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**Research Article**

The studies reported here were designed to determine if there is an apical-basolateral asymmetry to the release of prostaglandins by or to the biochemical effects of prostaglandins on the renal collecting tubule. Canine cortical collecting tubule (CCCT) cells were isolated by immunodissection and seeded at supraconfluent densities on Millipore filters. The resulting confluent monolayer of CCCT cells: (a) developed and maintained a transcellular potential difference of 1 mV (apical side negative); (b) exhibited a permeability to inulin that was the same as that obtained with similar monolayers of Madin-Darby canine kidney (MDCK) cells; and (c) released adenosine 3',5'-cyclicmonophosphate (cAMP) in response to arginine vasopressin (AVP) added to the basolateral but not the apical surface of the monolayer. These results indicate that confluent monolayers of CCCT cells on Millipore filters have characteristics of asymmetry that are seen with intact collecting tubules. Moreover, PGE2 added to either side of the CCCT cell monolayer crossed the monolayer at the same slow rate as inulin, which demonstrated the feasibility of examining the sidedness of the effects of and the release of PGE2. Although AVP caused cAMP release only when added to the basolateral side of CCCT cells, AVP caused the release of PGE2 when added to either the apical or basolateral surface. This result implies that there are at least two AVP receptor systems, […]



# Apical-Basolateral Membrane Asymmetry in Canine Cortical Collecting Tubule Cells

## Bradykinin, Arginine Vasopressin, Prostaglandin $E_2$ Interrelationships

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**Abstract.** The studies reported here were designed to determine if there is an apical-basolateral asymmetry to the release of prostaglandins by or to the biochemical effects of prostaglandins on the renal collecting tubule. Canine cortical collecting tubule (CCCT) cells were isolated by immunodissection and seeded at supraconfluent densities on Millipore filters. The resulting confluent monolayer of CCCT cells: (*a*) developed and maintained a transcellular potential difference of 1 mV (apical side negative); (*b*) exhibited a permeability to inulin that was the same as that obtained with similar monolayers of Madin-Darby canine kidney (MDCK) cells; and (*c*) released adenosine 3',5'-cyclicmonophosphate (cAMP) in response to arginine vasopressin (AVP) added to the basolateral but not the apical surface of the monolayer. These results indicate that confluent monolayers of CCCT cells on Millipore filters have characteristics of asymmetry that are seen with intact collecting tubules. Moreover, $PGE_2$ added to either side of the CCCT cell monolayer crossed the monolayer at the same slow rate as inulin, which demonstrated the feasibility of examining the sidedness of the effects of and the release of $PGE_2$.

Although AVP caused cAMP release only when added to the basolateral side of CCCT cells, AVP caused the release of $PGE_2$ when added to either the apical or basolateral surface. This result implies that there are at least two AVP receptor systems, one coupled to cAMP synthesis and one to $PGE_2$ formation. In contrast to the results observed with AVP, bradykinin caused $PGE_2$ release only when added to the apical surface of CCCT cells, which suggested that urinary but not blood borne kinins elicit $PGE_2$ formation by the canine collecting tubule. $PGE_2$ was released in comparable amounts on each side of the monolayer in response both to AVP and to bradykinin.

High concentrations ($\geq 10^{-8}$ M) of $PGE_2$ added to either side of the monolayer caused the release of cAMP. However, at concentrations ($10^{-10}$–$10^{-12}$ M) at which $PGE_2$ had no independent effect on cAMP release, $PGE_2$ inhibited the release of cAMP, which normally occurred in response to AVP. This inhibition occurred with $PGE_2$ added to either the apical or basolateral surface of the CCCT cell monolayer. $PGE_2$ ($10^{-11}$ M) also inhibited the AVP-induced accumulation of intracellular cAMP by CCCT cells seeded on culture dishes. This inhibition was only observed when the cells were preincubated with $PGE_2$ for $\geq$20 min. Our results are consistent with the concept that inhibition by prostaglandins of the hydroosmotic effect of AVP is due to inhibition of AVP-induced cAMP production. This inhibition does not appear to involve a direct physical interaction of $PGE_2$ with the AVP receptor which is coupled to adenylate cyclase, since CCCT cells must be preincubated with $PGE_2$ for 20 min for the inhibition to be observed, and since $PGE_2$ added to the apical surface of CCCT cells inhibits cAMP release in response to AVP acting from the basolateral surface.

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## Introduction

The renal collecting tubule is an attractive system for studying the physiological function and mechanism of action of pros-

taglandin $E_2$ ($PGE_2$).[1] The collecting tubule is the part of the renal tubule that exhibits the highest cyclooxygenase activity (1–6), and along with the vasculature (7), the medullary interstitial cells (8), and the glomeruli (9, 10) constitutes one of the four major sites of PG synthesis in the kidney (11). In addition, PG have been shown to have two potent effects on the transport properties of isolated collecting tubule segments. The first effect is to inhibit the water resorption that occurs in response to arginine vasopressin (AVP) (12, 13), and the second effect is to inhibit $Na^+$ resorption (14–17).

There is strong circumstantial evidence that PG inhibit the hydroosmotic effect of AVP in vivo as well as in vitro (18, 19). For example, indomethacin treatment and essential fatty acid deficiency, two regimens that inhibit renal PG production, increase urine osmolality (20–22). Thus, $PGE_2$ appears to have a physiological as opposed to simply a pharmacological action on the collecting tubule.

Recently, $PGE_2$ has been observed to inhibit AVP-induced adenosine 3′,5′-cyclic monophosphate (cAMP) accumulation in the rabbit cortical collecting tubule. This action of $PGE_2$ accounts for inhibition of the hydroosmotic effect of AVP (23). However, the molecular mechanisms underlying this inhibition have yet to be described. Moreover, the mechanism by which PG inhibit luminal to basolateral $Na^+$ movement is undefined.

To facilitate the study of the mechanisms of actions of PG on the collecting tubule, we developed a culture system of canine cortical collecting tubule (CCCT) cells (24). These cells exhibit many of the morphological and biochemical properties of collecting tubule cells in situ. We began the study reported here asking whether there is an apical-basolateral asymmetry either to the release of PG by CCCT cells or to the effects of PG on cAMP metabolism in CCCT cells. These questions were prompted in part by a report that $PGE_2$ acts only from the basolateral surface of rabbit collecting tubule segments to inhibit $Na^+$ resorption (14). This result indicated that $PGE_2$ is unable to traverse the collecting tubule and that there is an asymmetry to $PGE_2$ receptors in the collecting tubule. In performing studies on the sidedness of hormonal responses in CCCT cells, we noted that extremely low concentrations of $PGE_2$ ($10^{-12}$ M) would inhibit the release of cAMP that normally occurred in response to AVP, and that this effect occurred when $PGE_2$ was added to either side of the CCCT cell monolayer. In this report, we describe these experiments on the inhibition by $PGE_2$ of AVP-induced cAMP formation, and also describe complementary studies on the sidedness of the effects of AVP and bradykinin on cAMP and PG metabolism by CCCT cells.

