Dexmedetomidine Inhibits Osmotic Water Permeability in the Rat Cortical Collecting Duct

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ABSTRACT

The purpose of this study was to determine whether the selective alpha-2 agonist dexmedetomidine inhibits basic transport properties in the rat cortical collecting duct (CCD). Sprague-Dawley rat CCDs were isolated and perfused to allow measurement of osmotic water permeability (Pf), transepithelial voltage (Vt) and resistance (Rt). Arginine vasopressin (AVP) increases Pf, hyperpolarizes Vt, and decreases Rt in the CCD via stimulation of adenylyl cyclase. Dexmedetomidine at 100 nM added to the basolateral side of the CCD reduced AVP-stimulated Pf by 95% to 100%, and the alpha-2 antagonist atipamezole reversed the inhibition. In the presence of the protein kinase C inhibitor staurosporine, dexmedetomidine reduced AVP-stimulated Pf, by 70% to 75% compared with the complete inhibition without staurosporine. When Pf was increased by the use of the nonhydrolyzable analog of cAMP, 8-chlorophenylthio-cAMP, in lieu of AVP, dexmedetomidine inhibited Pf by ~35%. This demonstrated alpha-2-mediated inhibition of Pf despite the presence of constant cellular cAMP levels. Dexmedetomidine reversed AVP-induced effects on Vt and Rt, indicating inhibition of Na\(^+\) transport. Results confirm an alpha-2-mediated mechanism that reduces Na\(^+\) and water transport in the CCD and suggest that a cellular messenger other than cAMP is involved. This messenger could be protein kinase C.

AVP increases Pf and Na\(^+\) transport in the mammalian collecting duct by stimulating adenylyl cyclase and raising cellular levels of cAMP (Grantham and Burg, 1966; Reif et al., 1984; Schafer and Troutman, 1990; Star et al., 1988). Post-cAMP cellular events involve a complex mechanism in which water channels (referred to as aquaporins) and Na\(^+\) channels are inserted into the apical membrane, thereby increasing the epithelial permeability to Na\(^+\) and water (Handler, 1988). Alpha-2 adrenoceptors appear to play an important role in modulating AVP-stimulated salt and water transport in the collecting duct (Chen et al., 1991; Hawk et al., 1993; Krothapalli et al., 1983; Rouch et al., 1991).

The alpha-2 agonist clonidine partially inhibits AVP-stimulated Pf and Jlb in the isolated-perfused rat CCD (Chen et al., 1991). Clonidine also reverses the AVP-stimulated electrophysiological responses in the rat CCD to include the hyperpolarization in Vt, decrease in Rt and reduction in the apical-membrane fractional resistance of principal cells (Rouch et al., 1991). Epinephrine completely inhibited these transport properties in the rat CCD via an apparent alpha-2-mediated mechanism, since yohimbine, an alpha-2 antagonist, prevented the effect (Hawk et al., 1993).

The cellular mechanism underlying alpha-2-mediated modulation of transport in the CCD has been attributed to the reduction in adenylyl cyclase activity. Indeed, clonidine reduces AVP-induced elevation in cAMP in microdissected rat CCDs (Chabardès et al., 1988). Epinephrine produces the same effect, which is prevented by yohimbine but not by prazosin, an alpha-1 antagonist (Umemura et al., 1985). Because cAMP appears to be the key second messenger leading to the increase in salt and water transport in the CCD, the alpha-2-mediated reduction in cellular cAMP has been viewed as the functional evidence for the alpha-2-induced inhibition of transport in this nephron segment.

Interestingly, Hawk et al. (1993) reported that epinephrine partially, although significantly, reduced Pf and Jlb in the rat CCD when these transport properties were stimulated by either forskolin, a direct stimulator of adenylyl cyclase, or bromo adenosine cAMP, a nonhydrolyzable cAMP analog. These findings demonstrated that inhibition occurred even in the presence of constant cAMP levels, indicating the involvement of at least one other cellular second messenger. It was possible that as a nonselective adrenergic agonist epineph-

ABBREVIATIONS: CCD, cortical collecting duct; IMCD, inner medullary collecting duct; Jlb, lumen-to-bath Na\(^+\) flux; Pf, osmotic water permeability; Lp, hydraulic conductivity; Vt, transepithelial voltage; Rt, transepithelial resistance; PKC, protein kinase C; 8-CPT-cAMP, 8-chlorophenylthio-cAMP; AVP, arginine vasopressin.
rines acted on more than one receptor, which contributed to the transport inhibition in a post-cAMP as well as a pre-cAMP cellular event.

