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Am J Physiol Renal Physiol, December 1, 2008; 295 (6): F1715-F1724.

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Role of PGE₂ in α_2 -induced inhibition of AVP- and cAMP-stimulated H₂O, Na⁺, and urea transport in rat IMCD

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Rouch, Alexander J., and Lúcia H. Kudo. Role of PGE₂ in α_2 -induced inhibition of AVP- and cAMP-stimulated H₂O, Na⁺, and urea transport in rat IMCD. *Am J Physiol Renal Physiol* 279: F294–F301, 2000.—PGE₂ inhibits osmotic water permeability (P_f) in the rat inner medullary collecting duct (IMCD) via cellular events occurring after the stimulation of cAMP, i.e., post-cAMP-dependent events. The α_2 -agonists also inhibit P_f in the rat IMCD via post-cAMP-dependent events. The purpose of this study was to determine whether PGE₂ plays a role in α_2 -mediated inhibition of P_f , Na⁺, and urea transport in the rat IMCD. Isolated terminal IMCDs from Wistar rats were perfused to measure, in separate experiments, P_f , lumen-to-bath ²²Na⁺ transport (J_{lb}), and urea permeability (P_u). Transport was stimulated with 220 pM arginine vasopressin (AVP) or 0.1 mM 8-(4-chlorophenylthio)-cAMP (CPT-cAMP). Indomethacin was used to inhibit endogenous prostaglandin synthesis, and the α_2 -agonists clonidine, oxymetazoline, and dexmedetomidine were used to test the role of PGE₂ in the α_2 -mediated mechanism that inhibits transport. All agents were added to the bath. Indomethacin at 5 μ M significantly elevated CPT-cAMP-stimulated P_f , J_{lb} , and P_u , and subsequent addition of 100 nM PGE₂ reduced these transport parameters. Indomethacin reversed α_2 inhibition of CPT-cAMP-stimulated P_f , J_{lb} , and P_u , and subsequent addition of PGE₂ reduced transport in each case. Indomethacin partially reversed α_2 inhibition of AVP-stimulated P_f , J_{lb} , and P_u , and PGE₂ reduced transport back to the α_2 -inhibited level. These results indicate that PGE₂ is a second messenger involved in the mechanism of transport inhibition mediated by α_2 -adrenoceptors via post-cAMP-dependent events in the rat IMCD.

signaling pathways; second messengers; inner medullary collecting duct; α_2 -adrenoceptor; osmotic water permeability

SALT, WATER, AND UREA TRANSPORT in the inner medullary collecting duct (IMCD) play an important role in the renal regulation of salt and water excretion. Arginine vasopressin (AVP) stimulates these transport properties enhancing absorption in the IMCD, and α_2 -agonists inhibit AVP-dependent transport (20, 29). This inhibitory mechanism has been associated with α_2 -adrenoceptors coupling to an inhibitory G (G_i) protein that decreases adenylyl cyclase activity, reducing cellular levels of cAMP (8, 11, 20, 34, 36).

Evidence indicates that this inhibitory mechanism occurs in the presence of constant cellular cAMP levels. When water transport in collecting duct nephron segments is stimulated by nonhydrolyzable analogs of cAMP in lieu of AVP, α_2 -agonists still produce significant inhibition (12, 29). The mechanism therefore appears to be more complex than just reducing adenylyl cyclase activity and must involve other second messengers.

PGE₂ has been studied extensively as a potential regulator of renal salt and water excretion and has been shown to affect these transport properties in the collecting duct (2, 14, 15). Nadler et al. (22) reported that PGE₂ reduced osmotic water permeability (P_f) stimulated by a nonhydrolyzable cAMP analog in the rat IMCD, indicating that PGE₂ inhibits P_f via post-cAMP-dependent events, and inhibition of protein kinase C (PKC) by staurosporine prevented the PGE₂-induced inhibition. They also reported that PGE₂ increased intracellular calcium concentration ($[Ca^{2+}]_i$) levels. These findings suggest a role for the phospholipase C metabolites in controlling water permeability.

Because both α_2 -agonists and PGE₂ inhibit water permeability via post-cAMP-dependent events in the IMCD, we hypothesized that the α_2 -inhibitory mechanism involves PGE₂ as a second messenger. The purpose of the present study was to test this hypothesis on water, Na⁺, and urea transport in the isolated rat IMCD. Results indicate that PGE₂ indeed plays a role in the α_2 -inhibitory mechanism of these transport processes in the IMCD. The specific action of PGE₂ in this mechanism remains to be determined.

MATERIALS AND METHODS

IMCD segments were isolated and perfused by techniques previously described (4, 26, 28). Wistar rats weighing 120–125 g and fed a standard chow (184 meq Na/kg) were killed by decapitation, and the kidneys were rapidly removed and cut into small slices that were placed in chilled dissection solution of the same composition as the bathing solution or bath described below. IMCD segments were dissected and isolated from the terminal two-thirds of the inner medulla, i.e., the terminal IMCD (19, 30).

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After isolation, the IMCD was transferred to a perfusion chamber on the stage of an inverted microscope and mounted on concentric pipettes that suspended the tubule in the bath. One end of the tubule was drawn by suction into the tip of one of the outer pipettes. The tip of the inner pipette containing the luminal perfusion solution, or perfusate, was advanced into the lumen of the tubule, and perfusion was initiated via air pressure.