1. *Abbreviations used in this paper:* cAMP, adenosine 3′,5′-cyclic monophosphate; AVP, arginine vasopressin; DD-AVP, ($Asu^{1,6}$, $Arg^8$)-vasopressin; IBMX, 3-isobutyl-1-methylxanthine; PSN antibiotic mixture, 5 mg penicillin, 5 mg streptomycin, and 10 mg neomycin/100; PG, prostaglandin; $PGE_2$, $PGF_1$, and $PGF_2$, prostaglandins $E_2$, $F_{1\alpha}$, and $F_{2\alpha}$; Tx, thromboxane.

## Methods

*Materials.* [$^3$H]Inulin methoxy (2–3 Ci/mmol), [$^3$H]$PGE_2$ (160 Ci/mmol), [$^3$H]$PGF_{2\alpha}$ (150 Ci/mmol), [$^3$H]6-keto-$PGF_{1\alpha}$ (150 Ci/mmol), [$^3$H]thromboxane (Tx) $B_2$ (155 Ci/mmol), [$^{125}$I]cAMP (150 Ci/mmol), $^{22}Na$ (20 Ci/mmol), and radioimmunoassay supplies for the cAMP assay were all purchased from New England Nuclear, Boston, MA. Rabbit anti-$PGE_2$ serum was obtained from Miles Laboratories Inc., Elkhart, IN. Rabbit anti-$PGF_{2\alpha}$, rabbit anti-6-keto-$PGF_{1\alpha}$, and rabbit anti-$TxB_2$ sera and radioimmunoassay supplies for these three compounds were purchased from Seragen, Inc. $PGE_2$ was purchased from Upjohn Diagnostics Co., Kalamazoo, MI. SEP-PAK $C_{18}$ cartridges were purchased from Waters Associates, Milford, MA. Soluble calf skin collagen was obtained from Worthington Biochemical Corp., Freehold, NJ. Modified Eagle's medium (DMEM) containing D-glucose (4.5 g/l), 5 mg penicillin, 5 mg streptomycin, and 10 mg neomycin/100 ml (PSN antibiotic mixture) (100X), and fetal bovine serum were purchased from Gibco Laboratories, Grand Island, NY. ($Asu^{1,6}$, $Arg^8$) vasopressin (DD-AVP) was obtained from Beckman Instruments Inc., Palo Alto, CA. AVP was obtained from Calbiochem-Behring Corp., San Diego, CA. Bradykinin triacetate, 3-isobutyl-1-methylxanthine (IBMX) and L-glutamine were purchased from Sigma Chemical, St. Louis, MO. Silica gel 60 plates were obtained from E. Merck, Darmstadt, Federal Republic of Germany. Flurbiprofen and Ibuprofen were gifts from Dr. Udo Axen of the Upjohn Diagnostics Co.

*Isolation and growth of CCCT cells on Millipore filters.* CCCT cells were isolated by immunodissection as described previously (24). The isolated cells were grown on 100-mm culture dishes in MEM containing 10% decomplemented fetal bovine serum, PSN antibiotic mixture (1X), and 2 mM glutamine under a water saturated 7% $CO_2$ atmosphere. CCCT cells that were to be seeded on Millipore filters were grown 3–6 wk with weekly changes of culture medium. Typically, 3–10 × $10^6$ cells were obtained from each culture dish. CCCT cells were removed from dishes by trypsinization and seeded at supraconfluent densities (2 × $10^6$ cells/cm$^2$) on collagen-coated Millipore filters (0.45 μm; catalog number HAMK 024 12) that were bonded to hollow polycarbonate cylinders (24, 25) (Fig. 1). The cylinders containing the cells were incubated in 6-well culture dishes under the culture conditions described above. Cells were tested for the presence of a transmembrane potential difference beginning 4 d after seeding. CCCT monolayers were used only after they exhibited a potential difference and were found to be impermeable to inulin (see below). Most monolayers were also tested for and found to exhibit a sidedness in their response to AVP. Other types of cells used for comparison, which included Madin-Darby canine kidney (MDCK) cells and Swiss mouse 3T3 fibroblasts, were cultured as described previously (24) and were seeded on Millipore filters as described above for CCCT cells.

*Permeability of CCCT cells on Millipore filters.* The permeability of cell monolayers to inulin, $PGE_2$, and $Na^+$ was measured as described below. All monolayers were tested for their permeability to inulin. The medium was removed by aspiration from both the inside and outside of the polycarbonate cylinders under sterile conditions, and this chamber was placed in a fresh 6-well culture dish. A solution containing a radioactive solute was added to one side of the cell monolayer, and the same solution without the isotope was added to the other side. Typically, the solution was culture medium (MEM containing 10% decomplemented fetal bovine serum, PSN antibiotic mixture (1X) and 2 mM glutamine). There were no appreciable differences in the permeability of CCCT cells to inulin or $PGE_2$ when comparing the culture medium

with the same medium without serum or to Krebs-Ringer buffer (composition in mM: 118 NaCl, 25 $NaHCO_3$, 14 glucose, 4.7 KCl, 2.5 $CaCl_2$, 1.8 $MgSO_4$, and 1.8 $KH_2PO_4$), pH 7.4. 1 ml was usually added to the inside of the cylinder (apical side of the monolayer) and 3 ml was added to the culture well in which the chamber was immersed (Fig. 1). In most experiments, [$^3$H]inulin, [$^3$H]$PGE_2$, and $^{22}Na^+$ were used at concentrations of $10^{-7}$ M. After the desired incubation periods, 0.05-ml aliquots were removed from inside and outside of the cylinders and radioactivity on both sides was measured by scintillation counting (24, 25). Counts for each vial were normalized to the original volumes. Percentages of the total amount of radioactivity added were then calculated for each side at each time period measured. Percentage data could then be transformed, to obtain a normal distribution for further statistical analysis, by utilizing the angular (inverse sine) transformation (26).

