since specific alpha-adrenergic blockers have been shown to prevent the NE-induced diuresis (4). On the other hand, the infusion of the beta-adrenergic agonist, ISO, has been shown to be associated with a consistent antidiuresis (11, 18, 19). This antidiuretic effect with intravenously administered ISO is abolished by beta- but not alpha-adrenergic antagonists (20). Considerable controversy exists as to the mechanism by which the adrenergic agonists exert these effects (1). The catecholamines could, through changes in blood pressure and renal perfusion pressure, alter renal water excretion. On the other hand, the catecholamines could affect the release of endogenous vasopressin or directly modify the ac-

TABLE IV

Effect of ISO on AVP-mediated J_v and P_f in the CCT of Rabbit

AVP concentration	AVP		AVP + ISO 10^{-6} M		AVP + ISO 10^{-5} M	
	J_v	P_f	J_v	P_f	J_v	P_f
μU/ml						
100	1.04±0.10 (6)	202.6±17.2	1.04±0.10 (4)*	199.4±21.7*	0.88±0.10 (5)*	166.6±29.3*
10	1.06±0.18 (5)	193.4±27.7	0.88±0.18 (5)*	171.5±33.3*	1.12±0.27 (4)*	197.4±43.9*
2.5	0.60±0.07 (4)	100.7±24.7	0.45±0.03 (4)*	85.7±16.9*	0.41±0.03 (4)*	75.8±13.9*

All values are expressed as means±SE. Tubules were studied at 25°C with a 165-mosmol/kg H_2O transepithelial osmotic gradient. J_v is fluid volume reabsorption in nanoliters per minute per millimeter. P_f is the osmotic water permeability coefficient in $\times 10^{-4}$ centimeters per second. Number in parenthesis is the number of tubules. Mean length of the tubules is 1.7 mm. Perfusion rate is not different between the groups.

* P = not significant when compared with AVP alone.

TABLE V
Effect of ISO Alone on J_v and P_f in the CCT of Rabbit

	J_v	P_f	V_t
Control at 25°C	0.01±0.12 (4)	4.6±11.0	8.4±0.8
ISO 10^{-5} M at 25°C	−0.08±0.05*	−9.3±4.5*	8.1±0.7*
ISO 10^{-5} M at 37°C	−0.16±0.05*	−14.9±3.9*	8.1±0.8*

All values are expressed as means±SE. Tubules were studied with a 165-mosmol/kg H_2O transepithelial osmotic gradient. J_v is fluid volume reabsorption in nanoliters per minute per millimeter. P_f is the osmotic water permeability coefficient in $\times 10^{-4}$ centimeters per second. V_t is the perfusion rate in nanoliters per minute. Mean length of the tubules is 1.9 mm. Number in parenthesis is the number of tubules.
* P = not significant when compared with control.

tion of vasopressin in increasing the water permeability of the distal nephron. The present studies, therefore, were performed in isolated segments of the rabbit cortical collecting tubules to determine whether catecholamines directly affect water absorption in this segment of the nephron in the absence of changes in glomerular filtration rate, renal plasma flow, or renal innervation.

Using clearance studies in water loaded subjects, Fisher observed that the diuretic effect of NE occurred in those subjects with a low, but not high, dose of AVP (17). These results were interpreted as indicating an antagonism by NE of the effect of AVP on the renal tubule. Schrier and Berl, however, could not detect any diuretic effect when NE was infused intravenously in acutely hypophysectomized dogs (10). Moreover, the intrarenal infusion of NE also failed to increase renal water excretion (10). Their results indicated that the water diuresis associated with intravenous NE is mediated primarily by suppression of endogenous AVP release. Similarly, there was no evidence of water diuresis when intravenous NE was administered to the patients with central diabetes inspidus (21).