The opposite end of the tubule was held in the tip of another glass micropipette where the perfusate accumulated. The tip of this pipette was coated with a viscous silicone liquid (Sylgard 184, Dow Corning) to isolate the perfusate from the bath. Samples of collected perfusate were taken periodically during an experiment with a constant-volume or volumetric pipette. The bath composition was as follows (in mM): 115 NaCl, 25 NaHCO₃, 10 sodium acetate, 5 KCl, 1.0 CaCl₂, 1.2 MgSO₄, 1.2 NaH₂PO₄, and 5.5 glucose, pH = 7.4. The solution was continuously bubbled with 95% O₂-5% CO₂. All experiments were conducted at 37°C.

P_f was determined by measuring net fluid flux (J_v) in the presence of a lumen-to-bath osmotic gradient (80–90 to 295–300 mosmol/kgH₂O). The perfusion solution was made hypotonic to the bath by reducing NaCl concentration to 50 mM, and rapid perfusion rates of 20–30 nl/min were used to avoid osmotic equilibrium. [¹⁴C]inulin at 50–100 counts/min (cpm)/nl in the luminal perfusate was used as the volume marker. Perfusion rate (V_i) was calculated as $V_i = V_o(IN_i/IN_o)$, where IN_i and IN_o are the inulin cpm per nanoliter in the initial luminal perfusate and collected fluid, respectively, and V_o is the collection rate. V_o was determined directly by measuring the time to fill the volumetric pipette, and J_v (nl · mm⁻¹ · min⁻¹) was then calculated as $J_v = (V_i - V_o)/l$, where l is the tubule length measured with an eyepiece micrometer. Three timed fluid samples were collected in each experimental period. The P_f of each collection was calculated with established methods and equations (1), and the reported P_f for a given experimental period represents the average of the three determinations.

Lumen-to-bath ²²Na⁺ transport (J_{lb}) was determined by measuring the disappearance rate of ²²Na⁺ from the lumen. Perfusion and bath solutions were identical, and the composition was the same as the bath solution noted above. Three timed fluid samples were collected in each experimental period, and the reported J_{lb} for a given experimental period represents the average of the three determinations. J_{lb} (peq · cm² · s⁻¹) was calculated as

$$J_{lb} = \frac{[Na^+]}{A \cdot 60} V_i \left(1 - \frac{C_o}{C_i} \right)$$

where $[Na^+]$ is the $[Na^+]$ in the bath, A is the luminal area (cm²), and C_o and C_i are the collected and perfused activities of ²²Na⁺, respectively.

P_u was determined from the disappearance rate of [¹⁴C]urea (50–100 cpm/nl) from the luminal perfusate. As in the J_{lb} experiments, perfusion and bath solutions were identical.

Because there was no net fluid absorption, P_u was calculated with the following equation

$$P_u = \frac{V_i}{A} \ln \frac{C_i}{C_o}$$

where A is the inner surface area of the tubule, and C_i and C_o are the activities of [¹⁴C]urea (cpm/nl) in the initial luminal perfusate and collected fluid, respectively. The reported P_u values represent the averages of three individual samples taken in each experimental period.

Experimental protocols. Once the IMCD was mounted on concentric pipettes, perfusion was initiated and the bath temperature was raised to 37°C in 10–15 min. After an equilibration period of 30–35 min, the sampling procedure for the control period began. After three collections were taken, 220 pM AVP or 0.1 mM 8-(4-chlorophenylthio)-cAMP (CPT-cAMP) was added to the bath, followed by 15–20 min of equilibration and the sampling procedure. Other agents were added in subsequent experimental periods, followed by equilibration and the sampling procedure. The sequence of a given protocol is shown on the abscissa of Figs. 1–4 and described in their legends.

The α₂-agonists clonidine, oxymetazoline, and dexmedetomidine were used at 100 nM. In dose-response protocols, we found this to be the level that produced maximal or near-maximal inhibition (17, 29). Dexmedetomidine is the most potent of the three, with clonidine and oxymetazoline demonstrating equal potency.

Source of biochemicals. AVP, CPT-cAMP, oxymetazoline, and epinephrine were purchased from Sigma Chemical (St. Louis, MO). Clonidine and dexmedetomidine were kindly provided by Boehringer Ingelheim (Ridgefield, CT), and by Dr. Riku Aantaa, Chief of Research, Orion-Farmos Pharmaceutical, Turku, Finland, respectively. [¹⁴C]inulin was purchased from New England Nuclear (Boston, MA).

Statistical analysis. Data were analyzed with a single-factor ANOVA with repeated measures, and P values between treatments were determined by using the SuperAnova statistical package. $P < 0.05$ was considered significant.

RESULTS

Table 1 summarizes data showing that indomethacin increased CPT-cAMP-stimulated P_f , J_{lb} , and P_u and that subsequent addition of PGE₂ reversibly decreased each form of transport.

Figure 1 shows that clonidine, oxymetazoline, and dexmedetomidine inhibited CPT-cAMP-stimulated P_f by 25, 22, and 82%, respectively. In each protocol, addition of indomethacin increased P_f , and subsequent addition of PGE₂ lowered P_f back to the α₂-inhibited level in the clonidine and oxymetazoline protocols. In the dexmedetomidine protocol, PGE₂ reduced P_f signif-