In the present study, we tested the effect of the selective alpha-2 agonist dexmedetomidine on AVP, and cAMP-stimulated \( P_f \) in the rat CCD. Electrophysiological experiments were also conducted to determine the effect of dexmedetomidine on \( V_t \) and \( R_t \). Results confirm the presence of an alpha-2-mediated mechanism that inhibits water and Na transport and suggest that at least some post-cAMP cellular events are involved.

**Methods**

Male Sprague-Dawley rats weighing 50 to 150 g were obtained from barrier-maintained, pathogen-free colonies at Harlan Sprague-Dawley (Indianapolis, IN). The rats were shipped in filter cartons and on receipt were kept in a barrier-maintained room and fed standard rat chow ad libitum. All rats were used within 2 weeks of arrival.

CCDs were isolated and perfused using techniques previously described (Burg, 1972; Reif et al., 1984). Briefly, rats were killed by decapitation, both kidneys were rapidly removed and four or five coronal slices were cut from each kidney. Slices were placed in chilled Decapitation, both kidneys were rapidly removed and four or five 15–20°C bathing solution (described below) to which 6% purified coronal slices were cut from each kidney. Slices were placed in chilled perfusate and bathing solutions were made to simulate the composition of the tubular fluid normally entering the CCD and the renal interstitial fluid, respectively. The bathing solution contained 122 mM NaCl, 25 mM NaHCO\(_3\), 5 mM KCl, 5 mM Na-acetate, 2 mM Na-phosphate, pH 7.4, 1.5 mM CaCl\(_2\), 0.5 mM MgCl\(_2\), 8 mM glucose, 4 mM l-alanine and 6 mM urea. The osmolality averaged 309 ± 4.0 (±S.D.) mOsmol/kg of H\(_2\)O, and pH was adjusted to 7.4. The hypotonic perfusate contained 88 mM NaCl, 5 mM KCl, 2 mM Na-phosphate, pH 7.4, 1.5 mM CaCl\(_2\), 0.5 mM MgCl\(_2\) and 50 mM urea. The final osmolality averaged 210.4 ± 5.5 (±S.D.) mOsmol/kg of H\(_2\)O, and pH was adjusted to 6.5.

We added 30 to 50 mCr/ml of exhaustively dialyzed \( [\text{methoxy}-\text{H}] \)-H\(_2\)O to the perfusate to be used as the volume marker. The perfusion rate \( (V_t) \) was calculated as \( V_t = V_l \cdot (\text{H}^+ / \text{H}_2\text{O}) \), where \( \text{H}^+ \) and \( \text{H}_2\text{O} \) represent the \(^3\text{H}\)-inulin (cpm) in the collected fluid and perfusate, and \( V_t \) represents the collection rate that was measured directly by recording the time required to fill a constant-volume collection pipette. Net fluid flux \( (J_v; \text{nl/mm/min}) \) was calculated as \( J_v = (V_t - V_l)/(L \cdot V_t) \), where \( L \) is the tubule length measured with an eyepiece micrometer. \( P_f \) was determined from \( P_f = R_t \cdot L/(V_t + V_l) \), where \( V_t \) is the partial molar volume of water, \( R_t \) is the gas constant and \( L \) is the hydraulic conductivity coefficient, which was determined as \( L_p = 1/\text{TRGC}_p (V_t - V_l) + C_o \cdot V_l \cdot \ln (C_o / C_l \cdot V_l / C_o \cdot V_l - C_v / V_v)) \) (Du Bois et al., 1976). The luminal surface area (\( A_r \)) is represented by \( S \), and \( C_o \) and \( C_l \) represent perfusate and bath osmolality, respectively. \( V_l \) ranged from 15 to 20 nL/min. These high perfusion rates ensure that an effective osmotic gradient existed along the entire length of the tubule and that the effect of small net solute fluxes on luminal tonicity was minimal (Al-Awqati et al., 1976).

In some of the flux studies, \( V_t \) was measured by placing an Ag-AgCl wire in the perfusion pipette and connecting it to a high impedance electrometer. The circuit was completed using a NaCl-agar bridge in contact with the grounded bath via a calomel electrode, and \( V_t \) was continuously monitored on a chart recorder.

