Functional Characterization of the Alpha Adrenergic Receptor Modulating the Hydroosmotic Effect of Vasopressin on the Rabbit Cortical Collecting Tubule

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Abstract. To characterize the type of alpha adrenergic receptor, the effects of specific alpha adrenergic agonists and antagonists on antidiuretic hormone ([Arg²]-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 (Jv, nanoliters per minute per millimeter) was 1.39±0.09 and osmotic water permeability coefficient (Pf, \(10^{-4}\) centimeters per second) was 150.2±15.0. The addition of \(10^{-6}\) M phenylephrine (PE), an alpha adrenergic agonist, resulted in a significant decrease in \(J_v\) and \(P_f\) to 0.72±0.11 (\(P < 0.005\)) and 69.9±10.9 (\(P < 0.005\)). The addition of \(10^{-4}\) M prazosin (PZ), an alpha₁ adrenergic antagonist, did not cause any significant change in \(J_v\) and \(P_f\), which were 0.71±0.09 (\(P = \text{NS vs. AVP + PE}\)) and 67.8±9.5 (\(P = \text{NS vs. AVP + PE}\)), respectively. In a separate group of tubules, in the presence of AVP (100 μU/ml) and PE (\(10^{-6}\) M), \(J_v\) and \(P_f\) were 0.78±0.17 and 76.1±18.0, respectively. The addition of \(10^{-6}\) M yohimbine (Y), an alpha₂ adrenergic antagonist, resulted in a significant increase in \(J_v\) to 1.46±0.14 (\(P < 0.01\)) and \(P_f\) to 157.5±22.3 (\(P < 0.005\)). Y (\(10^{-4}\) M) or PZ (\(10^{-4}\) M) alone did not significantly affect \(J_v\) and \(P_f\) in the presence of AVP (100 μU/ml).

The effect of the natural endogenous catecholamine norepinephrine (NE) on \(J_v\) and \(P_f\) in the presence of AVP and propranolol (PR) was next examined. \(J_v\) and \(P_f\) were 1.53±0.07 and 176.3±5.2, respectively, in the presence of AVP (100 μU/ml) and PR (\(10^{-4}\) M). The addition of NE (\(10^{-8}\) M) resulted in a significant decrease in \(J_v\) to 1.19±0.11 (\(P < 0.05\)) and \(P_f\) to 127.0±11.3 (\(P < 0.02\)). Increasing the concentration of NE to \(10^{-6}\) M resulted in a further decrease in \(J_v\) and \(P_f\) to 0.70±0.10 (\(P < 0.01\) vs. NE \(10^{-8}\) M) and 68.5±10.6 (\(P < 0.01\) vs. NE \(10^{-8}\) M), respectively. The inhibitory effect of NE on AVP-induced water absorption was blocked by Y, but not by PZ.

The effect of the alpha₂ adrenergic agonist clonidine (CD) on \(J_v\) and \(P_f\) was also examined. In the presence of AVP (10 μU/ml), \(J_v\) and \(P_f\) were 1.65±0.04 and 175.1±13.1, respectively. The addition of CD (\(10^{-6}\) M) resulted in a significant decrease in \(J_v\) to 1.08±0.12 (\(P < 0.01\)) and \(P_f\) to 108.1±15.4 (\(P < 0.01\)). Increasing the concentration of CD to \(10^{-4}\) M resulted in a further significant decrease in \(J_v\) and \(P_f\) to 0.57±0.13 (\(P < 0.02\) vs. CD \(10^{-6}\) M) and 54.7±13.8 (\(P < 0.01\) vs. CD \(10^{-6}\) M), respectively. Similar results were obtained in the presence of AVP (100 μU/ml). The inhibitory effect of CD on AVP-induced water absorption was blocked by Y. CD did not significantly affect \(J_v\) and \(P_f\) in the presence of 8-bromo adenosine 3',5'-cyclic monophosphate.

These studies indicate that alpha adrenergic agonists directly inhibit AVP-mediated water absorption at the level of the renal tubule, an effect that can be blocked by specific alpha₂ adrenergic antagonists, but not by specific alpha₁ adrenergic antagonists. Alpha₂ adrenergic stimulation directly inhibits AVP-mediated water absorption at the level of the tubule, an effect that can be blocked by a specific alpha₂ adrenergic antagonist. This effect appears to be exerted at the level of activation of adenylate cyclase, since it is absent in the presence of cyclic AMP.
**Introduction**

There is considerable evidence to suggest that alpha adrenergic stimulation causes an increase in free water clearance (C_{H2O}) (1). The exact mechanism by which catecholamines modulate water excretion, however, is unclear. Some studies have suggested that they cause changes in water excretion by modifying the release of endogenous vasopressin (1). Other studies, however, have suggested that catecholamines modulate the action of antidiuretic hormone at the cellular level (1). Clonidine (CD), a known alpha_2 adrenergic agonist, has also been shown to cause water diuresis (2-8). The exact mechanism by which CD causes changes in water excretion is not definitely established, but both suppression of vasopressin release and modulation of its renal effect have been proposed (2-8).

Recently, we have demonstrated that the alpha adrenergic agonist phenylephrine (PE) directly inhibits antidiuretic hormone-induced water absorption at the tubular level (9). We have also shown that this effect can be blocked by phentolamine, a known alpha adrenergic antagonist (9). However, it was not known whether the natural catecholamine, norepinephrine (NE), would exert a similar effect on antidiuretic hormone-induced water absorption in the cortical collecting tubule (CCT) and whether the effect of the alpha adrenergic agonists is mediated by alpha_1 or alpha_2 adrenergic receptors or both. Therefore, the present studies were performed to characterize the specific alpha adrenergic receptor responsible for modulating the action of antidiuretic hormone on water absorption in isolated CCT of the rabbit, perfused in vitro. In addition, studies were conducted to directly examine the effects of NE and of CD on water absorption in the CCT.