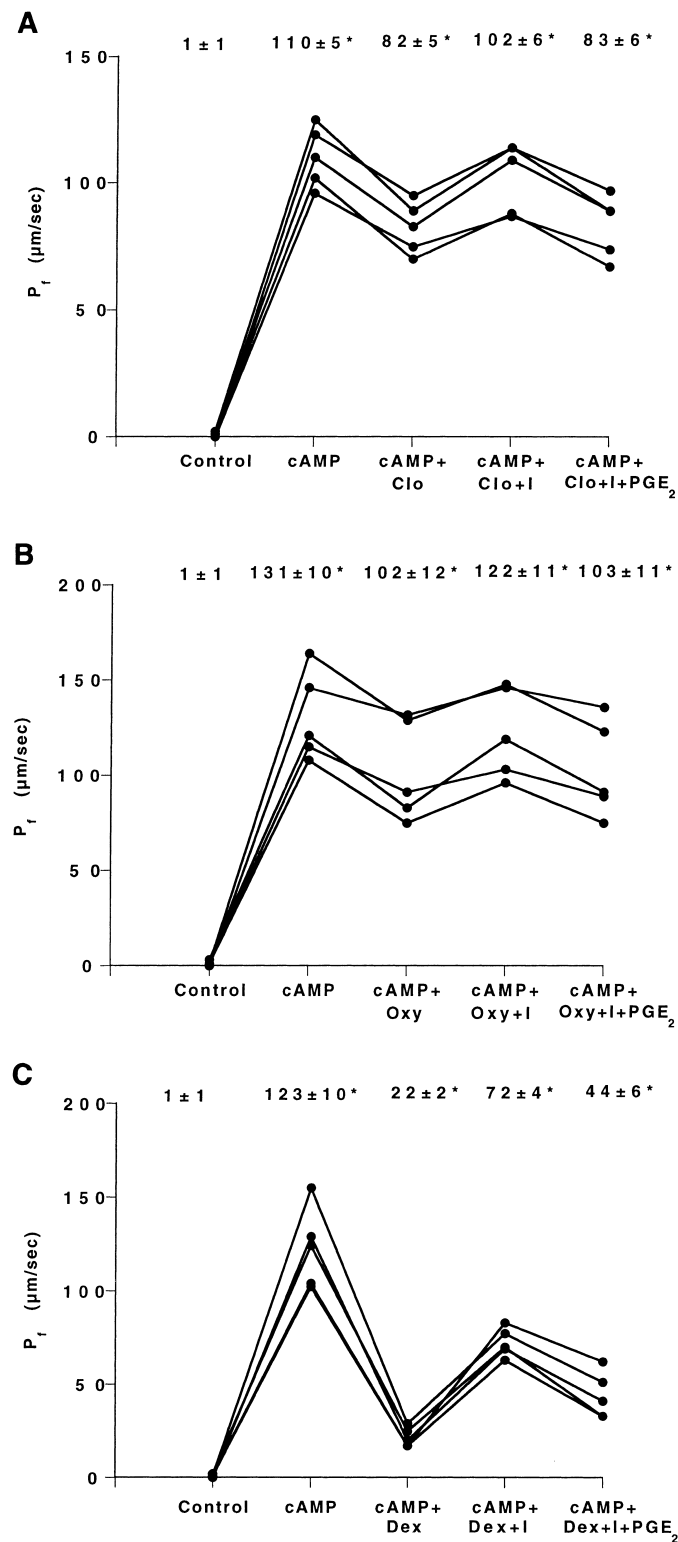
Table 1. Effect of indomethacin and PGE₂ on CPT-cAMP-stimulated P_f , J_{lb} , and P_u

Experiment Type	Control	cAMP	cAMP + I	cAMP + I + PGE ₂	cAMP + I
P_f , μm/s	2 ± 1	109 ± 5*	126 ± 5*	103 ± 5*	120 ± 5*
J_{lb} , peq · cm ² · s ⁻¹	543 ± 16	837 ± 25*	934 ± 15*	777 ± 17*	898 ± 13*
P_u , × 10 ⁻⁵ cm/s	13.7 ± 0.5	22.9 ± 1.6*	27.1 ± 1.0*	26.5 ± 0.7	30.6 ± 1.8*

Values are means ± SE; $n = 5$ in each set of experiments. P_f , osmotic water permeability; J_{lb} , lumen-to-bath ²²Na⁺ flux; P_u , urea permeability; 8-(4-Chlorophenylthio)-cAMP (CPT-cAMP; cAMP in table) was used at 0.1 mM, indomethacin (I) at 5 μM, and PGE₂ at 100 nM. *Significantly different from previous period.

icantly, although not completely back to the α_2 -inhibited level.

Figure 2A shows that PGE₂ prevented the indomethacin-induced increase in P_f reported in Table 1 and that the PKC inhibitor staurosporine failed to affect P_f with indomethacin and PGE₂ in the bath. In



another protocol, staurosporine slightly although significantly increased P_f with indomethacin, PGE₂, and the α_2 -agonist dexmedetomidine in the bath (Fig. 2B).

Figure 3 shows that clonidine, oxymetazoline, and dexmedetomidine inhibited CPT-cAMP-stimulated J_{lb} by 54, 56, and 77%, respectively. In each protocol indomethacin reversed the inhibition, and subsequent addition of PGE₂ lowered J_{lb} back to the α_2 -inhibited level.

Figure 4 shows that epinephrine at 100 nM and 1 μ M (A and B, respectively) inhibited CPT-cAMP-stimulated P_u . In both protocols, indomethacin significantly increased P_u and subsequent addition of PGE₂ lowered P_u . Clonidine and oxymetazoline slightly inhibited CPT-cAMP-stimulated P_u , as shown in Table 2.

