EFFECTS OF OSMOLALITY ON THE INTERACTION BETWEEN VASOPRESSIN AND PROSTAGLANDINS IN RAT RENAL PAPILLARY COLLECTING TUBULE CELLS IN CULTURE

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ABSTRACT
The effect of environmental tonicity on the interaction between arginine vasopressin (AVP) and prostaglandin (PG) E in the production of adenosine-3',5'-cyclic monophosphate (cAMP) was studied in cultured rat renal papillary collecting tubule cells. Both AVP and PGE_2 stimulated intracellular cAMP accumulation in a dose-dependent manner in an isotonic medium of 300 mOsm/kg H_2O in the presence of a phosphodiesterase inhibitor. Environmental tonicity modulated the effect of AVP, PGE, or of both, on cellular cAMP production. The hypertonic medium with 1,800 mOsm/kg H_2O enhanced the effect of 1×10^-9 M AVP on the cellular cAMP production 1.6-fold, but markedly reduced the effect of 2×10^-8 M PGE_1 or PGE_2 to one-sixth or one-third of that in the isotonic medium. The inhibitory effect of PGE_1 and PGE_2 on the action of AVP was not shown in the isotonic medium, but was seen in the hypertonic medium of 1,800 mOsm/kg H_2O. Synthesis of [^3H]PGE_2 was enhanced by hypertonicity, but not by AVP, when [^3H]arachidonic acid was incorporated in the medium. The results indicate that an environmental tonicity is an important factor in modulating the effect of PGE_1 or PGE_2 on AVP-mediated cAMP production in renal papillary collecting tubule cells.

Since the pioneering study by Orloff and Handler with the toad urinary bladder (28), interaction between arginine vasopressin (AVP) and prostaglandins (PGs) in water permeability has been investigated extensively (1, 10, 18, 24, 25, 27, 35). The action of AVP on the renal collecting tubule to increase water permeability is mediated by adenosine-3',5'-cyclic monophosphate (cAMP) (7, 16). The effects of PGs, however, are still in controversy. PGE, a major product of PGs in the kidney (32), has been reported to directly stimulate adenylate cyclase activity (10, 12, 14, 18, 25), to inhibit AVP-induced cAMP production in the collecting tubule (11, 24, 32), or to have little effect on the AVP action (8, 18, 34, 35). Similar conflicting results were also shown in amphibian epithelium (27, 28). Discrepancies among the investigators may depend upon factors such as differences in tissue preparations, nephron heterogeneity (26), environmental tonicity (21) and others. The effect of environmental tonicity on the interaction between PGE and AVP in cAMP metabolism has not yet been fully elucidated. Jackson et al. (18) and Torikai et al. (35) suggested that some cofactors required to generate cAMP may be lost in preparations of broken cells. Therefore, events in intact cells

Abbreviations: AVP, arginine vasopressin; PG, prostaglandin; cAMP, adenosine-3',5'-cyclic monophosphate; KRB, Krebs-Ringer buffered saline; Gpp(NH)p, 5'-guanylylimidodiphosphate; IMX, 3-isobutyl-1-methylxanthine
may not be reproduced in the cell-free system. This is particularly important for evaluating the effect of environmental osmolality.

The purpose of the present study is to explore a role of environmental tonicity in the interaction between AVP and PGE in the production of cAMP in cultured rat renal papillary collecting tubule cells. Culture system was developed by Grenier and Smith (12, 13) and by ourselves (17). Whether hypertonicity or AVP enhances conversion of arachidonic acid to PGs in cultured cells was also examined to determine a negative feedback loop between AVP and PGs in controlling cAMP production.