In vitro studies of the effect of alpha-adrenergic stimulation have also been conflicting. Using isolated papillae of the rat, Rayson et al. could not demonstrate any effect of NE on the diffusional water permeability of collecting ducts in the presence or absence of AVP (12). Base-line adenylate cyclase activity in the isolated cortical collecting tubule of the rabbit is not inhibited by alpha-adrenergic agonists (22). However, there is some in vitro evidence which suggests that alpha-adrenergic stimulation with NE antagonizes the effect of AVP on water transport (6, 8). It has been suggested that the antagonism by NE of the action of AVP in toad bladder may be mediated by interference with the effect of AVP to increase cAMP generation (6). Using suspensions of isolated cortical and medullary tubules of the rat, Kurokawa and Massry demonstrated that NE inhibits AVP-induced cAMP production by the medullary tubules, an effect that can be blocked by a specific alpha-adrenergic antagonist (7). Similarly, Beck et al. demonstrated that alpha-adrenergic activity inhibits the effect of AVP to stimulate cAMP generation in the dog kidney (23). More recently, a specific inhibitory effect of an alpha-adrenergic agonist on cAMP accumulation stimulated by AVP was shown in the isolated rabbit cortical collecting tubule (24). The results of our studies indicate that alpha-adrenergic stimulation with PE has a direct, dose-dependent effect on the cortical collecting tubule to inhibit AVP-mediated water absorption. Moreover, the alpha-adrenergic antagonist PH blocks the inhibitory effect of PE, but PH alone has no effect on AVP-mediated water absorption. The inhibition of water absorption by PE seems to be through the inhibition of production of cAMP since PE has no effect on 8-BrcAMP-mediated water absorption. These findings are similar to those of Handler et al., who demonstrated that alpha-adrenergic stimulation inhibited the hydro-osmotic effect of AVP, but not of exogenous cAMP in the isolated toad bladder (6). PE was chosen in our studies because of its pure alpha-adrenergic agonistic activity. The concentrations chosen were similar to those used in other studies (6, 7, 12, 23). We cannot ascertain from our studies whether the native catecholamines such as epinephrine and norepinephrine will exert a similar effect in vitro, in the low concentrations in which they exist in vivo. It is possible that the effect of PE seen in our studies may be more pharmacologic than physiologic.

In contrast to the effect of the alpha-adrenergic agonists, beta-adrenergic agonists have been shown to cause water antidiuresis both in man and experimental animals (11, 18, 19, 21). Levi et al. demonstrated that the intravenous infusion of ISO caused water antidiuresis in rats with congenital pituitary diabetes inspidus and that this effect could be abolished by propranolol, a specific beta blocker (9). However, Klein et al., in studies in the dog, could not detect any rise

in bioassayable AVP in the jugular vein following the systemic administration of ISO (25). In the toad bladder, ISO has been shown to augment the effect of AVP, although ISO alone has no effect on water absorption (6). Besides, beta-adrenergic agonists have been shown to enhance cAMP concentration in dog kidney slices (23) and kidney cortex homogenates (26). It has been inferred from the above studies that ISO has direct renal effect in modulating water excretion independent of changes in systemic levels of AVP. ISO-responsive adenylate cyclase activity was seen in the granular portion of the distal convoluted tubule as well as in both the granular and the light portions of the cortical collecting tubule of the rabbit (22). However, the response of the light portion of the cortical collecting tubule to ISO was much lower compared with the granular portion, and much lower with ISO than with AVP (22, 27). Low concentrations of AVP stimulated adenylate cyclase activity only in the light portions of the cortical collecting tubule and not in the granular portion (27). It was also shown that the respective effects of ISO and AVP on the cortical collecting tubule (light portion) adenylate cyclase activity were not additive when the two hormones were applied together (22). In another study, ISO has been shown to have no effect on AVP-induced cAMP generation in the rat medullary tubules (7). Thus, there seems to be no strong evidence to support enhancement by ISO of AVP-induced cAMP generation in the mammalian kidney. Other investigators also failed to detect a direct effect of ISO on renal tubular water absorption. Schrier et al., using intact dogs, demonstrated a specific antidiuretic effect of ISO (11). This effect was not seen in the acutely hypophysectomized dogs, suggesting that the beta-adrenergic effect is secondary to the release of endogenous AVP. In addition, the intrarenal infusion of ISO resulted in no antidiuresis, thereby again failing to provide evidence for a direct tubular effect of ISO. In vitro confirmation of these results has recently been provided by Rayson et al., who were unable to detect any effect of ISO on the diffusional water permeability of the collecting ducts or on the cAMP content of the isolated papillae of the rat (12). Similarly, it has been shown that ISO has no effect on basal osmotic water permeability in the isolated, perfused cortical collecting tubule of the rabbit (28). The results of our studies also demonstrate that the beta-adrenergic agonist ISO does not directly affect water absorption in the cortical collecting tubule of the rabbit. This lack of response is seen whether AVP is present in submaximal or maximal concentration. In addition, ISO alone does not increase water absorption when tubules are perfused while the bathing fluid temperature is maintained at 25 or 37°C.

In conclusion, the results of the present studies indicate that the alpha-adrenergic agonists directly inhibit AVP-mediated water absorption at the level of the renal tubule, an effect that can be blocked by a specific alpha-adrenergic antagonist. It appears that this effect is caused by inhibiting the AVP-induced cAMP production. An additional effect of alpha-adrenergic stimulation to modify release of endogenous vasopressin, however, cannot be excluded. On the other hand, the beta-adrenergic agonists do not directly enhance AVP-mediated water absorption at the level of the renal tubule. Beta-adrenergic stimulation may result in water antidiuresis either by modifying the release of endogenous vasopressin or by some other unknown mechanism.

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