*Effector-induced cAMP release.* Medium surrounding CCCT cells grown on Millipore filters was removed under sterile conditions. The chambers were washed with Krebs-Ringer buffer, pH 7.4, and transferred to 12-well culture dishes (Costar, Cambridge, MA). Each experiment involving only one effector (e.g. AVP) at a single concentration typically consisted of the exposure of each cell monolayer to three treatments: (*a*) no effector on either side (control), (*b*) effector present on the apical side, and (*c*) effector on the basolateral side. Because each chamber in a given experiment received all three treatments consecutively, the order of the treatments was randomized. For each treatment an effector in Krebs-Ringer buffer, pH 7.4, containing $10^{-4}$ M IBMX or only buffer with IBMX (no effector), was added to the appropriate sides and incubated at 37°C for different times, which typically were 1 h. Volumes were chosen so that the fluid levels were equal on both the inside and outside of the polycarbonate chambers: 0.5 ml for inside the chambers and 1.2 ml for outside the chamber (inside the culture dish). After the desired time, liquid on each side of the monolayer was collected and lyophilized. The lyophilized samples were resuspended in equal volumes (0.3–0.5 ml) of buffer and assayed for cAMP as described previously (24). Effector dose-response curves, time courses, and experiments involving more than one effector (e.g., AVP plus $PGE_2$) were performed using only one treatment per chamber. All other procedures were as described above.

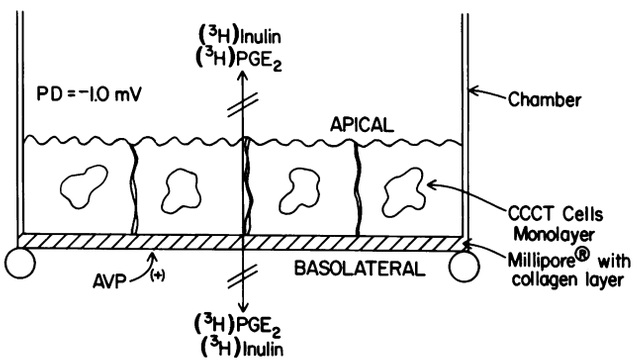


*Figure 1.* CCCT cell monolayer system for studying functional asymmetry of the cortical collecting tubule. CCCT cells ($2 \times 10^6$/cm$^2$) seeded on a Millipore filter that was bonded to a hollow polycarbonate cylinder form a confluent cell monolayer that has distinct apical and basolateral surfaces. The chamber stands on three legs in a culture dish. The three criteria routinely used to establish the functional polarity of these chambers (i.e., transcellular potential differences (PD), impermeability to inulin, and asymmetry of AVP-induced cAMP formation) are depicted.

*Effector-induced $PGE_2$ release.* The incubation procedures described above for measuring cAMP levels were used in experiments designed to measure PG formation. Krebs-Ringer buffer, pH 7.4, without IBMX was used in these experiments. The fluid containing PG released by the cells to either side of the cell monolayer was collected in individual tubes. The samples were immediately acidified to pH 4 with 1 M citric acid and were extracted twice with 2 vol of ethyl ether. The ether was evaporated under a stream of nitrogen; the samples were resuspended in an appropriate buffer and analyzed for immunoreactive $PGE_2$, as described previously (25).

*Characterization of PG released by CCCT cells.* The release of $PGE_2$, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, and $TxB_2$ by CCCT cells cultured on both Petri dishes and Millipore filters was quantitated. For CCCT cells seeded on culture dishes, radioimmunoassays were performed either directly on the medium or after extraction and purification. Analysis of the PG released by CCCT cells seeded on Millipore filters was performed only on either extracts of culture media. Purification of PG was performed as follows: media was extracted using SEP-PAK $C_{18}$ cartridges, as described by Powell (27). The methyl formate fractions containing the PG and Tx were evaporated, and each of the residues was resuspended in chloroform (circa 100 μl). These samples were chromatographed on silica gel 60 plates (E. Merck). The plates were developed twice in the organic phase of ethyl acetate/trimethylpentane/acetic acid/water (55:25:10:50; v/v/v/v)). PG standards were visualized with iodine vapor, and regions of the plates cochromatographing with the standards were scraped into individual tubes. The silica gel was extracted three times with 2 ml of chloroform/methanol (1:1; v/v). The solvent was evaporated under a stream of nitrogen and the samples were resuspended in buffer and subjected to radioimmunoassays (25).

*Effector-induced intracellular cAMP formation.* Treatment of monolayer cultures of nonconfluent CCCT cells with effectors (i.e., AVP, $PGE_2$, or both) was done in triplicate using 24-well culture dishes that were seeded at a density of $5 \times 10^4$ cells/well. Cells were rinsed free of media with Krebs buffer, pH 7.4, which contained $10^{-4}$ M IBMX; 0.3 ml of buffer alone or buffer containing $10^{-10}$ M $PGE_2$ was then added for a preincubation period of 60 min at 37°C. After this preincubation, the solutions were removed from the wells and 0.3 ml of buffer alone or buffer containing an effector was added to the cells. The monolayers were incubated for the desired time at 37°C. The solution in each well was removed and 500 μl of cold, 6% TCA was added. The wells were incubated at −80°C for 20 min, thawed at 24°C for 25 min, and incubated for 2 h at 0–4°C. The liquid in each well was then transferred to a test tube and extracted four times with 10 vol of diethyl ether. Any remaining ether was evaporated in a 60°C water bath for 10 min. The remaining aqueous phase was lyophilized. The lyophilized samples were resuspended in 0.125–0.2 ml of buffer and assayed for cAMP by radioimmunoassay as described previously (24).

*Statistical analysis.* All experiments involving an effector-induced response were done using a minimum of three replicates per treatment. A completely random analysis of variance was used to test for differences between sample means at $P < 0.05$ (26). Dunnett's test was used to compare differences between effector means with the control mean (26). In a few instances, when it was desired to compare all the effector means to each other and not only to the control mean, Student-Newman-Keuls test for all possible comparisons was used (26). Error bars on the figures are ±SE.