MATERIALS AND METHODS

Materials

AVP (grade VI), insulin, transferrin, triiodothyronine, 5'-guanlylimidodiphosphate [Gpp(NH)p], and 3-isobutyl-1-methylxanthine (IMX) were purchased from Sigma; hydrocortisone and forskolin were from Calbiochem-Behringer; collagenase (type II) was from Worthington; Dulbecco's modified Eagle's Minimal Essential Medium (MEM) was from Flow Laboratories; [3H]arachidonic acid (specific activity, 87.4 Ci/mmol) was from New England Nuclear. PGE₁ and PGE₂ were kindly provided by Ono Pharmaceutical, and indomethacin by Japan Merck. AcAMP RIA kit was obtained from Yamasa. Silica gel plates for thin layer chromatography were obtained from Toyo Biochemicals, culture tubes (15 ml) were from Corning, and tissue culture clusters (6- and 24-wells) were from Costar.

Cell Culture

Male Sprague-Dawley rats weighing 150-175 g were decapitated, and the abdominal wall was cut and the kidneys were removed under sterile conditions. Six kidneys from three rats were grouped together, and two or three groups were treated at each time. Renal papillary tissues were dissected out and minced using a sharp blade in 1 ml of collagenase (1 mg/ml) dissolved in Krebs-Ringer buffered saline (KRB; 128 mM NaCl, 5 mM KCl, 1.2 mM MgSO₄, 0.8 mM CaCl₂, 10 mM sodium acetate, 10 mM glucose, 10 mM Tris, and 2 mM NaH₂PO₄, pH 7.4). Minced tissues were transferred into 15-ml culture tubes containing 4 ml of collagenase in KRB (1 mg/ml), and kept at 37°C in a humidified incubator for 75 min. During incubation, tubes were gently shaken every 20 min to resuspend the precipitated tissues. Distilled water (3 ml) was added to disrupt red blood cells. The suspension was drawn up and down through a pipette to break any tissue clumps. Tubes were then centrifuged at 500 g for 4 min at room temperature, and pellets were resuspended in 10 ml of 10% bovine serum albumin dissolved in KRB and again centrifuged. Pellets were resuspended in Dulbecco's modified Eagle's MEM supplemented with 10% bovine fetal serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2.5 μg/ml fungizone. Dispersed cells and tubules were harvested into a total of 72 or 108 wells of 24-well tissue culture clusters with 1 ml Dulbecco's modified Eagle's MEM containing 10% bovine fetal serum, penicillin, streptomycin and fungizone, and kept in a humidified incubator under 90% air and 10% CO₂. On the second day of culture, the medium was changed to 1 ml 99% Dulbecco's modified Eagle's MEM containing 1% bovine fetal serum, 5 μg/ml insulin, 5 μg/ml transferrin, 5×10⁻⁸ M hydrocortisone, 5×10⁻⁸ M triiodothyronine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2.5 μg/ml fungizone, modified from Taub et al. (33).

Generation of cAMP in Response to AVP and Other Agents

On the fifth day of culture, cells were rinsed twice with 1 ml of KRB, and were incubated with 1 ml of the effector for varying periods in a humidified incubator. The medium osmolality was 300 mOsm/kg H₂O. The effector solution contained 5×10⁻⁴ M IMX. After incubation, the effector solution was aspirated off and cells were immediately immersed in 0.2 ml of 0.1 N HCl to stop the reaction. Cells were then collected into glass tubes (6×50 mm) with a rubber policeman, boiled for 5 min and then centrifuged at 2,500 g for 15 min at room temperature. The supernatants were decanted, and after adding 0.05 ml of 50 mM sodium acetate to each tube, cells were kept at −20°C until assay for cAMP. The pellets were dissolved in 0.2 ml of 1% sodium dodecyl sulfate and kept at 4°C until assay for protein. cAMP was assayed by radioimmunoassay (15). The lower limit of detection was 6.25 fmol/tube. There was no cross-reactivity with succinylated mono- and triphosphates and with other succinyl cyclic nucleotides. The recovery of cAMP from cultured cells was 93.2±5.9% (n=6). AVP, PGE₁, and PGE₂, and hyper-osmolality did not interfere with the cAMP radioimmunoassay. Protein was measured by the method of Lowry et al. (23).