To determine \( V_t \) (mV) and transepithelial resistance \( R_t \) (\( \Omega \text{ cm}^2 \)), CCDs were dissected as described above, transferred to a perfusion chamber and mounted on concentric pipettes using the Luigs-Neuman (Ratigen, Germany) in vitro perfusion system (Greger and Schlatter, 1983). We used perfusion and bathing solutions that were identical to avoid the complications of junction potentials and other artifacts, as described previously (Schlatter and Schafer, 1987). The solutions contained 160 mM Na\(^+\), 5 mM K\(^+\), 1.5 mM Ca\(^{++}\), 1.0 mM Mg\(^{++}\), 140 mM Cl\(^-\), 25 mM HCO\(_3\), 2 mM Na-phosphate, pH 7.4, 5 mM HEPES and 5 mM glucose. The osmolality of the solutions was 315 ± 4 (±S.D.) mOsmol/kg of H\(_2\)O, and the solutions were equilibrated with 95% O\(_2\)/5% CO\(_2\) before and during the experiments. The bathing solution flowed continuously through the chamber at a rate of 20 to 40 ml/min so that it could be exchanged rapidly. Luminal perfusion rate was maintained at 15 to 20 nL/min.

Perfusion pipettes made of theta glass (double-barreled) were used to allow simultaneous measurement of \( V_t \) and \( R_t \). \( V_t \) was measured between calomel electrodes connected to 4% agar bridges in the bath and luminal perfusate through one barrel of the perfusion pipette. A high impedance electrometer completed the circuit, and the output was continuously monitored on a chart recorder. \( R_t \) was determined by placing a silver wire in the other barrel of the perfusion pipette and passing current pulses of 30 to 80 nA, 800 msec in duration, into the lumen every 8 sec. The current-induced voltage deflections, measured at the perfusion and collection ends of the tubule, were used to determine \( R_t \) via cable analysis (Greger and Schlatter, 1983).

**Experimental Protocols**

After dissection and isolation, CCDs were transferred to the room-temperature bathing solution in the perfusion chamber and mounted onto concentric pipettes. The bathing solution temperature was then raised to 37°C to 38°C over a period of 10 to 15 min. Each protocol began with a control period during which the bath contained no AVP or dexmedetomidine.

**Flux Studies**

Effect of dexmedetomidine on AVP-stimulated \( P_f \). After three individual samples of collected fluid were taken in the control period, 220 pM AVP was added to the bath, and three additional samples were taken after an equilibration period of 15 to 30 min. This period was followed by the addition of 1 mM or 100 nM dexmedetomidine to the bath and a repeat of the collection procedure. The final period included the addition of 1 mM or 100 nM atipamezole to the bath. The recorded \( P_f \) in each period was the calculated average of the three collections.

Effect of PKC inhibition. This set of experiments was conducted beginning with the control period followed by the addition of 220 pM AVP to the bath. Dexmedetomidine at 100 nM was then added to the bath, and in the final period, atipamezole at 100 nM was added to the bath. In the final two periods, 10\(^{-8}\) M staurosporine, a PKC inhibitor, was also present in the bath.

Effect of dexmedetomidine on cAMP-stimulated \( P_f \). In lieu of AVP, the nonhydrolyzable cAMP analog 8-CPT-cAMP (at 10\(^{-4}\) M) was used to stimulate \( P_f \). This was followed by the addition of dexmedetomidine and, later, atipamezole as described in the first protocol above.

**Dose response.** This protocol examined the dose response of dexmedetomidine on \( P_f \). The control period was followed by the addition of AVP to the bath. Dexmedetomidine was then added to the bath in sequential periods at 10, 50 and 100 nM.

**Electrophysiology experiments.** Tubules were mounted on concentric pipettes using the Luigs-Neuman apparatus as described above. The bathing fluid temperature was raised to 37°C to 38°C, and current pulses were injected into the tubular lumen. This protocol was designed to determine whether dexmedetomidine would reverse the AVP-induced hyperpolarization in \( V_t \) and the AVP-induced reduction in \( R_t \). The sequence of periods was the same as those described for the \( P_f \) experiments. Each experimental period lasted 15
to 20 min, and measurements were recorded throughout each period. Data reported in Results are from the final 5 to 10 min of each period.