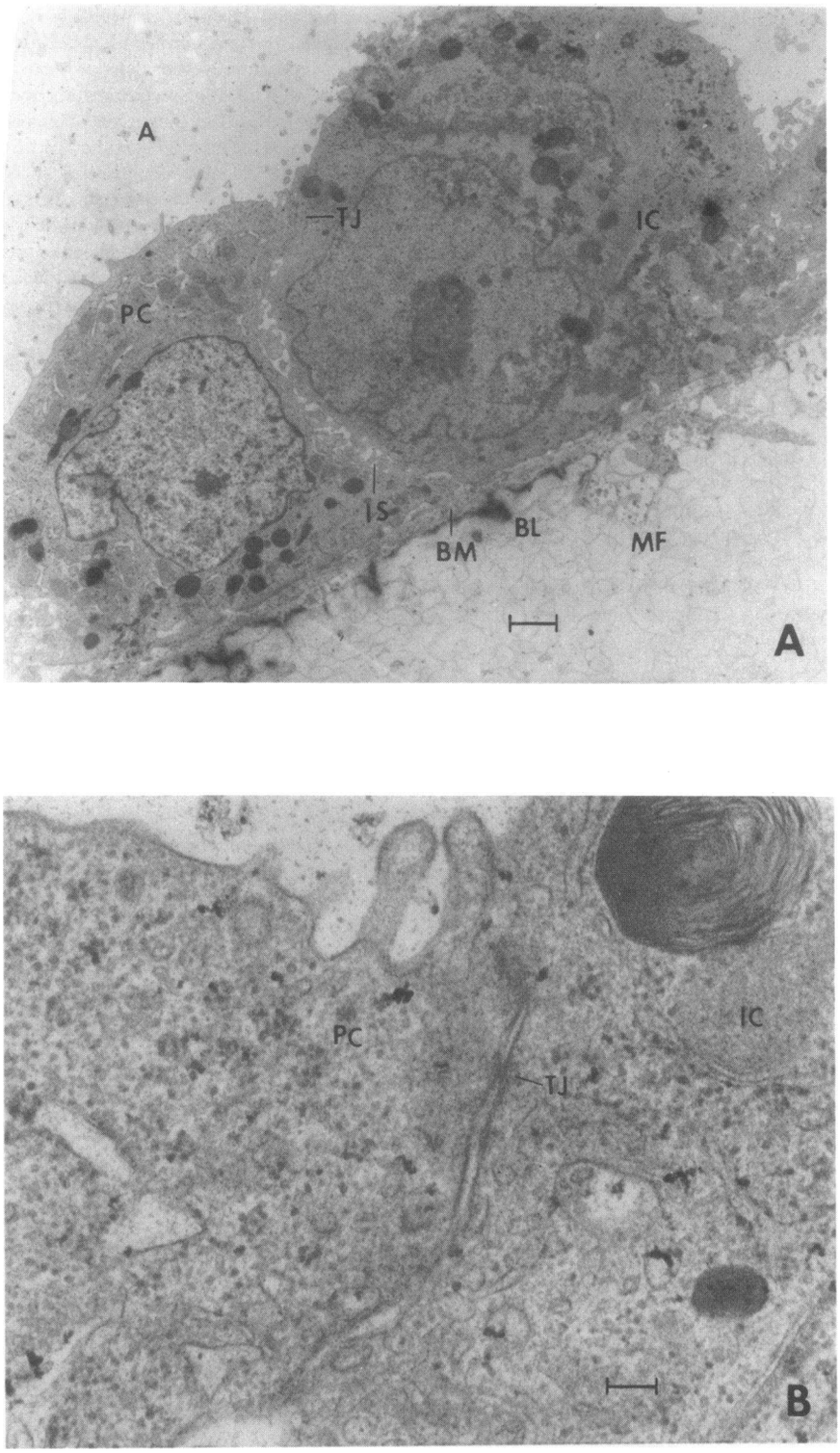


*Figure 2.* Electron photomicrographs of principal and intercalated CCCT cells seeded on a Millipore filter in chambers. (*A*) One principal and one intercalated cell. The basolateral sides of the cells show a flattened appearance against the filter, the presence of basement membrane, and loose junctional complexes between the cells. At the apical side, the cells have an abundance of microvilli and are joined by a tight junction. (*B*) Tight junction between the two cells shown in *A*. BM, basement membrane; PC, principal cell; IC, intercalated cell; A, apical side; BL, basolateral side; TJ, tight junction; IS, intercellular space; and MF, Millipore filter. Internal markers are 1 $\mu$m for *A* and 0.1 $\mu$m for *B*.

*Electron microscopy.* CCCT cells seeded on Millipore filters were examined by transmission electron microscopy (24, 25). Cells on the monolayers were fixed with 2% glutaraldehyde in 0.1 M sodium cacodylate, pH 7.4, for 24 h at 4°C. After fixation the chambers were washed thoroughly with 0.1 M sodium cacodylate, pH 7.4. The filters containing the fixed CCCT cells were separated from the polycarbonate cylinders. The filters were washed for 10 min in 0.1 M sodium cacodylate, pH 7.4, and postfixed in 1% $OsO_4$ in the same buffer for 4 h at 24°C and then overnight at 4°C. The filters were washed with sodium cacodylate buffer and dehydrated by sequential exposure to 25, 50, 70, 80, 95 (15 min each), and 100% (15 min, then 1 h, with a change in between) solutions of ethanol. The filters were exposed to decreasing proportions of ethanol/acetone: 2/1, 1/2, and finally 100% acetone (twice) for 15 min each. They were carefully cut into small pieces that were suitable for embedding and placed into glass vials for infiltration. Infiltration was as described previously (24). The sections obtained from the blocks were counterstained with 2% lead citrate in $H_2O$ and with 2% uranyl acetate in $H_2O$. They were examined and photographed using a Phillips model 201 transmission electron microscope. Kodak EM 4463 film (Eastman Kodak Co., Rochester, NY) was used for photography.

## Results

*Orientation of CCCT cells on Millipore filters.* In order to use CCCT cells on Millipore filters as a model to study the sidedness of the responses of collecting tubules to hormones, we first needed to determine if CCCT cells, when seeded at confluency on Millipore filters, were impermeable to small molecules and were morphologically, electrically, and biochemically asymmetric.

CCCT cells were seeded on Millipore filters at a density of $2 \times 10^6/cm^2$. Typically, these cells developed a transepithelial potential difference of 1±0.2 mV (Millipore filter side positive) 4–13 d after seeding and maintained this potential for up to an additional 21 d. As has been reported previously for MDCK cells (28), consistent results were obtained only with cells that had previously been grown and maintained at confluency on Petri dishes for at least 10 d before seeding on Millipore filters. As shown in Fig. 2, CCCT cells grown on Millipore filters exhibited typical intercellular tight junctions and had microvilli on their apical surfaces.