Table 3 summarizes data of the effect of PGE₂ on α_2 -mediated inhibition of AVP-stimulated P_f , J_{lb} , and P_u . The experimental protocol in these studies was the same as that in Figs. 1, 3, and 4, except that AVP not CPT-cAMP was used to stimulate transport. Clonidine and oxymetazoline inhibited AVP-stimulated P_f , and indomethacin reversed the inhibition by 51 and 25%, respectively. Subsequent addition of PGE₂ lowered P_f back to the α_2 -inhibited level in both protocols. Clonidine and oxymetazoline inhibited AVP-stimulated J_{lb} , and indomethacin reversed inhibition by 54 and 65%, respectively. Subsequent addition of PGE₂ reduced J_{lb} back to the α_2 -inhibited level in both protocols. Clonidine inhibited AVP-stimulated P_u by 27%. Indomethacin reversed this inhibition by 52%, and subsequent addition of PGE₂ lowered P_u back to the clonidine-inhibited level. The same trend occurred with oxymetazoline, but statistical significance was not achieved.

DISCUSSION

PGE₂ modulates transport in the collecting duct by affecting multiple cellular signaling pathways [see review by Hébert (13)]. With regard to this modulation, it is important to note species differences, segmental differences along the collecting duct, and experimental conditions. Sonnenburg and Smith (31) reported that in purified rabbit CCD cells PGE₂ elevated basal cAMP content and reduced AVP-stimulated cAMP accumulation; however, PGE₂ failed to affect AVP-stimulated cAMP in cultured cells. Using different techniques, Noland et al. (23) demonstrated that PGE₂ can inhibit

Fig. 1. Effect of PGE₂ on α_2 -inhibition of cAMP-stimulated osmotic water permeability (P_f). After the control period, 8-(4-chlorophenylthio)-cAMP (CPT-cAMP) at 0.1 mM (cAMP in figure) increased P_f ($P < 0.001$). Addition of 100 nM clonidine (Clo; A), oxymetazoline (Oxy; B), or dexmedetomidine (Dex; C) decreased P_f ($P < 0.001$). Addition of 5 μ M indomethacin (I) increased P_f in each protocol ($P < 0.01$), and addition of 100 nM PGE₂ reduced P_f back to the α_2 -inhibited level in A and B ($P < 0.01$) but not in C, although PGE₂ significantly decreased P_f in C ($P < 0.001$). The sequence of experimental periods is shown on the x-axis. All agents were added to the bath. P values are with respect to the previous period. Each line represents 1 experiment of 1 inner medullary collecting duct (IMCD) from 1 rat. Values (means \pm SE) are shown above each experimental period. *Significantly different from previous period.

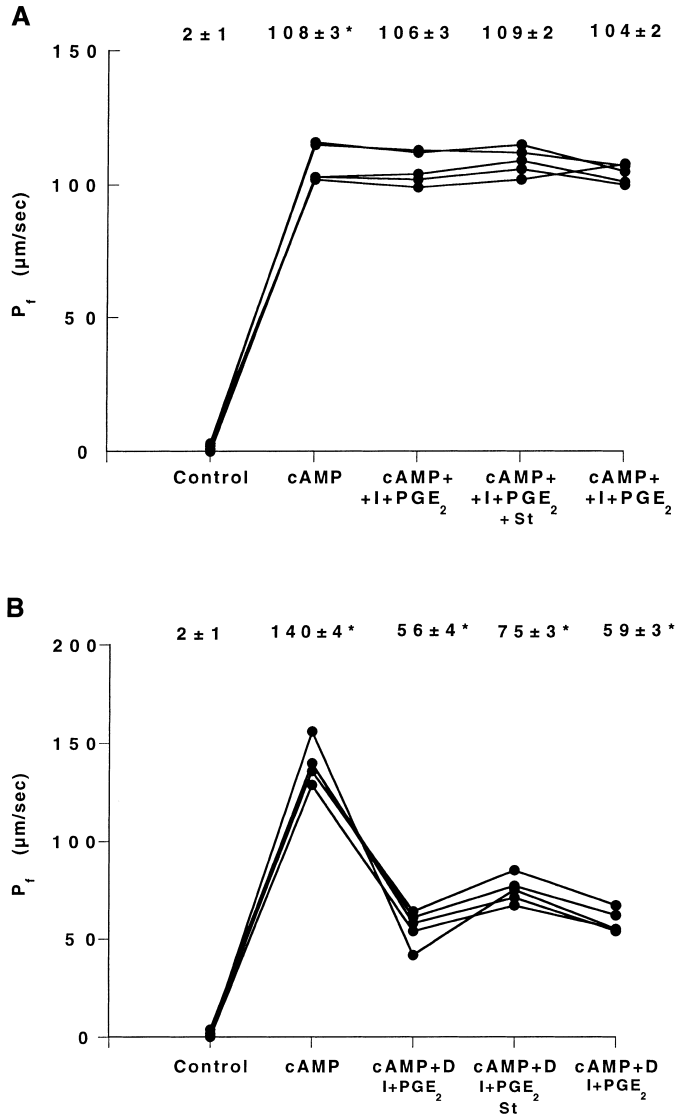


Fig. 2. Effect of staurosporine (St) on PGE₂ and α₂-inhibition of P_f. A: addition of CPT-cAMP at 0.1 mM to the bath increased P_f (P < 0.001). Addition of 5 μM indomethacin and 100 nM PGE₂ did not affect P_f, and P_f was not affected by subsequent addition of 10 nM staurosporine. B: addition of cAMP increased P_f (P < 0.001). Addition of 100 nM dexmedetomidine (D) with 5 μM indomethacin and 100 nM PGE₂ decreased P_f (P < 0.001). Addition of 10 nM staurosporine reversibly increased P_f by 22% (P < 0.01). (See legend of Fig. 1 for format.) *Significantly different from previous period.