The following experiments were performed.
1) A dose-dependent response of cAMP to AVP (1×10⁻¹¹-1×10⁻⁷ M) was studied. The incubation time was 10 min and intracellular cAMP was determined. 2) To determine the effect of incubation time on cAMP accumulation, cells were incubated for varying periods with a submaximal stimulatory concentration of AVP (1×10⁻⁹ M). Intra- and extracellular cAMP levels were determined. 3) Effects of parathyroid hormone, glucagon, sodium fluoride, Gpp(NH)p and forskolin on cAMP accumulation in renal papillary collecting tubule cells were examined. Cultured cells were incubated for 10 min with one of the effectors and intracellular cAMP levels were measured.

**Effects of Osmolality on the Interaction between PGE and AVP in Cellular cAMP Accumulation**

We examined whether osmolality of the medium modulates the effect of either AVP or PGE, or both, on cellular cAMP accumulation. Hypertonic solutions were prepared by adding sodium chloride and urea in a molar ratio of 1:2 to the standard KRB (300 mOsm/kg H₂O) (10). Four solutions with the osmolality of 300, 600, 1,200 or 1,800 mOsm/kg H₂O were used. PGE₂ or PGE₁ was dissolved in ethanol and then diluted with KRB. The final concentration of ethanol was 3.5×10⁻⁵ M. AVP was used at a concentration of 1×10⁻⁹ or 1×10⁻⁷ M. Cells were preincubated with one of the four solutions for at least 60 min prior to the start of experiment. After rinsing twice with an equiosmotic solution, cells were incubated for another 10 min with 1 ml of the effector, which contained AVP, PGE₁ or PGE₂, or both, and 5×10⁻⁴ M IMX in the equiosmotic solution. Control experiments were performed in the same manner with 3.5×10⁻⁵ M ethanol.

**Effects of Osmolality on Metabolism of [³H]Arachidonic Acid**

Dispersed cells and tubules from 6 rats were plated in 12 wells of 6-well tissue culture clusters. Cells on the fifth day of culture were used. After rinsing with 3 ml of KRB, cells were treated with 3 ml of either isotonic (300 mOsm/kg H₂O) or hypertonic solution (1,800 mOsm/kg H₂O) for at least 30 min prior to the start of experiments. Cells were incubated with 2 ml of one of the two solutions containing 2 μCi [³H]arachidonic acid for 60 min in a humidified incubator. Other groups of cells were incubated in the same manner with 1×10⁻⁷ M AVP in either the isotonic or the hypertonic medium. Also, cells treated with 2.2×10⁻⁴ M indomethacin before and during the experiments were studied. The supernatant decanted into glass tubes was immediately acidified to pH 3.0-3.5 with formic acid, and then extracted with 5 vol of ethyl acetate. The organic layer was evaporated to dryness under a stream of nitrogen. The pellet was resuspended in 0.1 ml of chloroform : methanol (2:1, v/v), and a 10 μl portion of the suspension was spotted on a silica gel plate. The authentic standard solutions of arachidonic acid, PGE₃, PGF₂α, PGA₂ and thromboxane B₂ were also spotted. The plate was developed in chloroform : methanol : acetic acid : water (90:8:1:0.8) to 15 cm, dried and treated with iodine vapor. Samples were cut into 5-mm strips, and their radioactivity was determined in an Aloka liquid scintillation spectrometer (model LSC-671). Cells in two wells treated similarly in the absence of [³H]arachidonic acid were used for protein measurement in each experiment. Through the experiment, the content of cell protein ranged from 47 to 60 μg/well.

**Statistics**

All values for cAMP and [³H]PGs were compared by analysis of variance, and by Student's t-test if necessary.