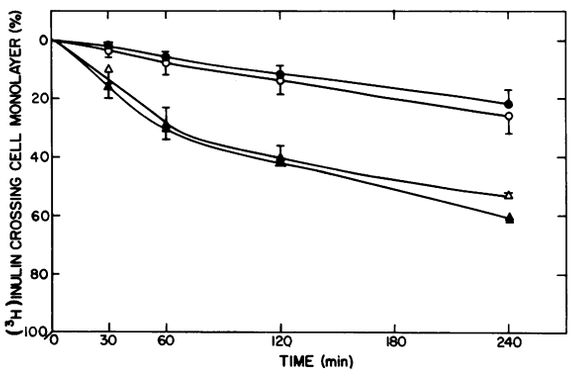


*Figure 3.* Permeability to [$^3$H]inulin of Millipore filters that were seeded with CCCT cells (○), MDCK cells (●), 3T3 cells (△), or no cells (▲). All chambers were seeded under identical conditions using $3 \times 10^6$ cells per chamber ($2 \times 10^6$ cells/cm$^2$). Points represent the mean values obtained from single experiments on nine different Millipore filters seeded with CCCT cells, and on six filters each seeded with MDCK cells, 3T3 cells, or no cells. [$^3$H]inulin (100 pmol) was added initially to the apical side. The amount of radioactivity found on each side of the monolayer at the indicated time periods was determined and used to calculate a percentage value. Identical results were obtained in similar experiments in which [$^3$H]inulin was added initially to the basolateral side.

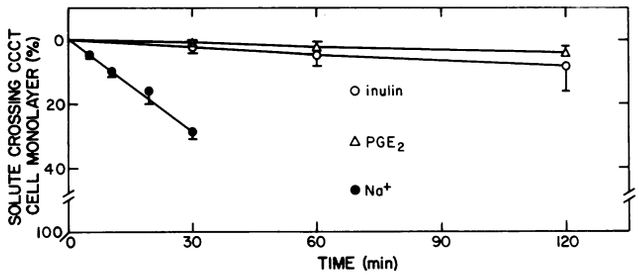


*Figure 4.* Permeability of confluent CCCT cell monolayers to [$^3$H]inulin (○), [$^3$H]$PGE_2$ (△), and $^{22}Na^+$ (●). The same six chambers were used to test the fluxes for each of the solutes. Solute fluxes were measured as described in text. Radiolabeled solute (100 pmol; $10^{-7}$ *M*) was added initially to the apical side in the experiments depicted in this figure. The transcellular flux of [$^3$H]$PGE_2$ was identical when it was added initially to the basolateral side.

Confluent monolayers of CCCT cells on Millipore filters were relatively impermeable to inulin in comparison with Swiss mouse 3T3 fibroblasts or with filters to which no cells had been added (Fig. 3). MDCK cells exhibited a permeability barrier that was quantitatively similar to that seen with CCCT cells. The flux of inulin across the monolayer was found to be the same in both directions.

As shown in Fig. 4, $PGE_2$, which is the major PG product of CCCT cells (see Tables I and II below), crossed the CCCT cell monolayer at the same rate as inulin. This suggests that the only route for the movement of $PGE_2$ across the monolayer is pericellular. It should also be noted that <10% of the $PGE_2$ added to one side of the CCCT cell monolayer is able to cross the monolayer in 60 min; 60 min was the longest treatment time used in the studies reported below. Neither bradykinin nor AVP affected the rates of inulin or $PGE_2$ movement across the cell monolayer (data not shown). (No metabolism of $PGE_2$ was observed in these experiments.) Our results on the movement of $PGE_2$ are consistent with the observations of other groups which indicate that PG do not freely diffuse through cell membranes (29, 30).

The sidedness of the effect of AVP on the release of cAMP by CCCT cell monolayers is shown in Fig. 5. Extracellular cAMP was measured in all cases because it was impractical to kill a monolayer for a single measurement of intracellular cAMP. AVP added to the basolateral surface of CCCT cells caused the release of cAMP, but even supramaximal concentrations of AVP ($10^{-6}$ M) added to the apical surface of CCCT cells failed to

*Table I. Release of PG by CCCT Cells on Culture Dishes**

| Treatment | Immunoreactive PG released (fmol/μg cell protein/60 min) | | | |
|---|---|---|---|---|
| | $iPGE_2$ | $iPGF_{2\alpha}$ | $iTxB_2$ | i-6-keto-$PGF_{1\alpha}$ |
| No effector | 5.4±0.2 | 1.9±0.1 | 1.4±0.1 | 0.5±0.2 |
| No effector + flurbiprofen | 4.3±0.1 | 1.0±0.1 | 1.0±0.1 | 0.4±0.2 |
| Bradykinin | 29.6±1.7‡ | 1.9±0.1 | 1.8±0.1 | 0.6±0.1 |
| Bradykinin + flurbiprofen | 4.8±0.1 | 1.2±0.2 | 1.3±0.3 | 0.4±0.1 |
| DD-AVP | 15.3±1.8 | 1.7±0.1 | 1.4±0.1 | 0.5±0.2 |
| DD-AVP + flurbiprofen | 5.9±0.2 | 1.4±0.1 | 1.2±0.1 | 0.4±0.1 |

* Nonconfluent CCCT cells ($1 \times 10^6$/60-mm culture dish) were incubated for 60 min in the presence of bradykinin ($10^{-6}$ *M*), DD-AVP ($10^{-6}$ *M*), or no effector at 37°C under a 7% $CO_2$ atmosphere. A 60-min incubation in the presence or absence of Flurbiprofen ($10^{-3}$ *M*) was performed before incubation with effectors. PG were extracted and purified as described in Methods. The data are expressed per microgram cell protein and represent the mean of four replicates per treatment.
‡ Significantly different from control values (i.e., absence of effector in the presence or absence of Flurbiprofen) ($P < 0.05$) ±SE.

elicit cAMP release. cAMP was released on both sides of the monolayer, a result consistent with the observation of elevated medullary cAMP levels that is noted when animals are treated with antidiuretic hormone (31). To determine the time course for cAMP release, CCCT cells were treated for 0, 15, 30, 60, or 120 min with AVP on either the apical or basolateral side of the cells. Maximal levels of cAMP in the medium were seen 60 min after addition of AVP to the basolateral surface; AVP added to the apical surface did not increase extracellular cAMP above those levels seen without AVP (data not shown). Results on the time course or release of cAMP are typical of those observed with cultured epithelial cells (32). Half-maximal release of cAMP occurred at a basolateral AVP concentration of $\sim 10^{-10}$ M (data not shown). This result is quantitatively similar to that seen with CCCT cells that are grown on plastic culture dishes

*Table II. Release of PG by CCCT Cells on Chambers*

| Treatment | Immunoreactive PG (pmol/60 min/chamber) | | | |
|---|---|---|---|---|
| | $iPGE_2$ | $iPGF_{2\alpha}$ | $iTxB_2$ | i-6-keto-$PGF_{1\alpha}$ |
| No effector | 3.5±0.1 | 2.7±0.8 | 2.8±0.7 | 1.8±0.3 |
| Bradykinin | 12.3±0.4* | 3.2±0.3 | 3.4±0.2 | 1.3±0.5 |
| DD-AVP | 8.6±0.7* | 2.4±0.2 | 3.1±0.1 | 2.1±1.1 |

CCCT cells cultured on chambers were incubated for 60 min in the presence of bradykinin ($10^{-6}$ *M*), DD-AVP ($10^{-6}$ *M*), or no effector at 37°C under a 7% $CO_2$ atmosphere. Medium from both sides of the monolayer was pooled and analyzed for immunoreactive PG without prior purification, as described in Methods. The data represent the mean of three replicates per treatment.
* Significantly different from control values (i.e., absence of effector) ($P < 0.05$) ±SE.