**Methods**

Segments of CCT were isolated and perfused in vitro as described by Burg et al. (10) with few modifications (9). 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 killed by decapitation and the right kidney was 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 millimolar): 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 of the solution was 7.4 and osmolality was 290 mosmol/kg H_2O. 5% (vol/vol) of 5 g/dl defatted bovine albumin (Calbiochem-Behring Corp., La Jolla, CA) solution was added to prevent adhesion of tubules to the dish. CCT (1.0-2.5 mm in length) 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 perfusate was advanced into the lumen of the tubule. Perfusion was initiated by a gravity flow-system at a rate of 5-15 nl/min. The luminal perfusion solution contained (in millimolar): 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. Excessively dialyzed [methoxy-3H]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 was determined by using an eyepiece micrometer. Tubules were discarded if any breaks or denuded areas were visible along the perfused length. Tubules were also discarded if there was a leakage rate of [H]inulin into the bathing solution in excess of 1% of the perfusion rate. The bathing solution was then replaced by another solution of the following composition (in millimolar): 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 g/dl defatted bovine albumin solution was added to the bathing solution. The pH of the bathing solution was maintained at 7.4 by continuous bubbling with 95% O_2/5% CO_2. The osmolality of the bathing solution was 290 mosmol/kg H_2O. 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). Bath temperature was maintained at 25-27°C in all the experiments.

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 Biofluor (New England Nuclear) and radioactivity was measured in a Tri-Carb liquid scintillation counter (Packard Instruments Co., Downers Grove, IL).

(10)-vasopressin (AVP) acetate was obtained from Calbiochem-Behring Corp; PE HCl, NE bitartrate, yohimbine (Y) HCl, and 8-bromo adenosine 3',5'-cyclic monophosphate (8-BrcAMP) were obtained from Sigma Chemical Co., St. Louis, MO; propranolol (PR) HCl was obtained from Ayerst Laboratories Inc., New York; CD HCl was a kind gift from Boehringer Ingelheim Ltd., Ridgefield, CT; and prazosin (PZ) HCl was a kind gift from Dr. Eugene Weiss of Pfizer Laboratories, New York. All solutions were prepared daily and added in desired concentration to the bathing solution only, just before commencement of the experiment. NE was kept in a dark container in a refrigerator until needed. The amount of PZ that was necessary to achieve the desired concentration was directly added to the bathing solution and the solution was mixed thoroughly for 1 hr using the ultrasonic sonicator (Heat Systems-Ultrasonics Inc., Plainview, NY) to ensure complete dissolution of PZ.

Perfusion of the tubules was initiated within 30 min from the time of decapitation in most of the experiments. In all the experiments, an initial equilibration period of 120-150 min was allowed to elapse from the time of decapitation to ensure the disappearance of the effects of endogenous vasopressin. In between the experimental periods, a stabilization period of 30-45 min was maintained. The following groups of experiments were performed.

**Group I. Effect of PE and PZ on AVP-induced water absorption**

After the initial equilibration period, AVP was added to the bathing solution in a concentration of 100 μM. After a stabilization period,
timed samples of collected fluid were obtained. PE (10^{-6} M), an alpha
adrenergic agonist, was then added to the bathing solution containing
AVP, and samples of collected fluid were obtained after another sta-
bilization period. PZ (10^{-4} M), an alpha, adrenergic antagonist, was
then added to the bathing solution containing AVP and PE. Collected
fluid samples were obtained again after the stabilization period. PZ and
PE were then removed from the bath and another set of collected fluid
samples was obtained in the presence of AVP only.

**Group II. Effect of PE and Y on AVP-induced water absorption.**
After the initial equilibration period, AVP (100 µU/ml) was added to
the bathing solution and collected fluid samples were obtained as in
group I. PE (10^{-6} M) was then added to the bathing solution and samples
of collected fluid were obtained. Y (10^{-6} M), an alpha; adrenergic an-
tagonist was then added to the bathing solution containing AVP and PE
and timed samples of collected fluid were obtained. The effect of Y
(10^{-5} M) was similarly studied in a fourth experimental period.

**Group III. Effect of NE on AVP-induced water absorption in PR-
treated tubules.** To be sure that the natural endogenous catecholamines
exerted the same effect as PE, this group of experiments was performed
using NE. After the initial equilibration period, AVP (100 µU/ml) and
PR (10^{-3} M) were added to the bathing solution. Timed samples of
collected fluid were obtained after a stabilization period. NE (10^{-6} M)
was then added to the bathing solution and collected fluid samples were
depicted. The dose-response relationship of NE to AVP-induced water
absorption in PR-treated tubules was examined by adding progressively
increasing concentrations of NE (from 10^{-6} M to 10^{-3} M) to the bathing
solution. When NE (10^{-4} M) was added to the bathing solution, irre-
sversible cellular damage occurred.

**Group IV. Effect of NE and PZ on AVP-induced water absorption.**
The experimental protocol was same as in group I except that NE
(10^{-4} M) was used in this group instead of PE (10^{-6} M).

**Group V. Effect of NE and Y on AVP-induced water absorption.**
The experimental protocol was the same as in group II except that NE
(10^{-6} M) was used in this group instead of PE (10^{-6} M).

**Group VI. Effect of CD on AVP-induced water absorption.** (a) After
the initial equilibration period, AVP (10 µU/ml) was added to the bathing
solution and timed samples of collected fluid were obtained after a stabilization
period. CD (10^{-6} M) was then added to the bathing solution and collected fluid samples were again obtained after the stabilization
period. The effect of CD (10^{-5} and 10^{-4} M) on AVP (10 µU/ml)-induced
water absorption was similarly studied in subsequent experimental per-
iods. CD was then removed from the bathing solution and collected fluid samples were obtained in the presence of AVP (10 µU/ml) only.
(b) The experimental protocol was same as in group VIa except that
AVP was used in a concentration of 100 µU/ml.

**Group VII. Effect of CD and Y on AVP-induced water absorption.**
After an initial equilibration period, AVP was added to the bathing
solution in a concentration of 100 µU/ml. After obtaining collected
fluid samples, CD (10^{-4} M) was added to the bathing solution containing
AVP. After another stabilization period, collected fluid samples were
obtained. Y (10^{-3} M) was then added to the bathing solution containing
AVP and CD and samples of the collected fluid were obtained. The effect of Y (10^{-4} M) was similarly examined in a fourth experimental
period.