AVP-induced cAMP accumulation in cultured rabbit CCD cells. Chabardès et al. (5) reported that PGE₂ reduced AVP-stimulated cAMP in dissected rabbit CCDs but not in rat CCDs, and Chen et al. (6) provided transport data consistent with these findings in that PGE₂ inhibited AVP-stimulated P_f and J_{lb} in the rabbit but not rat CCD.

Although PGE₂ modulates transport in the rabbit but not the rat CCD and the effect appears to be related to PGE₂ regulation of cAMP, PGE₂ plays a role in the rat IMCD by an apparent cAMP-independent mechanism. Maeda et al. (20) reported that PGE₂ did not affect cAMP content in the rat IMCD

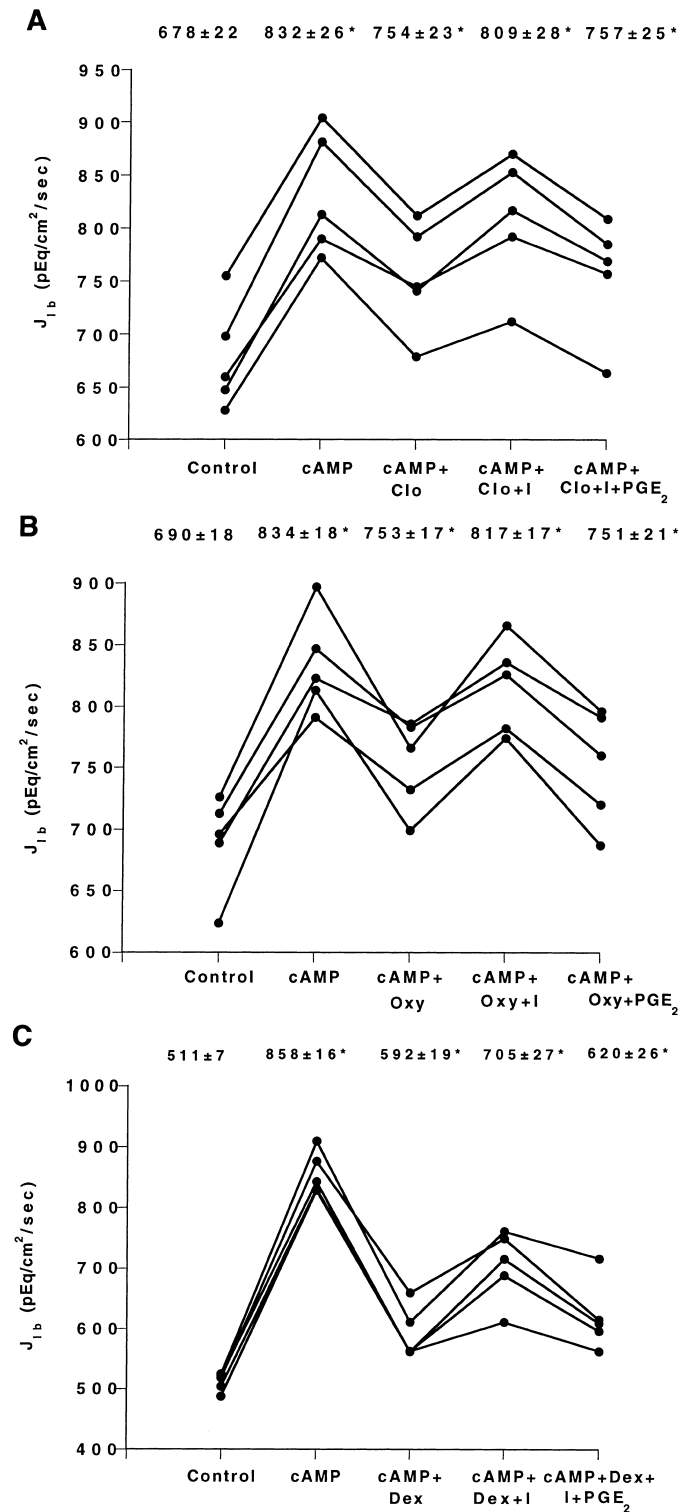


Fig. 3. Effect of PGE₂ on α₂-inhibition of cAMP-stimulated lumen-to-bath ²²Na⁺ transport (J_{lb}). After the control period, CPT-cAMP at 0.1 mM increased J_{lb} (P < 0.001). Addition of 100 nM clonidine (A), oxymetazoline (B), or dexmedetomidine (C) decreased J_{lb} (P < 0.001). Addition of 5 μM indomethacin increased J_{lb} in each protocol (P < 0.001), and addition of 100 nM PGE₂ reduced P_f back to the α₂-inhibited level (P < 0.001). (See legend of Fig. 1 for format.) *Significantly different from previous period.