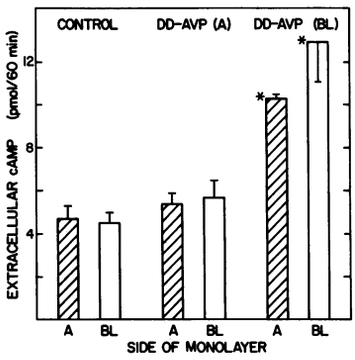


*Figure 5.* Sidedness of DD-AVP effect on cAMP release from CCCT cell monolayers on Millipore filters. Indicated on the horizontal axis is the side of release of cAMP: A, apical, and BL, basolateral. The bars indicate the release in response to no effector or to $10^{-6}$ *M* DD-AVP added to either the apical (A) or basolateral (BL) side of the monolayer. The data represent the mean of six chambers. All treatments were at 37°C for 60 min in the presence of $10^{-4}$ *M* IBMX. Radioimmunoassays for extracellular cAMP were performed as described in text. *Significantly different from control values (i.e., absence of DD-AVP) ($P < 0.05$).

(24). These results suggest that AVP can act only from the basolateral surface of CCCT cell monolayers to cause cAMP formation.

*PG synthesis by CCCT cells.* We reported previously that immunoreactive $PGE_2$ was formed in response to AVP and bradykinin by CCCT cells that were cultured on Petri dishes (24). To determine if $PGE_2$ was the major PG product, analyses of the PG formed by CCCT cells were performed. PG were extracted from the media surrounding CCCT cells that were treated with no effector or with AVP or bradykinin. The PG were separated by thin-layer chromatography and the materials in the regions cochromatographing with $PGE_2$, $PGF_{2\alpha}$, $TxB_2$, and 6-keto-$PGF_{1\alpha}$ standards were eluted from the silica gel and analyzed by specific radioimmunoassays (Table I). The results indicate that the major product formed both in the presence and absence of AVP and bradykinin was $PGE_2$. Small amounts of other prostanoids were also detected, but, unlike $PGE_2$, the amounts of these other products did not increase after treatment of CCCT cells with AVP or bradykinin. Almost identical data were obtained when medium surrounding the cells was analyzed by radioimmunoassays without prior extraction (data not shown).

PG radioimmunoassays were also performed on media pooled from the basolateral and apical surfaces of CCCT cells on Millipore filters after addition of AVP or bradykinin to both sides of the cell monolayer. Again, $PGE_2$ was found to be the major PG product (Table II). $PGE_2$ has now been found to be the primary PG formed by preparations of rabbit, canine and rat cortical, and papillary collecting tubules (6, 25, 33).

The effects on $PGE_2$ release of AVP and of bradykinin added to either the apical or basolateral side of CCCT cells on Millipore filters was examined. As shown in Fig. 6, AVP caused an increase in the release of $PGE_2$ when added to either the apical or basolateral side of the cells. Half-maximal increases in $PGE_2$ release were observed at $\sim 10^{-10}$ M AVP; the half-maximal concentration for AVP-induced $PGE_2$ release was the same for AVP

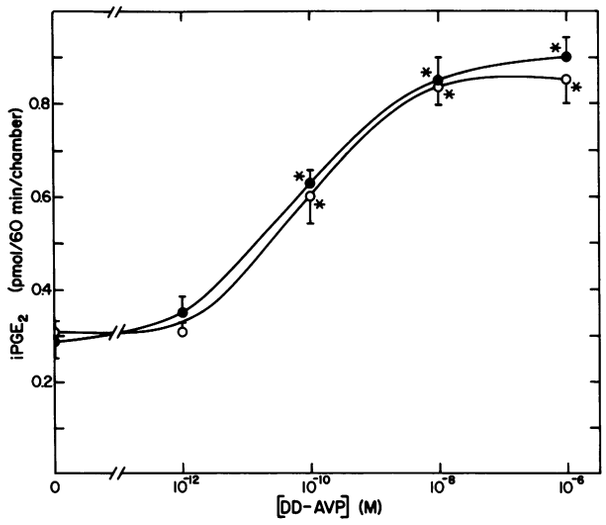


*Figure 6.* Concentration dependence for the DD-AVP-induced release of $iPGE_2$ by CCCT cells on Millipore filters. The chambers were incubated at 37°C for 60 min with the indicated concentrations of DD-AVP added to either the basolateral (○) or apical (●) side of the CCCT cell monolayers. Media from both sides of the monolayer were pooled and analyzed for $iPGE_2$ as described in text. Each point represents the mean of triplicate chambers. *Significantly different from control values (i.e., absence of DD-AVP) ($P < 0.05$).

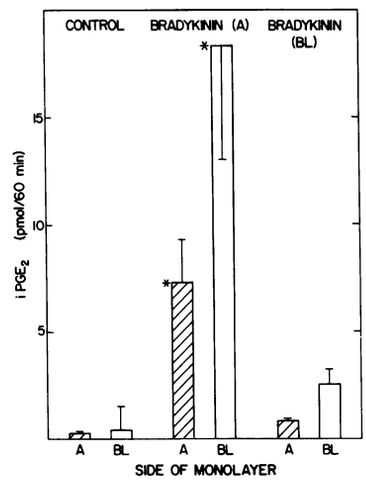


*Figure 7.* Sidedness of bradykinin effect on the release of $iPGE_2$ from CCCT cells on Millipore filters. Each bar indicates the amount of $iPGE_2$ measured on the apical (A) or basolateral (BL) side of cell monolayers after treatment with no effector or with bradykinin ($10^{-6}$ *M*) added to either the apical (A) or basolateral (BL) side of the monolayer. All treatments were performed at 37°C for 60 min. Radioimmunoassays for $PGE_2$ were as described in text. *Significantly different from control values ($P < 0.05$).

added to either side of the cell monolayer, and was about the same as that observed for the release of cAMP (occurring when AVP was added to the basolateral side of the cells). The amounts of $PGE_2$ found on the apical and on the basolateral side of the cells were comparable both in the case of treatment with no effector or with AVP added to either the apical or basolateral surface (data not shown). Thus, $PGE_2$ was released on both sides of the monolayer in response to the addition of AVP to either side of the monolayer.