**Group VIII. Effect of CD on cyclic AMP-induced water absorption.**
In this group of tubules, 8-BrcAMP (10^{-6} M) was added to the bathing
solution initially. After obtaining the collected fluid samples, CD
(10^{-3} M) was then added to the bathing solution containing 8-BrcAMP
and collected fluid samples were obtained. After obtaining samples of the collected fluid in the presence of AVP (100 µU/ml), as in the other groups, PZ (10^{-4} M), PR (10^{-4} M), and
Y (10^{-4} M) were added separately to the bathing solution containing
AVP during the subsequent experimental periods. Collected fluid samples
were obtained in each experimental period.

**Calculations.** Net fluid absorption \((J)\) was calculated using the for-
mula: \(J(nl/min/mm) = (V_t - V_0)/L\), where \(V_t\) is the perfusion rate in
nanoliters per minute, \(V_0\) is the collection rate in nanoliters per minute,
and \(L\) is the length of the tubule in millimeters.

\[
V_{t} = V_{o} + V_{a} (\frac{[^{3}H_{a}]}{[H_{a}]})
\]

where \(V_t\) was calculated from the formula: \(V_t = V_o + \frac{^{3}H_a}{H_a}\), where \(^{3}H_a\) and \(^{3}H_a\) are the inulin counts per nanoliter in the collected and perfused
fluids, respectively.

The osmotic water permeability coefficient, \(P_f (cm \cdot s^{-1})\) was calculated
using the formula (11):

\[
P_f = \frac{V_{o}C_{o} - C_{t}}{A \cdot P_{fT} \ln \left(\frac{C_{o} - C_{t}}{C_{o} - C_{b}}\right)}
\]

where \(V_t\) is the perfusion rate in cubic centimeters per second, \(A\) is the
tubule luminal surface area (calculated from the measured length and an
arbitrarily assumed diameter of 20 µm), and \(P_{fT}\) is the partial molar
volume of water. \(C_o, C_t,\) and \(C_b\) are the osmolalities of the perfusate,
bath, and collected fluid, respectively. Osmolality of the perfusate and
bath were measured. Osmolality of the collected fluid was calculated from
the measured perfuse osmolality and the relative increase in the
concentration of the volume marker measured in the collected fluid.

All values represent the mean of two or more collections for each
experimental period. The data are shown as means±SE. Statistical analysis
was performed by using the t test for paired data (two experimental
periods) or by analysis of variance (more than two experimental periods
in the same tubule) (12, 13).

**Results**

**Group I.** The effect of the alpha adrenergic agonist PE on AVP-
mediated \(J_{w}\) and \(P_f\) and the effect of the alpha, adrenergic an-
tagonist PZ on the decrease in \(J_{w}\) and \(P_f\) caused by PE are shown in
Table I. In this group of tubules (n = 4), when AVP was present in the
bathing solution in a concentration of 100 µU/ml, \(J_{w}\) averaged 1.39±0.09
nl.min^{-1}·cm^{-1} (P < 0.005 vs. AVP alone) and 150.2±15.0
× 10^{-4} cm/s. The addition of PE (10^{-4} M) resulted in a
significant decrease of \(J_{w}\) and \(P_f\) to 0.72±0.11
nl.min^{-1}·cm^{-1} (P < 0.005 vs. AVP alone) and 69.9±10.9
× 10^{-4} cm/s (P < 0.005 vs. AVP alone), respectively. When
PZ (10^{-4} M) was added to the bathing solution containing AVP
(100 µU/ml) and PE (10^{-4} M), there was no significant change in
\(J_{w}\) and \(P_f\) when \(P_f\) were 0.71±0.09 nl.min^{-1}·cm^{-1} (P = NS
vs. AVP + PE) and 67.8±9.5 × 10^{-4} cm/s (P = NS vs. AVP
+ PE), respectively. \(J_{w}\) and \(P_f\) increased to 1.34±0.11
nl.min^{-1}·cm^{-1} (P < 0.005 vs. AVP + PE, P < 0.005 vs. AVP
+ PE + PZ) and 144.5±17.1 × 10^{-4} cm/s (P < 0.01 vs. AVP
+ PE, P < 0.005 vs. AVP + PE + PZ), respectively, when both
PZ and PE were removed from the bath and the tubules were
exposed to AVP (100 µU/ml) alone. These values are not sig-
nificantly different from the values obtained during the initial
experimental period with AVP alone.
Table I. Effect of PE and PZ on AVP-mediated J, and P in the CCT of Rabbit

<table>
<thead>
<tr>
<th></th>
<th>AVP 100 μU/ml</th>
<th>AVP 100 μU/ml + PE 10⁻⁴ M</th>
<th>AVP 100 μU/ml + PE 10⁻⁴ M + PZ 10⁻⁴ M</th>
<th>AVP 100 μU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>J</td>
<td>P</td>
<td>V₁</td>
<td>J</td>
<td>P</td>
</tr>
<tr>
<td>1.57</td>
<td>183.3</td>
<td>10.3</td>
<td>0.83</td>
<td>81.8</td>
</tr>
<tr>
<td>1.16</td>
<td>113.0</td>
<td>12.9</td>
<td>0.43</td>
<td>40.0</td>
</tr>
<tr>
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<td>8.9</td>
<td>0.92</td>
<td>89.6</td>
</tr>
<tr>
<td>1.47</td>
<td>162.4</td>
<td>8.5</td>
<td>0.71</td>
<td>68.2</td>
</tr>
<tr>
<td>Mean</td>
<td>1.39</td>
<td>150.2</td>
<td>10.2</td>
<td>0.72*</td>
</tr>
<tr>
<td>±SE</td>
<td>0.09</td>
<td>15.0</td>
<td>1.0</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Tubules were studied at 25°C with a 165 mosmol/kg H₂O transepithelial osmotic gradient. J, is given in nanoliters per minute per millimeter; P, in X 10⁻⁴ centimeters per second; and V₁, in nanoliters per minute. Mean length of the tubules is 2.2 mm. * P < 0.005 vs. AVP alone. ‡ P = not significant vs. AVP alone. § P = not significant vs. AVP + PE 10⁻⁴ M. † P < 0.005 vs. AVP + PE 10⁻⁴ M + PZ 10⁻⁴ M.