**Source of Biochemicals**
AVP and 8CPT-cAMP were obtained from Sigma Chemical Co. (St. Louis, MO). AVP was added to the bath from a stock solution of 220 μM in water, and 8CPT-cAMP was added from aliquots of 50 mg/ml in water. Dexmedetomidine and atipamezole were kindly provided by Dr. Riku Aantaa (Research Department, Orion-Farmos Pharmaceuticals, Tukey, Finland); they were added to the bath from stock solutions in water.

**Statistical Analysis**
Data were analyzed using a one-way analysis of variance with repeated measures. Comparisons between experimental periods were made with Schefe’s test.

**Results**

**Effect of dexmedetomidine on AVP-stimulated Pf.** Figure 1 shows that either 1 μM (fig. 1A) or 100 nM (fig. 1B) dexmedetomidine inhibited AVP-stimulated Pf by 95% to 100% (P < .001). Atipamezole at the same concentration as dexmedetomidine reversed the inhibition. Figure 2 shows that dexmedetomidine did not affect basal Pf. The addition of AVP to the bath in the third period raised Pf slightly, and the addition of atipamezole in the final period elevated Pf markedly.

**Effect of dexmedetomidine on AVP-stimulated Pf in the presence of staurosporine.** Figure 3 shows that 100 nM dexmedetomidine significantly reduced AVP-stimulated Pf when 10^-8 M staurosporine was present in the bath. In this experimental condition, dexmedetomidine reduced Pf 70% to 75% compared with the virtual 100% inhibition without staurosporine.

**Dose response of dexmedetomidine on Pf.** Figure 4 shows that dexmedetomidine at 10, 50 and 100 nM reduced AVP-stimulated Pf by 54%, 85% and 100%, respectively.

**Effect of dexmedetomidine on 8CPT-cAMP-stimulated Pf.** Figure 5 shows that 100 nM dexmedetomidine inhibited Pf stimulated by 10^-2 M 8CPT-cAMP. Atipamezole reversed this effect. It should be noted that the degree of inhibition was partial at ~35% and a higher number of measurements was required to achieve statistical significance compared with the AVP-stimulated condition.

**Effect of dexmedetomidine on Vi & Rt.** Figure 6A shows that dexmedetomidine depolarized the AVP-induced hyperpolarization in Vi. Dexmedetomidine also increased AVP-induced reduction in Rt (fig. 6B). Atipamezole reversed the effect on Vi. Additional results on Vi recorded from flux studies of figure 1B are provided in figure 7A. These data also show the depolarizing effect of dexmedetomidine on AVP-induced hyperpolarization. Figure 7B shows the chart recording of one of these experiments. The effect of dexmedetomidine occurred within minutes of the addition of the agent to the bath.

**Discussion**
The major findings of this study were that in the perfused rat CCD, the selective alpha-2 agonist dexmedetomidine reduced (1) AVP-stimulated Pf by 95% to 100%, (2) AVP-stimulated Pf in the presence of the PKC inhibitor staurosporine by 70% to 75% and (3) 8CPT-cAMP-stimulated Pf by 25% to 35%. Dexmedetomidine significantly reversed the AVP-induced effects on Vi and Rt. Overall, these findings indicate an alpha-2-mediated mechanism that modulates AVP-dependent Na^+ and water transport in the rat CCD, and the mechanism appears to include post-cAMP events in addition to the pre-cAMP event of alpha-2-induced inhibition of adenylyl cyclase.

Although the precise physiological function of renal alpha-2 adrenoceptors is not yet defined, it is well known that alpha-2 agonists enhance urine output. Blandford and Smyth (1988, 1989) and Smyth et al. (1992) demonstrated that this effect is mediated in at least two separate sites in
the kidney: one is non-AVP dependent, in which urine output is secondary to increased osmolar clearance, and the other is AVP dependent, in which urine output is secondary to increased free water clearance. The latter site occurs in the collecting duct in which AVP increases epithelial water permeability and plays a critical role in regulating salt and water balance (Al-Zahid et al., 1977; Ausiello et al., 1987; Grantham and Burg, 1966; Schafer et al., 1991; Tomita et al., 1985). Dexmedetomidine alone did not affect $P_f$ (fig. 2), indicating that the alpha-2 effect in the collecting duct indeed depends on the presence of AVP.