Although AVP was effective in eliciting $PGE_2$ release when added to either side of the cell monolayer, bradykinin caused $PGE_2$ release only when added to the apical surface of the CCCT cell monolayer (Fig. 7). As shown in Table III, $PGE_2$ was found on both sides of the monolayer at different times after the addition of bradykinin to the apical side, which indicates that $PGE_2$ was released on both sides of the monolayer. After bradykinin treatment, the amount of $PGE_2$ found on the basolateral side of the monolayer tended to be 1.5–2 times greater than that found on the apical side (Fig. 7); however, this difference was not statistically significant, and the concentration of $PGE_2$ on each side of the monolayer was the same (circa $4 \times 10^{-9}$ M).

*$PGE_2$-induced release of cAMP from CCCT cells.* $PGE_2$ was tested at a variety of concentrations and on either side of CCCT cell monolayers for its ability to elicit the release of cAMP. Significant cAMP release was observed with $10^{-8}$ M $PGE_2$, and there was no difference in the dose-response curves for $PGE_2$ when it was added to either the basolateral or apical surfaces (Fig. 8). Thus, $PGE_2$, unlike AVP, can act from either side of the CCCT cell to cause cAMP release.

*Inhibition of AVP-induced cAMP release by $PGE_2$.* At concentrations of $10^{-10}$ M or less, $PGE_2$ had no significant effect on cAMP release (Fig. 8). Therefore, we used three concentrations to test the effects of $PGE_2$ on AVP-induced cAMP release from CCCT cells. In the first experiment, $PGE_2$ was added to both the apical and basolateral side of the cell monolayer at a concentration of $10^{-10}$ M on both sides 1 h before the addition of AVP. Monolayers used as no effector controls or monolayers treated with AVP alone were preincubated for 60 min with buffer that did not contain $PGE_2$. The preincubation medium was removed after 60 min; then, after a further 1-h incubation

*Table III. Time Course of Bradykinin-induced Release of $PGE_2$ by CCCT Cells on Millipores Filters**

| Treatment | Immunoreactive $PGE_2$ (pmol/chamber) | |
|---|---|---|
| | Apical side | Basolateral side |
| No effector (60 min) | 2.3±0.2 | 3.0±0.5 |
| Bradykinin (0 min) | 2.3±0.3 | 2.1±0.2 |
| Bradykinin (2 min) | 2.1±0.3 | 4.3±1.5 |
| Bradykinin (4 min) | 5.9±2.0‡ | 4.5±0.5‡ |
| Bradykinin (10 min) | 4.6±1.2‡ | 5.5±0.7‡ |
| Bradykinin (60 min) | 4.7±0.7‡ | 5.7±1.2‡ |

* Millipore filters seeded with $3 \times 10^6$ CCCT cells per chamber ($2 \times 10^6$ cells/cm$^2$) were incubated for the indicated times in the presence of bradykinin ($10^{-6}$ *M* on the apical surface) or with no effector at 37°C under a 7% $CO_2$ atmosphere. After the indicated incubation time, medium was removed from each side of the monolayer and assayed for immunoreactive $PGE_2$, as described in Methods. The data represent the mean of two replicates per treatment.
‡ Significantly different from control values (i.e., after 60 min in the absence of effector) ($P < 0.05$) ±SE.

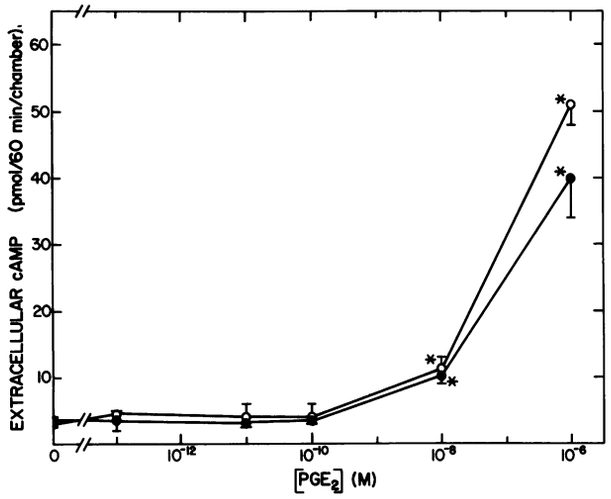


*Figure 8.* Concentration dependence for the $PGE_2$-induced release of cAMP from CCCT cells monolayers on Millipore filters. $PGE_2$ was added at the indicated concentrations to either the apical (○) or basolateral (●) side of the CCCT cell monolayer. Incubations were performed at 37°C for 60 min in the presence of IBMX ($10^{-4}$ *M*). Media from both sides of the monolayer were pooled and analyzed for cAMP as described in text. Each point represents the mean of triplicate chambers. *Significantly different from control values (i.e., absence of $PGE_2$) ($P < 0.05$).

in the presence of no effector, AVP ($10^{-8}$ M) alone or both AVP ($10^{-8}$ M) and $PGE_2$ ($10^{-10}$ M), the medium was assayed for cAMP. Under these conditions, $PGE_2$ completely blocked AVP-induced cAMP release (Fig. 9).

Fig. 10 shows the sidedness and the concentration dependence for $PGE_2$ as an antagonist of the release of cAMP that occurs in response to AVP ($10^{-8}$ M). Significant inhibition of AVP-induced cAMP release was noted at concentrations of $PGE_2$ as low as $10^{-12}$ M. Interestingly, the dose-response curves for inhibition of AVP-induced cAMP release were the same for $PGE_2$ when it was added to either side of the CCCT cell monolayer. One cannot ascribe the inhibition obtained with $10^{-12}$ M $PGE_2$ to diffusion of $PGE_2$ across the monolayer, since a maximum of 10% of the $PGE_2$ could have crossed the monolayer during the 60-min preincubation period (Fig. 4). Thus, $PGE_2$ apparently can act from either the apical or basolateral surface of CCCT cells to inhibit AVP-induced cAMP release, even though AVP appears to act only from the basolateral surface to cause cAMP formation (Fig. 5).

The concentration of $PGE_2$ that surrounds the CCCT cell monolayers after a 60-min preincubation (Fig. 6) ranges from $10^{-9}$ to $10^{-10}$ M due to endogenous synthesis of the PG. When endogenous synthesis is blocked with either Ibuprofen or aspirin, a potentiation of the AVP response is observed (Table IV). Nevertheless, exogenous $PGE_2$ ($10^{-11}$ M) completely suppressed AVP-induced cAMP release in the presence or absence of cyclooxygenase inhibitors. Thus, the effect of newly formed PG, while measurable, does not appear to have an overriding influence on this system. This appears to be due to two factors. First, the inhibitory effect of $PGE_2$ requires a prolonged incubation with this PG (see Table V), and the $PGE_2$ arising from the cells themselves is not present at elevated concentrations during the entire preincubation period. Second, after the preincubation period, the medium surrounding the cells is removed and replaced with fresh medium to begin the 60-min treatment (e.g. with AVP).