Group II. The effect of PE on AVP-mediated J, and P, and the effect of the α₁ adrenergic antagonist Y on the decrease in J, and P, caused by PE are depicted in Fig. 1. In this group of tubules (n = 4), J, and P, averaged 1.48±0.08 nl·min⁻¹·mm⁻¹ and 157.2±12.8 X 10⁻⁴ cm/s, respectively, in the presence of AVP (100 μU/ml) alone. As in group I, the addition of PE (10⁻⁴ M) resulted in a significant decrease in J, and P, to 0.78±0.17 nl·min⁻¹·mm⁻¹ (P < 0.005 vs. AVP alone) and 76.1±18.0 X 10⁻⁴ cm/s (P < 0.005 vs. AVP alone), respectively. However, the addition of Y (10⁻⁶ M) to the bathing solution containing AVP and PE resulted in a significant increase in J, to 1.46±0.14 nl·min⁻¹·mm⁻¹ (P < 0.01 vs. AVP + PE, P = NS vs. AVP alone) and P, increased to 157.5±22.3 X 10⁻⁴ cm/s. (P < 0.005 vs. AVP + PE, P = NS vs. AVP alone). When the concentration of Y was increased to 10⁻⁵ M there was no further decrease in J, and P, which were 1.50±0.08 nl·min⁻¹·mm⁻¹ (P = NS vs. Y 10⁻⁶ M) and 161.2±14.1 X 10⁻⁴ cm/s (P = NS vs. Y 10⁻⁶ M), respectively.

Group III. In this group of tubules (n = 4), we examined whether NE, a native catecholamine, has a similar effect as that of PE on AVP-induced J, and P. To block the beta adrenergic effects of NE, PR was added throughout the experiment to the bathing solution in a concentration of 10⁻⁴ M. As shown in Table II, J, and P, were 1.53±0.07 nl·min⁻¹·mm⁻¹ and 176.3±5.2 X 10⁻⁴ cm/s, respectively, in the presence of PR (10⁻⁴ M) and AVP (100 μU/ml). The addition of NE (10⁻⁵ M) to the bathing solution containing PR and AVP resulted in a significant decrease in J, to 1.19±0.11 nl·min⁻¹·mm⁻¹ (P < 0.05 vs. PR + AVP) and in P, to 127.0±11.3 X 10⁻⁴ cm·s⁻¹ (P < 0.02 vs. PR + AVP). Increasing the concentration of NE to 10⁻⁷ M resulted in a further but not significant decrease in J, and P, to 0.95±0.13 nl·min⁻¹·mm⁻¹ (P = NS vs. NE 10⁻⁸ M, P < 0.01 vs. PR + AVP) and 98.0±14.5 X 10⁻⁴ cm/s (P = NS vs. NE 10⁻⁸ M, P < 0.005 vs. PR + AVP), respectively. Increasing the concentration of NE to 10⁻⁸ M, however, resulted in a significant decrease in J, to 0.70±0.10 nl·min⁻¹·mm⁻¹ (P < 0.01 vs. NE 10⁻⁸ M) and in P, to 68.5±10.6 X 10⁻⁴ cm/s (P < 0.01 vs. NE 10⁻⁸ M). J, and P, decreased further to 0.44±0.07 nl·min⁻¹·mm⁻¹ (P < 0.01 vs. NE 10⁻⁷ M) and 40.7±6.9 X 10⁻⁴ cm/s (P < 0.01 vs. NE 10⁻⁷ M), respectively, when the concentration of NE was increased to 10⁻⁸ M. With the addition of NE in a concentration of 10⁻⁶ M, irreversible cellular damage occurred.

Group IV. The effect of PZ on NE-induced decrease in J, and P, was examined in this group of tubules (n = 4). As shown in Table III, in the presence of AVP (100 μU/ml), J, and P, were 1.46±0.06 nl·min⁻¹·mm⁻¹ and 154.0±4.1 X 10⁻⁴ cm/s, respectively. The addition of NE (10⁻⁶ M) resulted in a significant decrease in J, to 0.54±0.01 nl·min⁻¹·mm⁻¹ (P < 0.005 vs. AVP alone) and in P, to 50.5±1.4 X 10⁻⁴ cm/s (P < 0.001 vs. AVP alone).
Table II. Effect of NE on AVP-mediated $J_4$ and $P_f$ in the CCT of Rabbit in the Presence of PR(10^{-4} M)

<table>
<thead>
<tr>
<th>PR + AVP 100 μU/ml</th>
<th>PR + AVP 100 μU/ml + NE 10^{-4} M</th>
<th>PR + AVP 100 μU/ml + NE 10^{-5} M</th>
<th>PR + AVP 100 μU/ml + NE 10^{-6} M</th>
<th>PR + AVP 100 μU/ml + NE 10^{-7} M</th>
</tr>
</thead>
<tbody>
<tr>
<td>$J_4$</td>
<td>$P_f$</td>
<td>$V_1$</td>
<td>$J_4$</td>
<td>$P_f$</td>
</tr>
<tr>
<td>1.47</td>
<td>180.8</td>
<td>8.2</td>
<td>0.90</td>
<td>96.5</td>
</tr>
<tr>
<td>1.59</td>
<td>180.9</td>
<td>9.4</td>
<td>1.39</td>
<td>150.8</td>
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<td>1.69</td>
<td>182.7</td>
<td>12.3</td>
<td>1.31</td>
<td>133.5</td>
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<td>1.36</td>
<td>160.6</td>
<td>8.8</td>
<td>1.17</td>
<td>127.1</td>
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<tr>
<td>Mean</td>
<td>1.53</td>
<td>176.3</td>
<td>9.7</td>
<td>1.19*</td>
</tr>
<tr>
<td>±SE</td>
<td>0.07</td>
<td>5.2</td>
<td>0.9</td>
<td>0.11</td>
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</table>

Tubules were studied at 25°C with a 165 mosmol/kg H2O transepithelial osmotic gradient. $J_4$ is given in nanoliters per minute per millimeter; $P_f$, in $\times 10^{-4}$ centimeters per second; and $V_1$, in nanoliters per minute. Mean length of the tubules was 2.4 mm.