**RESULTS**

During 10 min of incubation, cAMP in renal papillary collecting tube cells reached 167.6±18.6 fmol/µg protein (Fig. 1). AVP at concentrations higher than 1×10⁻¹¹ M caused a significant increase in intracellular cAMP in a dose-dependent manner. In the presence of 1×10⁻⁹ M AVP, intracellular cAMP increased rapidly and reached a peak after 10 min, followed by a gradual decrease (Fig. 2). In contrast, extracellular cAMP did not change during 5 to 30 min (Fig. 2). Further experiments were therefore performed with an incubation time of 10 min.

As shown in Table 1, parathyroid hormone significantly increased intracellular cAMP at 0.05 U/ml, but not at 0.5 U/ml. Glucagon significantly augmented intracellular cAMP. Sodium fluoride and Gpp(NH)p did not stimulate cAMP accumulation in the intact, cultured renal papillary collecting tubule cells (data not shown). In contrast, forskolin markedly stimulated the cAMP production in cells.

Osmolality of the medium greatly affected the intracellular cAMP level in response to PGE₂ (Fig. 3). With osmolality at 300 mOsm/kg H₂O, PGE₂ stimulated the cAMP accumulation in a
Fig. 1 Effects of AVP on intracellular cAMP accumulation in cultured rat renal papillary collecting tubule cells. Values are the mean ± SEM (n=6).

dose-dependent manner. Increase in osmolality augmented the basal accumulation of cAMP. The value of 241.9±20.4 fmol/μg protein/10 min in cells incubated in a hypertonic medium (1,800 mOsm/kg H₂O) was significantly greater than in the isotonic medium (P<0.05). In contrast, increase in osmolality apparently reduced the responsiveness of cells to PGE₂. In the hypertonic medium of 1,800 mOsm/kg H₂O, only the cellular cAMP accumulation in
Table 1  Intracellular cAMP Accumulation in Response to AVP, Parathyroid Hormone (PTH), Glucagon, and Forskolin

<table>
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<tr>
<th></th>
<th>n</th>
<th>cAMP (fmol/μg protein/10 min)</th>
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<tbody>
<tr>
<td>Vehicle</td>
<td>6</td>
<td>216.8 ± 28.7</td>
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<tr>
<td>AVP, 1×10^-9 M</td>
<td>6</td>
<td>472.9 ± 33.4**</td>
</tr>
<tr>
<td>AVP, 1×10^-7 M</td>
<td>6</td>
<td>1,010.5 ± 87.5**</td>
</tr>
<tr>
<td>PTH, 0.05 U/ml</td>
<td>6</td>
<td>351.2 ± 18.9*</td>
</tr>
<tr>
<td>PTH, 0.5 U/ml</td>
<td>6</td>
<td>238.5 ± 20.6</td>
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<tr>
<td>Glucagon, 1×10^-8 M</td>
<td>6</td>
<td>356.2 ± 36.9*</td>
</tr>
<tr>
<td>Glucagon, 1×10^-6 M</td>
<td>6</td>
<td>487.5 ± 23.9**</td>
</tr>
<tr>
<td>Forskolin, 2.4×10^-7 M</td>
<td>6</td>
<td>1,879.0 ± 258.6**</td>
</tr>
<tr>
<td>Forskolin, 2.4×10^-6 M</td>
<td>6</td>
<td>3,341.4 ± 384.7**</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM. *P<0.05 vs. vehicle. **P<0.01 vs. vehicle.

Fig. 3  Effects of PGE_2 on cAMP accumulation in cultured rat renal papillary collecting tubule cells under varying osmolality. ○ No PGE_2 added; ● 1×10^-9 M AVP; △ 2×10^-8 M PGE_2; ▲ 2×10^-7 M PGE_2; ▼ 2×10^-6 M PGE_2. Values are the mean ± SEM (n=6). *, the difference in each group; values are compared to those in the 300 mOsm/kg H_2O medium (*P<0.05, **P<0.01). † and ††, the difference between the groups; the values are compared to ○ at each osmolality (†P<0.05, ††P<0.01).