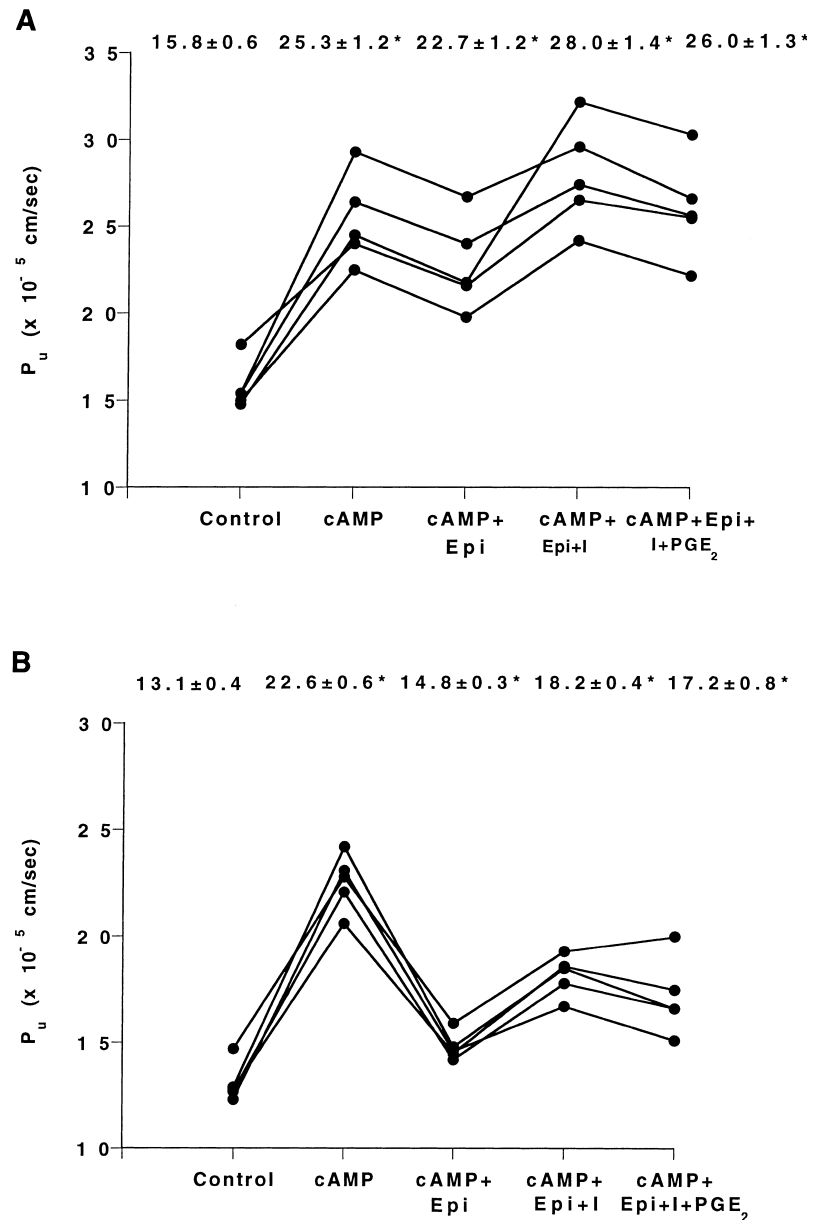


Fig. 4. Effect of PGE₂ on epinephrine-induced inhibition of cAMP-stimulated urea permeability (P_u). Addition of 0.1 mM CPT-cAMP increased P_u ($P < 0.001$). Epinephrine (Epi) added at 100 nM (A) or 1 μ M (B) reduced P_u by 27 and 82%, respectively. Addition of 5 μ M indomethacin raised P_u in both protocols ($P < 0.01$), and subsequent addition of 100 nM PGE₂ decreased P_u ($P < 0.05$). (See legend of Fig. 1 for format.) *Significantly different from previous period.

with or without AVP. Nadler et al. (22) reported that 100 nM PGE₂ reversibly inhibited CPT-cAMP-stimulated P_f in the isolated rat IMCD by ~40%, and the PKC inhibitor staurosporine prevented this inhibition. They concluded that in the rat IMCD PGE₂ inhibits P_f via post-cAMP-dependent events that involve PKC.

Evidence regarding the effects of α_2 -agonists on transport in the collecting duct also requires close

attention to the species studied. Chen et al. (6) reported that the α_2 -agonist clonidine inhibited AVP-stimulated P_f and J_{1b} in the rat but not the rabbit CCD. Chabardès et al. (5) reported that clonidine reduced AVP-stimulated cAMP accumulation in the rat but not the rabbit CCD. Maeda et al. (20) demonstrated α_2 -inhibition of both AVP-stimulated cAMP accumulation and AVP-stimulated P_u in the rat IMCD. Edwards et al. (9) reported that α_2 -agonists inhibited AVP-stimu-

Table 2. Effect of PGE₂ on α_2 -mediated inhibition of cAMP-stimulated P_u

α_2 -Agonist	Control	cAMP	cAMP + Ag	cAMP + Ag + I	cAMP + Ag + I + PGE ₂
Clo	11.7 ± 1.0	23.1 ± 0.7*	19.0 ± 1.1*	20.7 ± 0.9	19.7 ± 0.0
Oxy	11.2 ± 0.9	20.5 ± 0.5*	18.3 ± 0.4*	19.7 ± 0.4*	18.2 ± 0.5*

Values are means \pm SE expressed as $\times 10^{-5}$ cm/s; $n = 5$ in each set of experiments. Clo, clonidine (100 nM); Oxy, oxymetazoline (100 nM); Ag, α_2 -agonist. CPT-cAMP was used at 0.1 mM, indomethacin at 5 μ M, and PGE₂ at 100 nM. *Significantly different from previous period.