AVP alone. The addition of PZ (10^{-4} M) to the bathing solution containing AVP and NE did not result in any significant change in $J_4$ and $P_f$, which were 0.60±0.08 $nL\cdot min^{-1}\cdot mm^{-1}$ (P = NS vs. AVP + NE) and 56.3±7.9 $\times 10^{-4}$ cm/s (P = NS vs. AVP + NE), respectively. However, $J_4$ and $P_f$ increased to 1.34±0.07 $nL\cdot min^{-1}\cdot mm^{-1}$ (P < 0.005 vs. AVP + NE + PZ) and 137.8±6.11 $\times 10^{-4}$ cm/s (P < 0.001 vs. AVP + NE + PZ), respectively, when NE and PZ were removed from the bathing solution and the tubules were studied in the presence of AVP alone. These values are not statistically different from those obtained during the initial experimental period, with AVP alone.

Group V. The effect of Y on NE-induced decrease in $J_4$ and $P_f$ was examined in this group of tubules (n = 4). As shown in Table IV, the presence of AVP (100 μU/ml) in the bathing solution resulted in a $J_4$ of 1.48±0.05 $nL\cdot min^{-1}\cdot mm^{-1}$ and $P_f$ of 156.2±8.3 $\times 10^{-4}$ cm/s. As in group IV, the addition of NE (10^{-6} M) resulted in a significant decrease in $J_4$, to 0.70±0.09 $nL\cdot min^{-1}\cdot mm^{-1}$ (P < 0.01 vs. AVP alone) and in $P_f$ to 67.8±10.5 $\times 10^{-4}$ cm/s (P < 0.005 vs. AVP alone). However, the addition of Y (10^{-5} M) to the bathing solution resulted in a significant increase in $J_4$ and $P_f$ to 1.41±0.09 $nL\cdot min^{-1}\cdot mm^{-1}$ (P < 0.02 vs. AVP + NE, P = NS vs. AVP alone) and 147.8±11.0 $\times 10^{-4}$ cm/s (P < 0.01 vs. AVP + NE, P = NS vs. AVP alone), respectively. Increasing the concentration of Y to $10^{-3}$ M did not result in any further significant increase in $J_4$ and $P_f$, which were 1.54±0.09 $nL\cdot min^{-1}\cdot mm^{-1}$ and 163±7.3 $\times 10^{-4}$ cm/s, respectively.

Group VI. The effect of the alpha_2 adrenergic agonist CD on AVP-induced $J_4$ and $P_f$ was examined in this group of tubules. As shown in Table V, in the presence of maximal concentrations (10 μU/ml) of AVP (Group VIa, n = 5) in the bathing solution, $J_4$ and $P_f$ were 1.65±0.04 $nL\cdot min^{-1}\cdot mm^{-1}$ and 175.1±13.1 $\times 10^{-4}$ cm/s, respectively. When CD was added to the bathing solution in a concentration of 10^{-8} M, there was a significant

Table III. Effect of NE and PZ on AVP-induced $J_4$ and $P_f$ in the CCT of Rabbit

<table>
<thead>
<tr>
<th>AVP 100 μU/ml</th>
<th>AVP 100 μU/ml + NE 10^{-4} M</th>
<th>AVP 100 μU/ml + NE 10^{-5} M + PZ 10^{-4} M</th>
<th>AVP 100 μU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>$J_4$</td>
<td>$P_f$</td>
<td>$V_1$</td>
<td>$J_4$</td>
</tr>
<tr>
<td>1.38</td>
<td>150.4</td>
<td>10.7</td>
<td>0.56</td>
</tr>
<tr>
<td>1.37</td>
<td>144.4</td>
<td>10.7</td>
<td>0.50</td>
</tr>
<tr>
<td>1.48</td>
<td>158.0</td>
<td>10.0</td>
<td>0.56</td>
</tr>
<tr>
<td>1.61</td>
<td>163.2</td>
<td>9.6</td>
<td>0.54</td>
</tr>
<tr>
<td>Mean</td>
<td>1.46</td>
<td>154.0</td>
<td>10.3</td>
</tr>
<tr>
<td>±SE</td>
<td>0.06</td>
<td>4.1</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Tubules were studied at 25°C with a 165 mosmol/kg H2O transepithelial osmotic gradient. $J_4$ is given in nanoliters per minute per millimeter; $P_f$, in $\times 10^{-4}$ centimeters per second; and $V_1$, in nanoliters per minute. Mean length of the tubules was 2.1 mm.

R. K. Krothapalli and W. N. Suki
Table IV. Effect of NE and Y on AVP-induced J, and P in the CCT of Rabbit

<table>
<thead>
<tr>
<th>AVP 100 µU/ml</th>
<th>AVP 100 µU/ml + NE 10⁻⁶ M</th>
<th>AVP 100 µU/ml + NE 10⁻⁴ M + Y 10⁻⁵ M</th>
<th>AVP 100 µU/ml + NE 10⁻⁴ M + Y 10⁻⁵ M</th>
</tr>
</thead>
<tbody>
<tr>
<td>J</td>
<td>P</td>
<td>V</td>
<td>J</td>
</tr>
<tr>
<td>1.42</td>
<td>135.9</td>
<td>12.4</td>
<td>1.49</td>
</tr>
<tr>
<td>1.60</td>
<td>172.4</td>
<td>9.1</td>
<td>1.60</td>
</tr>
<tr>
<td>1.40</td>
<td>150.1</td>
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<td>1.16</td>
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<tr>
<td>1.50</td>
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<tr>
<td>Mean</td>
<td>1.48</td>
<td>156.2</td>
<td>10.3</td>
</tr>
<tr>
<td>±SE</td>
<td>0.05</td>
<td>8.3</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Tubules were studied at 25°C with a 165 mosmol/kg H₂O transepithelial osmotic gradient. J, is given in nanoliters per minute per millimeter; P, in × 10⁻⁶ centimeters per second; and V, in nanoliters per minute. Mean length of the tubules is 2.0 mm. * P < 0.01 vs. AVP alone. ** P < 0.005 vs. AVP alone. § P = not significant vs. AVP alone.