The effect of alpha-2 agonists on AVP-dependent transport function in the collecting duct has been studied previously. Chen et al. (1991) reported that 1 μM clonidine induced 30% to 40% inhibition of AVP-stimulated $P_f$, $J_{H^+}$, and $V_i$ in the isolated rat CCD. Krothapalli and Suki (1984) reported that norepinephrine significantly reduced AVP-stimulated $P_f$ in the rabbit CCD and that the alpha-2 antagonist yohimbine blocked this effect, whereas prazosin, an alpha-1 antagonist, did not. These authors also reported that clonidine significantly reduced AVP-stimulated $P_f$ in the rabbit CCD but did not affect $P_f$ stimulated by 8-bromo adenosine cAMP. In an earlier study, Krothapalli et al. (1983) showed that the non-selective adrenergic agonist phenylephrine did not affect

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**Fig. 2.** Dexmedetomidine does not affect basal $P_f$. In this protocol shown on the horizontal axis, 100 nM dexmedetomidine added to the bath did not affect $P_f$ compared with control values. The subsequent addition of 220 pM AVP raised $P_f$ slightly, and the addition of 100 nM atipamezole raised $P_f$ significantly ($P < .001$). Each line represents a single experiment, and the mean ± S.E. values are shown above each experimental period.

**Fig. 3.** Inhibition of $P_f$ by dexmedetomidine in the presence of staurosporine. AVP at 220 pM added to the bath raised $P_f$ compared with control values ($P < .001$). Dexmedetomidine at 100 nM inhibited $P_f$ by 70% to 75% in the presence of $10^{-6}$ M staurosporine (St), a PKC inhibitor. This period (AVP+Dex+St) was different from the control period ($P < .01$). Atipamezole at 100 nM significantly reversed the dexmedetomidine-induced inhibition ($P < .005$). Each line represents a single experiment, and the mean ± S.E. values are shown above each experimental period.

**Fig. 4.** Dose response of dexmedetomidine-induced inhibition of AVP-stimulated $P_f$. AVP at 220 pM raised $P_f$ compared with control levels ($P < .001$). Dexmedetomidine added to the bath at 10, 50 and 100 nM reduced $P_f$ by 54%, 86%, and 100%, respectively. Each line represents a single experiment, and the mean ± S.E. values are shown above each experimental period.

**Fig. 5.** Dexmedetomidine inhibits cAMP-stimulated $P_f$. The set of experiments followed the same protocol as shown in figure 1 except that $10^{-4}$ M 8-CPT-cAMP (cAMP) was used in lieu of AVP. The addition of cAMP raised $P_f$ ($P < .001$), and dexmedetomidine at 100 nM significantly reduced $P_f$ ($P < .01$). Atipamezole in the final period reversed the inhibition ($P < .001$). Each line represents a single experiment, and the mean ± S.E. values are shown above each experimental period.
reversed these effects but prazosin did not, indicating an alpha-2-mediated mechanism. Ribeiro et al. (1987) demonstrated that the alpha-2-mediated inhibition of Pᵢ in the rabbit CCD was attenuated by pretreatment with pertussis toxin, which inactivates the inhibitory G protein via ADP ribosylation. Taken together, these findings demonstrate that alpha-2 adrenoceptors in the CCD are coupled to an inhibitory G protein that inhibits adenyl cyclase when activated and thereby reduces cellular cAMP levels.

Evidence suggesting that this alpha-2-inhibitory transport mechanism could involve post-cAMP dependent events in the rat CCD came from Hawk et al. (1993). They studied the effects of epinephrine on transport properties in CCDs from Sprague-Dawley rats as well as Dahl salt-sensitive and salt-resistant rats. They demonstrated that 100 nM epinephrine inhibited by 80% to 100% the AVP-stimulated Jᵢₖ and Pᵢ from all strains and that yohimbine reversed the inhibition.

These rats were treated with deoxycorticosterone, which induces mineralocorticoid effects on Na⁺ transport and synergistically increases the AVP-stimulated Jᵢₖ in the rat CCD (Chen et al., 1990). Thus, epinephrine significantly inhibited not only the cAMP-dependent component of Jᵢₖ stimulated by AVP but also the component stimulated by deoxycorticosterone, which is cAMP independent. This suggested that the alpha-2 inhibitory mechanism utilized cellular events in addition to reducing cAMP accumulation.