Table 3. Effect of PGE₂ on α_2 -mediated inhibition of AVP-stimulated P_f , J_{lb} , and P_u

α_2 -Agonist-Experiment Type	Control	AVP	AVP + Ag	AVP + Ag + I	AVP + Ag + I + PGE ₂
Clo- P_f , $\mu\text{m/s}$	1 \pm 1	216 \pm 9*	177 \pm 8*	197 \pm 8*	171 \pm 7*
Oxy- P_f , $\mu\text{m/s}$	1 \pm 1	253 \pm 21*	142 \pm 9*	170 \pm 13*	134 \pm 8*
Clo- J_{lb} , $\text{peq} \cdot \text{cm}^2 \cdot \text{s}^{-1}$	735 \pm 13	837 \pm 20*	781 \pm 14*	831 \pm 15*	785 \pm 14*
Oxy- J_{lb} , $\text{peq} \cdot \text{cm}^2 \cdot \text{s}^{-1}$	671 \pm 15	851 \pm 6*	760 \pm 12*	819 \pm 12*	752 \pm 13*
Clo- P_u , $\times 10^{-5} \text{ cm/s}$	11.7 \pm 0.5	22.6 \pm 0.9*	19.7 \pm 0.6*	21.2 \pm 0.6*	19.6 \pm 0.4*
Oxy- P_u , $\times 10^{-5} \text{ cm/s}$	12.3 \pm 1.0	23.9 \pm 2.6*	22.1 \pm 2.8	23.3 \pm 2.9	21.5 \pm 2.6

Values are means \pm SE; $n = 5$ in each set of experiments. AVP, arginine vasopressin (220 pM). Indomethacin was used at 5 μM , PGE₂ at 100 nM, clonidine at 100 nM, and oxymetazoline at 100 nM. *Significantly different from previous period.

lated cAMP accumulation in rat but not in dog, pig, monkey, or human IMCD.

The classic mechanistic explanation related to α_2 -adrenoceptors is that they couple to G_i proteins and inhibit adenylyl cyclase activity (8, 11, 25). However, evidence indicates that α_2 inhibition of AVP-stimulated transport in the IMCD occurs via post-cAMP-dependent events. We reported that the α_2 -agonists dexmedetomidine, clonidine, and oxymetazoline reduced CPT-cAMP-stimulated P_f in the rat IMCD (17, 29). These findings indicate that the α_2 -inhibitory mechanism in the IMCD involves unidentified second messengers. Because both PGE₂ and α_2 -agonists inhibit AVP-stimulated P_f via post-cAMP-dependent events, we hypothesized that PGE₂ is one of those messengers associated with α_2 -induced inhibition.

To examine this hypothesis, we tested the ability of the cyclooxygenase inhibitor indomethacin to reverse α_2 -inhibition of AVP- and CPT-cAMP-stimulated P_f , J_{lb} , and P_u in the isolated rat IMCD. In addition, exogenous PGE₂ was added to determine whether it would decrease the indomethacin-induced reversal of α_2 -inhibition. We used the α_2 -agonists dexmedetomidine, clonidine, and oxymetazoline, which inhibit AVP-stimulated P_f with dose-dependent profiles (17, 29). Dexmedetomidine is nonselective with respect to the α_2 -subtypes (α_{2A} , α_{2B} , and α_{2C}) (24), clonidine is selective to both α_2 - and imidazoline receptors (3, 10) and appears to bind to α_{2B} -adrenoceptors in the collecting duct (16, 36), and oxymetazoline is selective to the α_{2A} -adrenoceptor (35). We used these agonists because we knew of their inhibitory capability, and, because they demonstrate different pharmacological binding characteristics, there could be distinguishing characteristics with regard to cellular signaling.

We tested the effects of indomethacin and PGE₂ on CPT-cAMP-stimulated P_f , J_{lb} , and P_u . Table 1 summarizes these data. Indomethacin increased CPT-cAMP-stimulated transport in all three protocols, and subsequent addition of PGE₂ reversibly reduced the transport properties. These results expand on the findings of Nadler et al. (22) and demonstrate that endogenous PGE₂ plays a role in regulating water, sodium, and urea transport via post-cAMP-dependent events.

Figure 1 contains results from three separate protocols showing that indomethacin reversed α_2 -induced inhibition caused by clonidine, oxymetazoline, and dexmedetomidine (Fig. 1, A, B, and C, respectively) of

CPT-cAMP-stimulated P_f . PGE₂ added in the final period reduced P_f back to the α_2 -inhibited level in (Fig. 1, A and B but not in C) although PGE₂ still significantly reduced P_f . These results indicate a role for PGE₂ in α_2 -mediated inhibition of P_f .

In the P_f experiments we tested the effect of the PKC inhibitor staurosporine. Figure 2A shows that PGE₂ added to the bath with indomethacin did not affect CPT-cAMP-stimulated P_f . PGE₂ prevented the indomethacin-induced increase in CPT-cAMP-stimulated P_f (see Table 1). Subsequent addition of 10 nM staurosporine, the same concentration shown to block PGE₂-induced inhibition of CPT-cAMP-stimulated P_f reported by Nadler et al., did not affect P_f . Figure 2B, however, shows that dexmedetomidine added with indomethacin and PGE₂ reduced CPT-cAMP-stimulated P_f by 60% and the addition of staurosporine reversibly increased P_f by 22%. This suggests a role for PKC in the α_2 -mediated inhibition of P_f .

The three α_2 -agonists inhibited CPT-cAMP-stimulated J_{lb} (Fig. 3, A, B, and C). In all three cases, indomethacin reversed α_2 -inhibition and the subsequent addition of PGE₂ reduced J_{lb} back to the α_2 -inhibited level. Dexmedetomidine inhibited J_{lb} (77%) more than clonidine (56%) or oxymetazoline (56%). PGE₂ accounted for the major portion of the clonidine- and oxymetazoline-induced inhibition (70 and 79%, respectively), whereas it accounted for only 42% in the dexmedetomidine-induced inhibition. Rocha and Koda (27) reported that PGE₂ did not affect bath-to-lumen Na⁺ flux.