The effect of CD on J, and P in the presence of supramaximal concentrations (100 µU/ml) of AVP is shown in Table VI (Group VIb, n = 5). J, and P were 1.66 ±0.03 nm·min⁻¹·mm⁻¹ and 215.0±12.7 × 10⁻⁴ cm/s, respectively, in the presence of AVP alone. The addition of CD (10⁻⁴ M) to the bathing solution in the presence of AVP resulted in an insignificant decrease in J, to 1.50±0.03 nm·min⁻¹·mm⁻¹ (P = NS vs. AVP alone) and a significant decrease in P to 188.7±16.1 × 10⁻⁴ cm/s (P < 0.05 vs. AVP alone). Increasing the concentration of CD to 10⁻³ M resulted in a further significant decrease in J, and P to 1.28±0.12 nm·min⁻¹·mm⁻¹ (P < 0.05 vs. CD 10⁻³ M) and 155.2±16.6 × 10⁻⁴ cm/s (P < 0.025 vs. CD 10⁻³ M), respectively. When CD (10⁻⁴ M) was added to the bathing solution, there was a further significant decrease in J, to 0.86±0.11 nm·min⁻¹·mm⁻¹ (P < 0.01 vs. CD 10⁻³ M) and in P, to 98.1±18.4 × 10⁻⁴ cm/s (P < 0.005 vs. CD 10⁻³ M). J, increased to 1.49±0.06 nm·min⁻¹·mm⁻¹ (P < 0.005 vs. AVP + CD 10⁻⁴ M, P = NS vs. AVP alone), when CD was removed from the bathing solution and the tubules were studied in the presence of AVP alone.

Table V. Effect of CD on AVP-induced J, and P in the CCT of Rabbit

<table>
<thead>
<tr>
<th>AVP 10 µU/ml</th>
<th>AVP 10 µU/ml + CD 10⁻⁴ M</th>
<th>AVP 10 µU/ml + CD 10⁻⁴ M</th>
<th>AVP 10 µU/ml + CD 10⁻⁴ M</th>
</tr>
</thead>
<tbody>
<tr>
<td>J</td>
<td>P</td>
<td>V</td>
<td>J</td>
</tr>
<tr>
<td>1.82</td>
<td>226.9</td>
<td>11.9</td>
<td>1.38</td>
</tr>
<tr>
<td>1.56</td>
<td>162.0</td>
<td>12.2</td>
<td>1.28</td>
</tr>
<tr>
<td>1.60</td>
<td>157.4</td>
<td>14.1</td>
<td>1.12</td>
</tr>
<tr>
<td>1.66</td>
<td>168.3</td>
<td>12.6</td>
<td>0.79</td>
</tr>
<tr>
<td>1.62</td>
<td>161.0</td>
<td>10.8</td>
<td>0.81</td>
</tr>
<tr>
<td>Mean</td>
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<td>12.3</td>
</tr>
<tr>
<td>±SE</td>
<td>0.04</td>
<td>13.1</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Tubules were studied at 25°C with a 165 mosmol/kg H₂O transepithelial osmotic gradient. J, is given in nanoliters per minute per millimeter; P, in × 10⁻⁶ centimeters per second; and V, in nanoliters per minute. Mean length of the tubules is 2.0 mm. * P < 0.001 vs. AVP alone. ** P < 0.005 vs. AVP and CD 10⁻⁴ M. §§ P < 0.05 vs. AVP and CD 10⁻⁴ M.
of AVP alone. Similarly, P1 increased to 193.9±24.3×10⁻⁴ cm/s (P < 0.001 vs. AVP + CD 10⁻⁴ M, P = NS vs. AVP alone).

**Group VII.** To determine whether the effect of CD was specific, the effect of the alpha₂ adrenergic antagonist Y on CD-induced decrease in water absorption was studied in this group of tubules (n = 4). As depicted in Fig. 2, J and P1 were 1.64±0.10 nl·min⁻¹·mm⁻¹ and 190.2±12.1×10⁻⁴ cm/s, respectively, in the presence of AVP (100 µU/ml) alone. Addition of CD (10⁻⁴ M) resulted in a significant decrease in J, to 0.42±0.04 nl·min⁻¹·mm⁻¹ (P < 0.001 vs. AVP alone) and in P1 to 38.7±4.3×10⁻⁴ cm/s (P < 0.001 vs. AVP alone). The addition of Y (10⁻⁴ M) resulted in a significant increase in J, and P1 to 1.01±0.06 nl·min⁻¹·mm⁻¹ (P < 0.02 vs. AVP + CD 10⁻⁴ M) and 102.3±7.4×10⁻⁴ cm/s (P < 0.01 vs. AVP + CD 10⁻⁴ M), respectively. Increasing the concentration of Y to 10⁻³ M resulted in a further significant increase in J, to 1.45±0.11 nl·min⁻¹·mm⁻¹ (P < 0.02 vs. Y 10⁻⁶ M, P = NS vs. AVP alone) and in P1 to 160.4±15.3×10⁻⁴ cm/s (P < 0.02 vs. Y 10⁻⁶ M, P = NS vs. AVP alone). Addition of Y (10⁻⁴ M) did not result in any further significant increase in J, and P1, which were 1.60±0.06 nl·min⁻¹·mm⁻¹ (P = NS vs. Y 10⁻³ M) and 182.1±8.2×10⁻⁴ cm/s (P = NS vs. Y 10⁻⁵ M), respectively.

**Group VIII.** The effect of CD on cyclic AMP-induced water absorption was examined in this group of tubules (n = 4). The presence of 8-BrCAMP (10⁻⁴ M) in the bathing solution resulted in a J, of 1.48±0.08 nl·min⁻¹·mm⁻¹ and P1 of 166.9±16.1×10⁻⁴ cm/s. The addition of CD (10⁻⁴ M) to the bathing solution containing 8-BrCAMP resulted in no change in J, and P1, which were 1.41±0.07 nl·min⁻¹·mm⁻¹ and 155.3±17.0×10⁻⁴ cm/s, respectively (Table VII).