The authors also showed that epinephrine partially inhibited Jᵢₖ and Pᵢ stimulated by 8-bromo adenosine cAMP. Although the level of inhibition was relatively small (~20–30%) compared with that of the AVP-stimulated transport (~80–100%), data indicated that epinephrine produced inhibition despite the presence of constant cellular cAMP levels. This suggested that a second-messenger system other than that involved with cAMP played a role. In addition, epinephrine could have acted on more than one adrenergic adrenoceptor.

In the present study, we tested the effect of the selective alpha-2 agonist dexmedetomidine on Pᵢ in the isolated rat CCD. Dexmedetomidine was at least as effective as epinephrine in reducing AVP-stimulated Pᵢ (Hawk et al., 1993), with significant inhibition observed at 10 nM (fig. 4). When we stimulated Pᵢ with 8CPT-cAMP, dexmedetomidine significantly reduced Pᵢ although to a lesser degree than that observed with AVP. Snyder et al. (1992) reported that 8CPT-cAMP was a potent activator of protein kinase A in CCD cells and suggested that it should be the cAMP analog of choice in functional studies like the isolated-perfused tubule technique. Atipamezole reversed the inhibitory effects, demonstrating its effectiveness as an alpha-2 antagonist in the rat CCD.

It should be noted that obtaining statistical significance with alpha-2-mediated inhibition of cAMP-stimulated Pᵢ was difficult in our study as well as in the study of Hawk et al. (1993), who reported that epinephrine significantly reduced 8-bromo adenosine cAMP-stimulated Pᵢ in the Dahl salt-sensitive and salt-resistant rat CCDs but not in the Sprague-Dawley rat CCD, although in the latter, inhibition was observed in four of six experiments. The partial inhibitory effect of dexmedetomidine and the relatively large variability associated with the Pᵢ measurement contribute to this difficulty.

We showed that dexmedetomidine reduced 8-CPT-cAMP-stimulated Pᵢ in 10 of 13 experiments (fig. 5), ranging from
10% to 85% with an average inhibition of 35%. Although a higher number of experiments was required to obtain statistical significance, we interpret our results coupled with those from Hawk et al. (1993) to indicate that at least a portion of the alpha-2-mediated inhibition in the rat CCD occurs via post-cAMP-dependent events.

These findings now lead to the question of what other second messengers are involved in this mechanism. The commonly studied cellular messengers related to salt and water transport in the CCD include PKC, calcium and prostaglandins (Ando et al., 1987; Hays et al., 1987; Jones et al., 1988; Rouch et al., 1993). Findings shown in figure 3 suggest that PKC might be one of these messengers. Dexmedetomidine inhibited AVP-stimulated P_f by 70% to 75% in the presence of the PKC inhibitor staurosporine in contrast to the 100% inhibition by dexmedetomidine in the absence of staurosporine.

Mori et al. (1989) reported that epinephrine, via alpha-2 adrenoreceptor-mediated action, stimulated phosphoinositide turnover in platelets. It is well-known that phosphoinositide turnover leads to the production of inositol trisphosphate and diacylglycerol; the former increases intracellular calcium, and the latter stimulates PKC. These authors suggested that two types of alpha-2 adrenoceptors exist in platelets: one is coupled to the inhibition of adenylyl cyclase, and the other is coupled to the phosphoinositide system. Although additional experiments are required to confirm the involvement of PKC, calcium or other messengers in the alpha-2-mediated inhibitory mechanism, it is interesting to note the evidence reported by Nadler et al. (1992) demonstrating that prostaglandin E_2 inhibited P_f via a post-cAMP event in the IMCD and that staurosporine prevented this effect.

It should also be recognized that the collecting duct is structurally and functionally heterogeneous as demonstrated by the differences between the CCD and IMCD (Jacobson, 1981; Madsen and Tisher, 1986; Ridderstrale et al., 1987). It now appears this heterogeneity is further demonstrated by the alpha-2-mediated inhibitory mechanism of AVP-stimulated transport. We recently reported that dexmedetomidine completely inhibited AVP-stimulated as well as dibutyryl cAMP-stimulated P_f in the rat IMCD (Rouch and Kudo,
1996). These findings provide stronger evidence that alpha-2 agonists inhibit AVP-stimulated transport in the collecting duct via post-cAMP-dependent events. However, our data in the present study indicate that although a portion of the alpha-2-mediated inhibition occurs via a post-cAMP process, the mechanism in the CCD is predominately pre-cAMP.