Fig. 4  Effects of osmolality on the interaction between PGE_1 and AVP in cAMP accumulation in cultured rat renal papillary collecting tubule cells. ○ No agent added; ○ 1×10^-9 M AVP; ▲ 2×10^-8 M PGE_1; ▼ 1×10^-9 M AVP and 2×10^-6 M PGE_2. Values are the mean ± SEM (n=6). *, the difference in each group; values are compared to those in the 300 mOsm/kg H_2O medium (*P<0.05, **P<0.01). † and ††, the difference between the groups; the values are compared to ○ at each osmolality (†P<0.05, ††P<0.01).

Table 2  shows the effect of osmolality on the interaction between 2×10^-9 M PGE_2 and AVP in cellular cAMP production. Increase in AVP-induced cellular accumulation of cAMP was enhanced by the exposure of cells to the hypertonic media. Simultaneous addition of PGE_2 and AVP significantly increased cAMP in cells exposed to the medium with either 300 or 1,200 mOsm/kg H_2O. However, in the 1,800 mOsm/kg H_2O medium the presence of 2×10^-9 M PGE_2 significantly diminished cAMP increase in response to 1×10^-9 M AVP, as compared to its absence (P<0.05). Combination of 2×10^-9 M PGE_2 and 1×10^-7 M AVP gave a similar result.

Fig. 4 shows the interaction between PGE_1 and AVP in cellular cAMP accumulation in varying osmolality. The findings were essentially similar to those presented in Table 2. When 2×10^-8 M PGE_1 was used, the cellular cAMP accumulation induced by PGE_1 alone or by the response to 2×10^-6 M PGE_2 was significantly higher than the basal value.
Table 2  Effects of Osmolality on Interaction between AVP and PGE₂ in cAMP Production in Cultured Rat Renal Papillary Collecting Tubule Cells

<table>
<thead>
<tr>
<th>Osmolality of the medium</th>
<th>cAMP Accumulation in cells (fmol/μg protein/10 min)</th>
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<tbody>
<tr>
<td>a) Vehicle</td>
<td>301.6± 48.2</td>
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<tr>
<td>b) 1 x 10⁻⁹ M AVP</td>
<td>573.3± 50.5**</td>
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<tr>
<td>c) 1 x 10⁻⁷ M AVP</td>
<td>581.9± 72.6*</td>
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<tr>
<td>d) 2 x 10⁻⁹ M PGE₂</td>
<td>942.7±107.1**</td>
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<tr>
<td>e) 1 x 10⁻⁹ M AVP+</td>
<td>1,039.0±183.9**</td>
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<tr>
<td>f) 1 x 10⁻⁷ M AVP+</td>
<td>1,603.5±183.9**</td>
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<tr>
<td></td>
<td>b vs. e P&lt;0.05</td>
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<td></td>
<td>c vs. f P&lt;0.05</td>
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</table>

In each group, six determinations were made. Values are the mean±SEM. *P<0.05 vs. vehicle. **P<0.01 vs. vehicle.

combination of PGE₁ and 1 x 10⁻⁹ M AVP was significantly reduced in the medium of 600 or 1,200 mOsm/kg H₂O compared to those in the isotonic medium.

As shown in Table 3, extracellular cAMP concentration in the vehicle group was significantly higher at 1,800 mOsm/kg H₂O than at 300 mOsm/kg H₂O. PGE₂-induced extracellular cAMP concentration, however, was significantly less in the hypertonic medium than in the isotonic medium. The amount of the total cAMP in response to the vehicle or 1 x 10⁻⁹ M AVP was significantly greater in the hypertonic medium than in the isotonic medium. In contrast, the amount of the total cAMP in response to 2 x 10⁻⁸ M PGE₂ or to PGE₂ plus AVP was significantly less in the hypertonic medium than in the isotonic medium.