**Group IX.** In this group of tubules (n = 2), in the presence of AVP (100 µU/ml) J, and P1 were 1.64±0.09 nl·min⁻¹·mm⁻¹ and 177.7±18.5×10⁻⁴ cm/s, respectively. Addition of PZ (10⁻⁴ M), in the presence of AVP did not result in any significant change in J, and P1, which were 1.57±0.01 nl·min⁻¹·mm⁻¹ (P = NS vs. AVP alone) and 167.7±4.5×10⁻⁴ cm/s (P = NS vs. AVP alone), respectively. Similarly, the addition of PR (10⁻⁴ M) or Y (10⁻⁴ M) did not result in any significant change in J, or P1. In the presence of PR (10⁻⁴ M), J, was 1.55±0.01 nl·min⁻¹·mm⁻¹ (P = NS vs. AVP alone) and P1 was 163.9±4.5×10⁻⁴ cm/s (P = NS vs. AVP alone). In the presence of Y (10⁻⁴ M), J, was 1.57±0.08 nl·min⁻¹·mm⁻¹ (P = NS vs. AVP alone) and P1, 166.4±13.6×10⁻⁴ cm/s (P = NS vs. AVP alone).

**Discussion**

Catecholamines modulate several aspects of renal function including the renal blood flow, glomerular filtration, tubular...
transport, and the release of renin (1). The effect of catecholamines on water absorption has drawn the attention of several investigators. There is evidence to suggest that alpha adrenergic stimulation increases \( C_{\text{H}_{2}O} \) (14-16). On the other hand, beta adrenergic stimulation has been shown to cause diuresis (1, 17). The exact mechanism by which catecholamines cause changes in water excretion, however, is unclear. Some of the previous studies have suggested that these agents cause changes in water excretion by modifying the release of endogenous vasopressin, whereas others have suggested that these agents modify the action of AVP at the cellular level (1). Using isolated segments of the rabbit CCT that were perfused in vitro, we have recently demonstrated that the alpha adrenergic agonist PE directly inhibits AVP-mediated water absorption at the tubular level, an effect that can be blocked by a specific alpha adrenergic antagonist phenolamine (9). The inhibition of water absorption by PE seems to be through the inhibition of AVP-mediated cyclic AMP production (9).

The alpha adrenergic receptors can be subdivided into alpha\(_1\) and alpha\(_2\) adrenergic receptors (18, 19). The recognition of the subtypes was preceded by the appreciation that they had a role in modulating the amount of catecholamine release from the nerve terminals. In general, alpha\(_2\) receptors include typical postsynaptic receptors mediating smooth muscle contraction, whereas alpha\(_1\) receptors include not only the presynaptic autoregulatory alpha adrenergic receptors but also the postsynaptic receptors that are shown on the platelets (18). Because of the difficulties in using the anatomical classification, alpha adrenergic receptors are classified into subtypes based on their differences in affinity for various selective alpha adrenergic agonists and antagonists (19). In radioligand-binding studies, alpha\(_1\) adrenergic receptors can be identified by using an alpha\(_1\) adrenergic selective antagonist, \([^{3}H]PZ\), whereas alpha\(_2\) adrenergic receptors can be identified by using an alpha\(_2\) adrenergic selective antagonist, \([^{3}H]Y\) (20, 21). Similarly, the role of the specific receptors can be defined by using the specific agonists and antagonists in functional studies (22).

In the kidney, both alpha\(_1\) and alpha\(_2\) receptors have been identified, with an apparent dominance of alpha\(_2\) adrenergic receptors by a factor of three to one (22). This work suggested that alpha\(_2\) adrenergic receptors are located on the vasculature and mediate the renal vasoconstriction. On the other hand, alpha\(_1\) adrenergic receptors are located on the renal tubules (22). Similarly, radioligand binding has indicated that the alpha adrenergic receptors on the guinea pig proximal tubule are of alpha\(_2\) adrenergic type (23, 24). The presence of alpha\(_2\) adrenergic receptors coupled to inhibition of parathyroid hormone-stimulated adenylate cyclase has been demonstrated in homogenates of rat renal cortex (25, 26). However, the functional role of these receptors and the specific type of alpha adrenergic receptors (alpha\(_1\) or alpha\(_2\) or both) involved in modulating AVP-induced water absorption, have not been identified. Using isolated rat CCT, Chabardes et al. (27) have shown in a preliminary report that the inhibition of AVP-induced cyclic AMP accumulation by nonspecific alpha adrenergic agonists can be blocked by Y but not by PZ. Therefore, the present studies were performed in isolated segments of rabbit CCT to characterize functionally the specific subtype of alpha adrenergic receptors involved in the modulation of the hydroosmotic effect of AVP. As in our previous studies (9), the results of the present studies indicate that alpha adrenergic stimulation with PE has a direct effect on the CCT to inhibit AVP-mediated water absorption. The present studies demonstrate further that this effect can be blocked only by the specific alpha\(_2\) adrenergic antagonist Y. On the other hand the alpha\(_1\) adrenergic antagonist PZ had no effect on the inhibition of AVP-mediated water absorption by PE. Neither Y nor PZ alone had any effect on AVP-mediated water absorption. The failure of high concentrations of PZ to antagonize the alpha adrenergic effect of PE is in conflict with the radioligand studies that show displacement of \([^{3}H]CD\) by high concentrations of PZ (23, 24). The absence of a functional parallel to the radioligand-binding studies underscores the importance of functional studies in determining the biological meaning of physicochemical observations. It must be added, in this regard, that PZ did not block the inhibition of antidiuretic hormone-stimulated cyclic AMP accumulation in rat CCT induced by alpha adrenergic agonists (27), an observation that is in agreement with ours.