In the P_u experiments we used the nonselective adrenergic agonist epinephrine because of our earlier study, which showed that dexmedetomidine is not an effective P_u inhibitor (29). Indomethacin reversed epinephrine-induced inhibition of CPT-cAMP-stimulated P_u , and PGE₂ reduced this effect (Fig. 4). These results demonstrate that urea transport can be inhibited via post-cAMP-dependent events and PGE₂ plays a role in modulating urea transport. Further data summarized in Table 2 show that clonidine and oxymetazoline significantly lowered CPT-cAMP-stimulated P_u . Indomethacin and PGE₂ produced small effects that were significant in the oxymetazoline but not the clonidine experiments.

In addition to our results on CPT-cAMP-stimulated transport, we also tested the effect of PGE₂ on α_2 -mediated inhibition of AVP-stimulated P_f , J_{lb} , and P_u .

These results are summarized in Table 3. Indomethacin reversed clonidine- and oxymetazoline-induced inhibition of AVP-stimulated P_f , and PGE₂ reduced P_f back to the α_2 -inhibited level. The same pattern was observed with clonidine- and oxymetazoline-induced inhibition of AVP-stimulated J_{1b} . Indomethacin reversed clonidine-induced inhibition of AVP-stimulated P_u , and PGE₂ reduced P_u back to the clonidine-inhibited level. Oxymetazoline did not reduce AVP-stimulated P_u with statistical significance. Again, results with α_2 -mediated inhibition of P_u are not as consistent as with P_f and J_{1b} , but it still appears that α_2 -adrenoceptors are involved in the modulation of AVP-stimulated P_u .

Reversal of α_2 -induced inhibition of transport by indomethacin was observed regardless of the method of transport stimulation (AVP or CPT-cAMP) and of the α_2 -agonist used. No major distinguishing differences between clonidine- and oxymetazoline-induced inhibition were observed other than clonidine inhibited AVP-stimulated P_u whereas those results with oxymetazoline failed to produce statistical significance (Table 3). One observation worth noting is that indomethacin partially reversed clonidine- and oxymetazoline-induced inhibition of AVP-stimulated P_f and J_{1b} ; i.e., the AVP period was significantly higher than the AVP+ α_2 -agonist+indomethacin period whereas it completely reversed clonidine- and oxymetazoline-induced inhibition of CPT-cAMP-stimulated P_f and J_{1b} and the CPT-cAMP period was not significantly different from the CPT-cAMP+ α_2 -agonist+indomethacin period. Endogenous production of cAMP via AVP likely provided a more effective transport stimulus that indomethacin at 5 μ M did not block completely. AVP increases $[Ca^{2+}]_i$ levels in the rat IMCD (32); thus it is also possible $[Ca^{2+}]_i$ plays a role in α_2 -inhibitory mechanism. We did not measure $[Ca^{2+}]_i$ levels, but a zero-calcium bath did not reduce α_2 -mediated inhibition of AVP-stimulated P_f in the rat IMCD (results not shown). We are unaware of any results with regard to the effect of α_2 -agonists on phosphoinositide hydrolysis in the collecting duct; however, α_2 -adrenoceptors have been shown to activate multiple signal transduction pathways, including those associated with arachidonic acid and the phosphoinositide system (7, 18, 21, 33).

Indomethacin partially reversed dexmedetomidine-induced inhibition of CPT-cAMP-stimulated P_f and J_{1b} (Figs. 1C and 3C). Dexmedetomidine, which as stated earlier is nonselective for the α_2 -adrenoceptor subtypes, produces greater inhibition of AVP-stimulated transport than either clonidine or oxymetazoline. This could be due to higher potency, efficacy, or both. Evidence has been conflicting as to which α_2 -adrenoceptor subtypes exist in the IMCD. Some results demonstrate the α_{2B} over the α_{2A} whereas other results suggest the opposite (34, 36). Our results could suggest that multiple adrenoceptors are responsible for the higher inhibition produced by dexmedetomidine. Because PGE₂ accounts for a smaller portion of the inhibition produced by dexmedetomidine compared with clonidine and oxymetazoline, other second messengers could be

involved in dexmedetomidine-induced inhibition. Future studies are required to determine these other messengers as well as α_2 -adrenoceptor subtypes.

Finally, it is recognized that indomethacin can influence other cellular events besides cyclooxygenase inhibition, and thus it is conceivable that another indomethacin-induced event occurred in our experiments. We used indomethacin because it has been the most commonly used agent in these kinds of experiments. In addition to indomethacin we have tested naproxen and ketorolac, two other potent inhibitors of cyclooxygenase, on dexmedetomidine-induced inhibition of CPT-cAMP-stimulated P_f by using the same protocol as in Fig. 1C (i.e., replacing indomethacin with naproxen or ketorolac). Both agents reversed α_2 -mediated inhibition to the same degree as indomethacin, and subsequent addition of PGE₂ significantly reduced P_f (these data are not shown). Thus we think it is unlikely that endogenous prostaglandins were not the major cellular messengers being affected in this study.

In summary, results of this study indicate that α_2 -adrenoceptors in the rat IMCD play a role in regulating water, sodium, and urea transport via a cellular mechanism that involves post-cAMP dependent events, which involve, among other second messengers, PGE₂. PKC appears to be involved as well. This mechanism could involve multiple α_2 -adrenoceptors and signaling pathways.

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