Cultured cells converted [³H]arachidonic acid to [³H]PGE₂ and to [³H]PGF₂α. Exposure of cells to the hypertonic medium enhanced the conversion, as [³H]PGE₂ amounted to 0.549±0.089 pM vs. 0.311±0.048 pM (P<0.05), and [³H]PGF₂α amounted to 0.554±0.086 pM vs. 0.334±0.030 pM (P<0.05) in the hypertonic medium and in the isotonic medium, respectively (Fig. 5). [³H]PGE₂ and [³H]PGF₂α were decreased to 0.158±0.023 pM (n=5) and to 0.133±0.014 pM (n=5) in cells treated with 2.2 x 10⁻⁴ M indo-methacin (P<0.05). Addition of 1 x 10⁻⁷ M AVP did not enhance the conversion of [³H]-arachidonic acid to [³H]PGE₂ or [³H]PGF₂α (0.362±0.032 pM or 0.345±0.052 pM in the isotonic medium, n=6; 0.419±0.043 pM or 0.403±0.032 pM in the hypertonic medium, n=6). The third peak of [³H]PGA₂ was found; PGA₂ is thought to be a dehydration product of PGE₂.

DISCUSSION

In the rat renal papillary collecting tubule cultured by a modified method of Grenier and Smith (12, 13), the effect of AVP on intracellular cAMP accumulation was demonstrated in a dose-dependent manner. But neither sodium fluoride nor Gpp(NH)p caused an increase in cAMP in intact cells. Adenylate cyclase in membrane preparations is directly stimulated by sodium fluoride or Gpp(NH)p mediated by guanyl nucleotide regulatory unit (29). In contrast, forskolin can stimulate cellular cAMP production in such intact cells as we have used, and thus is a unique diterpene activator of adenylate cyclase (31). AVP was most effective in stimulating cAMP accumulation in cultured cells. Glucagon, but not parathyroid hormone at a high concentration, stimulated cAMP accumulation in these cells. These findings were similar to those in the isolated papillary collecting tubule published from other laboratories (10, 26).

As shown in Tables 2 and 3 and Fig. 4, hypertonic media increased the basal and AVP-induced intracellular and total cAMP concentration substantially. Since rats can maximally concentrate their urine to at least 3,000 mOsm/kg H₂O after severe dehydration (8), the medium with the osmolality of 1,800 mOsm/kg H₂O was chosen as the maximal hypertonic environment for papillary collecting tubule cells. We used the hypertonic medium with NaCl and urea follow-
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<td><strong>1,800 mOsm/kg H₂O</strong></td>
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<td>n.s.</td>
<td>&lt;0.001</td>
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Table 3: The cAMP Level as Affected by AVP and/or PGE₂ and by Osmolality
ing the study by Edwards et al. (10), but did not use other osmotic solutions. Increase in cAMP production by hypertonicity was observed in a similar preparation of rats in the absence of the phosphodiesterase inhibitor (30), in the isolated papillary collecting tubule (10), and in the kidney slices (6). Edwards et al. (10) have demonstrated that in the isolated papillary collecting tubule both adenylyl cyclase and phosphodiesterase activities are inhibited by hyperosmolality of 1,400–2,000 mOsm/kg H2O, and also that cAMP increased significantly with an increase in osmolality from 300 to 2,000 mOsm/kg H2O. Since these enzymes were assayed in cell-free preparations exposed to varying osmolality, it is not clear that such effects can also be found in intact cells. The present study, performed in the presence of a phosphodiesterase inhibitor, suggests that hyperosmolality enhances the basal and AVP-induced cAMP production in cells. This osmolality effect may be exerted at the site behind the AVP receptor, since the basal cAMP accumulation was also augmented under the hypertonic environment.