In considering the effects of the catecholamines on renal function, it should be emphasized that NE is the neurotransmitter released at the sympathetic effector sites, whereas epinephrine is the principal circulating adrenergic hormone (28, 29). To determine whether the natural endogenous catecholamine NE exerts the same effect as that of PE, we examined the effect of NE on AVP-induced water absorption in CCT that were treated with PR to block the beta adrenergic effects of NE. Our results indicated that alpha adrenergic stimulation with NE also had a direct dose-dependent effect to inhibit AVP-mediated water absorption. PR alone had no effect on AVP-mediated water absorption, indicating that the effect seen in our experiments

| Table VII. Effect of CD on 8-BrCAMP-mediated Jv and P1 in the CCT of Rabbit |
|-------------------|-----------------|-----------------|-------------------|-----------------|
| 8-BrCAMP 10⁻⁴ M   | 8-BrCAMP 10⁻⁴ M + CD 10⁻⁴ M |                   |
| Jv   | P1   | V1 | Jv   | P1   | V1 |
| 1.57 | 198.8 | 7.7 | 1.59 | 202.2 | 7.7 |
| 1.65 | 181.2 | 7.3 | 1.43 | 147.9 | 7.8 |
| 1.27 | 123.5 | 11.1 | 1.24 | 120.9 | 10.4 |
| 1.43 | 164.0 | 8.2 | 1.36 | 150.3 | 8.5 |
| Mean | 1.48 | 166.9 | 8.6 | 1.41* | 155.3* | 8.6* |
| ±SE  | 0.08 | 16.1 | 0.9 | 0.07 | 17.0 | 0.6 |

Tubules were studied at 25°C with a 165 mosmol/kg H₂O transepithelial osmotic gradient. Jv is given in nanoliters per minute per millimeter; P₁, in X 10⁻⁴ centimeters per second; and V₁, in nanoliters per minute. Mean length of the tubules is 1.9 mm. * P = not significant vs. AVP alone.
was indeed the result of the alpha adrenergic effect of NE. Also, the inhibitory effect of NE was blocked by Y, but not by PZ, pointing to activation of an alpha2 adrenergic receptor.

It may be argued that the effect of NE seen in our studies may be more pharmacologic than physiologic. Using fluorescence histochemical and electron microscopic techniques, adrenergic nerve terminals have been shown to be in direct contact with the basement membranes of the proximal and distal tubules in monkey, rat, and dog kidneys, and the thick ascending limb of Henle’s loop in the rat kidney (30–33). More recently, L. Barajas (personal communication) demonstrated that the adrenergic nerve terminals are in close contact with the basement membranes of the CCT, also in the rat kidney. In view of this evidence, it can be assumed that the concentrations of NE (10−8–10−7 M) used in our studies may be physiologic since they fall in the range of concentrations present at nerve endings (34). It is quite possible, however, that the inhibitory effect of NE on water absorption becomes evident physiologically only in the conditions where there is maximal release of catecholamines in the kidney.

CD is a known alpha2 adrenergic agonist. Previous studies in anesthetized dogs (4, 5) and rats (3) have shown that the intravenous administration of CD causes increase in renal water excretion. This effect could be the result of a central inhibition of AVP release or inhibition of action of AVP on the renal tubule. Humphreys and Reid (4) could not detect any water diuresis when CD was infused intravenously into acutely hypophysectomized dogs. Their results indicated that CD causes water diuresis through inhibition of AVP release, possibly via an indirect pathway mediated by the alpha adrenergic effects of CD on the circulation. Reid and Keil (6) have shown decreased concentrations of plasma AVP in response to the administration of CD. Other investigators (7, 8), however, have failed to find any detectable changes in plasma AVP concentrations after the administration of clonidine. Solez et al. (8) have shown that intravenous administration of CD causes an increase in C18:0, even when it was administered into rabbits that were pretreated with vaspressin tannate in oil, suggesting that CD inhibits the antidiuretic action of AVP at the tubular level. The results of our studies would indicate that alpha2 adrenergic stimulation with CD has a direct, dose-dependent effect on the CCT to inhibit AVP-mediated water absorption, in the presence of maximal as well as supramaximal concentrations of AVP. This effect can be blocked by the specific alpha2 adrenoceptor antagonist Y, while Y alone has no effect on AVP-induced water absorption. These studies, therefore, provide the first evidence that an alpha2 adrenoceptor can modulate renal tubular function. The inhibitory effect of CD on water absorption may not be of physiologic importance to man, since it has not been shown to cause water diuresis in man. However, these observations definitely add strength to our studies, which demonstrate the specific role of alpha2 adrenergic receptors in inhibiting AVP-mediated water absorption.

Alpha2 adrenergic stimulation has been shown to inhibit adenylate cyclase activity in the renal cortex and platelets (18, 25). The inhibition of water absorption by CD appears to be through the inhibition of AVP-induced cyclic AMP generation, since CD has no effect on 8-Br-cyclic AMP-mediated water absorption. These findings are similar to those of our previous observations where PE was shown to have no effect on cyclic AMP-mediated water absorption. Alternatively, it is possible that alpha adrenoceptor agonists may exert their inhibitory effect on the hydroosmotic response to antidiuretic hormone via an increase in cytosolic calcium. Two lines of evidence argue against this possibility. First, the effect of changes in cytosolic calcium on the hydroosmotic response of epithelia to antidiuretic hormone is controversial with both increases and decreases in cytosolic calcium causing depressed response (35, 36). Second, in the rabbit collecting tubule low peritubular sodium concentration, which raises cytosolic calcium activity, inhibited the hydroosmotic response to 8-[(p-chlorophenylthio)-cyclic 3',5'-adenosine monophosphate (37), whereas in our previous published study (9) and in the present study adrenoceptor agonists did not.

In conclusion, the results of the present studies confirm our previous observation that alpha adrenergic stimulation directly inhibits AVP-mediated water absorption in the CCT. The native catecholamine NE exerts a similar effect to that of PE in vitro, even in the low concentrations in which it exists in vivo. The inhibitory effects of alpha adrenergic stimulation on AVP-mediated water absorption can be blocked only by specific alpha2 adrenergic antagonists, but not by alpha1 adrenergic antagonists, suggesting that the alpha2 adrenergic receptors are responsible for the inhibition of AVP-mediated water absorption, at the tubular level. In addition, alpha2 adrenergic stimulation with the specific agonist clonidine directly inhibited AVP-mediated water absorption, an effect that can be blocked by a specific alpha2 adrenergic antagonist. It appears that this effect was caused by inhibiting AVP-induced cyclic AMP production. An additional effect of alpha2 adrenergic stimulation to modify the release of endogenous vasopressin, however, cannot be excluded from our studies.

Acknowledgments

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References