Although we did not directly measure Na\(^+\) transport in this study, the results shown in figures 6 and 7 illustrate that dexametomidine reversed the AVP-stimulated effects on V\(_t\) and R, and are consistent with alpha-2-mediated inhibition of Na\(^+\) transport. AVP increases Na\(^+\) transport in the CCD by stimulating adenyl cyclase, and cAMP appears to be the key messenger as it is in the AVP-stimulated P\(_f\) response (Schafer and Troutman, 1990). Post-cAMP events related to Na\(^+\) transport remain controversial. Two major hormones increase Na\(^+\) transport in the CCD: AVP and aldosterone. Evidence suggests that AVP-stimulated Na\(^+\) transport results from the insertion of Na\(^+\) channels into the apical membrane from cytoplasmic vesicles, whereas the steroid-stimulated increase in Na\(^+\) transport results from the opening of silent, or “cryptic,” channels in the apical membrane (Kleyman et al., 1989; Li et al., 1982; Ling et al., 1990; Marunaka and Eaton, 1991). Our data suggest that the alpha-2 agonist reduces the apical-membrane Na\(^+\) conductance, which is consistent with other studies (Hawk et al., 1993; Rouch et al., 1991).

Another important issue deserving attention relates to the three characterized alpha-2 adrenoceptor subtypes: alpha-2A, alpha-2B and alpha-2C (Bylund et al., 1994). It is possible that dexametomidine couples with more than one alpha-2 subtype and activates two separate second-messenger systems, one inhibiting adenyl cyclase and the other activating PKC, as suggested by Morii et al. (1989). The specific adrenoceptor subtypes in the CCD have yet to be identified, although recently, experiments reported by Wilborn et al. (1996) showed that multiple alpha-2 adrenergic isoforms exist in the rat CCD. Thus, it is possible that the dexametomidine-induced inhibition of transport observed in this study involves a cAMP-dependent and a cAMP-independent mechanism mediated by different alpha-2 adrenoceptor subtypes.

Evidence indicates that dexametomidine and atipamezole are nonselective as an alpha-2 agonist and antagonist, respectively (Codd et al., 1995). Future studies should focus on the subtype-selective compounds identified from pharmacological binding studies. For example, preliminary data from our laboratory indicate that oxymetazoline, a relatively selective alpha-2A agonist (Bylund et al., 1994; Codd et al., 1995), partially inhibits AVP-stimulated P\(_f\) in the rat CCD (data not shown). Additional studies are needed to correlate pharmacological binding data with functional data related to transport inhibition.

Imidazoline receptors offer another possibility. Allan et al. (1993) reported that the imidazoline agonist moxonidine, when infused intrarenally into the rat, increased Na\(^+\) and water excretion. The higher urine output was accompanied with an increase in osmolar clearance but not free water clearance, and the addition of a V\(_2\) vasopressin receptor antagonist did not alter the imidazoline-induced effect. This is consistent with the increase by moxonidine of Na\(^+\) and water excretion via a mechanism other than the inhibition of AVP-stimulated transport and suggests that the site of action is not in the collecting duct. Nevertheless, the data presented in our study do not exclude a role for imidazoline receptors.

Dexametomidine can now be added to the list of adrenergic agonists that inhibit transport in the CCD; others include clonidine, phenylephrine, epinephrine and norepinephrine. Dexametomidine and epinephrine are the most effective at inhibiting AVP-stimulated transport in the CCD. Atipamezole can be added as an effective antagonist along with yohimbine and phentolamine. The other tested antagonist prazosin fails to reverse the agonist-induced inhibition of AVP-stimulated P\(_f\) (Krothapalli and Suki, 1984) or AVP-stimulated increase in cAMP in the CCD (Umemura et al., 1985).

In summary, this study confirms an alpha-2-mediated mechanism that inhibits AVP-stimulated Na\(^+\) and water transport in the rat CCD. The selective alpha-2 agonist dexametomidine completely inhibited AVP-stimulated P\(_f\) and partially, although significantly, inhibited SCPT-cAMP-stimulated P\(_f\). These findings indicate that inhibition of water transport in the CCD involves pre-cAMP as well as post-cAMP cellular events.

References


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