PGE1 or PGE2 alone had a distinct stimulatory effect on cellular cAMP accumulation in cultured papillary collecting tubule cells at relatively low osmolality. This is compatible with the previous finding of Grenier et al. (12). In these situations, however, PGE1 or PGE2 had no or little effect on the AVP-stimulated cAMP level in cultured cells. Similar findings were reported in the isolated medullary and papillary collecting tubule (10, 35). The present study further demonstrated that under a high osmotic environment of 1,800 mOsm/kg H2O, the presence of PGE1 or PGE2 blunts the AVP-induced cellular cAMP accumulation. Inhibition by PGE1 or PGE2 of the AVP-induced cellular cAMP accumulation is not due primarily to the leakage of cAMP from cells as speculated previously (19). Both the cellular or total content of cAMP was reduced in the presence of PGE2. Since the study was performed in the presence of the phosphodiesterase inhibitor, PGE1 or PGE2 is suggested to blunt cellular cAMP production in the hypertonic environment by modifying some intracellular events. The present results indicate that the environmental osmolality is an important factor for PGE-modulated cAMP production by AVP. In this regard, it should be noted that the regional differences in PG production are of sufficient magnitude to explain that the major PG's effects are on the papillary collecting tubule rather than on the cortical collecting tubule (22, 32).

In the present study, cultured renal papillary collecting tubule cells produced PGE2 and PGF2α, a finding in concert with the studies by Grenier et al. (13) and Currie et al. (5). The hypertonic medium but not the isotonic medium enhanced conversion of exogenous arachidonic acid to PGE2 and PGF2α. Craven and DeRubertis have recently demonstrated that the hypertonic NaCl or mannitol stimulates immunoreactive PGE production by slices of the renal
inner medulla, whereas urea inhibits this process (3). However, urea did not alter the conversion of exogenous arachidonic acid to PGE, indicating urea suppresses PGE synthesis through an inhibition of acyl hydrolase before a step of synthesis of arachidonic acid (4). The addition of AVP did not stimulate production of PGE₂ or PGF₂α in the isotonic medium. This finding is compatible with the studies from other laboratories (5, 13). Similar results were obtained in the hypertonic environment. Kirschenbaum and his associates (20), however, showed that in the isolated cortical collecting tubule an anti-diuretic action of AVP stimulated PG synthesis from arachidonic acid. The reason for this discrepancy remains unknown. As described above, PGE₁ or PGE₂ inhibits the cellular production of cAMP by AVP under the hypertonic environment. Thus, we could not demonstrate that PG synthesis modulates the anti-diuretic action of AVP by a closed feedback loop on the papillary collecting tubule. In fact, there is in vivo evidence that the administration of AVP markedly increased urinary excretion of PGE₂ which mainly depended on renal medullary production of PGs (9, 32). A possible explanation for this discrepancy is that renal medullary interstitial cells also produce PGE (36), that is stimulated by the vascular effect of AVP (2). Since PGE is easily leaked out of cells and PGF₂α receptors are located on the plasma membrane of renal epithelial cells, PGE derived from renal interstitial cells may antagonize the effect of AVP on the collecting tubule. Though we did not measure immunoreactive PGE₂ without the addition of exogenous arachidonic acid, the osmotic effect may also affect the synthesis of arachidonic acid from phospholipid, as suggested by Craven and her associates (3).

In summary, environmental osmolality modulates the effect of AVP, PGE, or both, on cellular cAMP production. Hypertonicity enhances the effect of AVP on cellular production of cAMP, but markedly reduces the effect of PGE₁ or PGE₂. The inhibitory effect of PGE₁ or PGE₂ on the action of AVP is not shown in the isotonic medium, but is shown under the hypertonic condition which pertains to the papillary tissue of the kidney. These results therefore indicate that an environmental toxicity is an important factor for PGE₁ or PGE₂-modulated production of cAMP by AVP. However, the interaction between AVP and PGs in cAMP metabolism by a closed feedback loop to regulate water permeability in the collecting tubule remains to be determined.

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