*Inhibition of AVP-induced accumulation of intracellular cAMP by $PGE_2$.* In studies on CCCT cells that were seeded on Millipore filters, only cAMP release was monitored. To determine if $PGE_2$ inhibited AVP-induced accumulation of intracellular cAMP, experiments were performed using CCCT cells that were grown as nonconfluent monolayers on plastic culture dishes. As shown in Table V, $10^{-11}$ M $PGE_2$ suppressed the AVP-induced accumulation of intracellular cAMP at 2- and 3-min time points. This inhibition also occurred when endogenous $PGE_2$ formation was blocked (data not shown). Curiously, inhibition was only observed after a 20-min preincubation of $PGE_2$ with the CCCT cells (Table V). A similar time dependence was observed in the inhibition of AVP-induced cAMP release from CCCT cell monolayers on Millipore filters. This time dependence suggests that $PGE_2$ is causing a chain of metabolic events to occur that prevents AVP-induced cAMP accumulation.

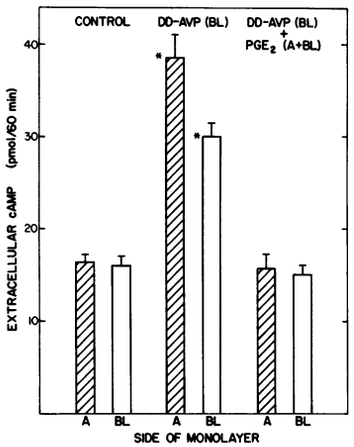


*Figure 9.* Inhibition by $PGE_2$ of DD-AVP-induced cAMP release from CCCT cell monolayers on Millipore filters. Each bar represents the amount of cAMP measured on the apical (A) or basolateral (BL) side of the CCCT cell monolayer after treatment with (*a*) $10^{-8}$ *M* DD-AVP added to the basolateral side; (*b*) $10^{-8}$ *M* DD-AVP (added to the basolateral side) plus $10^{-10}$ *M* $PGE_2$ (added to both sides); or (*c*) no effector. All samples were preincubated for 60 min at 37°C before the addition of DD-AVP. In samples treated with $PGE_2$, the $PGE_2$ was included at the beginning of the 60-min preincubation period at 37°C. After a 60-min incubation at 37°C with the indicated effectors, the media from the two sides were removed and assayed for cAMP as described in the text. All treatments were performed with triplicate chambers and in the presence of $10^{-4}$ *M* IBMX. *Significantly different from control values ($P < 0.05$).

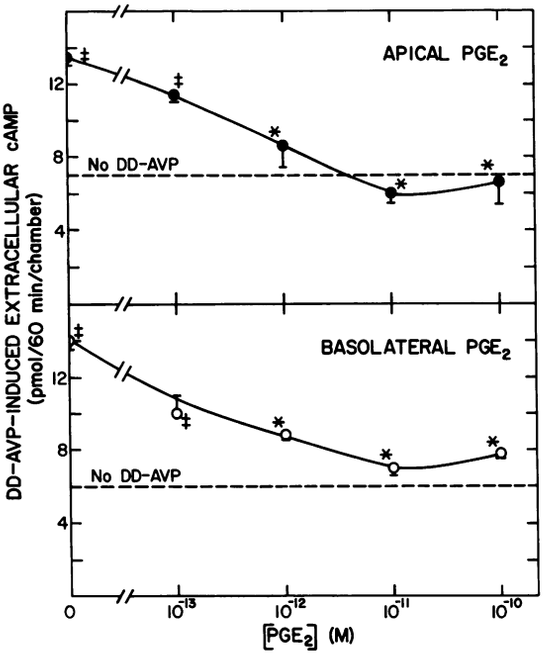


*Figure 10.* Concentration dependence for the inhibition by $PGE_2$ of DD-AVP-induced release of cAMP. CCCT cells on Millipore filters were preincubated for 60 min at 37°C with the indicated concentrations of $PGE_2$ on the apical or basolateral side of the monolayers. The chambers were then incubated for a second 60-min period at 37°C with the same concentrations of $PGE_2$ plus DD-AVP ($10^{-8}$ *M*). DD-AVP was added only to the basolateral side. Media from both sides of the monolayer were pooled and assayed for cAMP as described in the text. All treatments were performed in the presence of $10^{-4}$ *M* IBMX. Each point represents the mean of duplicate chambers. ‡Significantly different from control values (i.e., no effectors [-----]; *significantly different from values of samples treated with DD-AVP alone ($P < 0.05$).

Torikai and Kurokawa (23) suggested that $PGE_2$ may exert its inhibitory effect on AVP-induced cAMP formation by activating cAMP phosphodiesterase activity. Were this true in the CCCT cell system, one would expect $10^{-10}$–$10^{-12}$ M $PGE_2$ to decrease basal levels of intracellular cAMP. However, these concentrations of $PGE_2$ had no effect on intracellular cAMP levels in the presence of $10^{-4}$ M IBMX (data not shown).

## Discussion

*CCCT cells on Millipore filters.* The initial goal of the studies reported here was to test the concept that there is a sidedness to the release of PG from CCCT cells and to the effects of PG on cAMP metabolism by CCCT cells. This hypothesis developed from the observations of Stokes and Kokko (14) that $PGE_2$ inhibited $Na^+$ resorption when added to the basolateral but not the luminal surface of segments of rabbit cortical collecting tubule. The CCCT cell-Millipore filter system was developed to examine the sidedness of PG biosynthesis and function. The criteria that have qualified this CCCT cell system for use in these studies are as follows: (*a*) CCCT cells were morphologically asymmetric as determined by transmission electron microscopy; (*b*) CCCT cells exhibited a transcellular potential difference of the appropriate sign; (*c*) CCCT cells were impermeable to both inulin and $PGE_2$; and (*d*) CCCT cells formed cAMP in response to AVP that was added to the basolateral but not to the apical surface of the cell monolayer. Although MDCK cells also exhibit some of these properties (28, 32–34, 35), this is the first time that a sidedness to the response of cells on Millipore filters to a hormone (AVP) has been demonstrated.

*Sidedness of $PGE_2$ release by CCCT cells.* Depicted in Fig. 11 are our concepts of bradykinin, AVP, and $PGE_2$ interrelationships developed from the results of the experiments described in this report. Our studies on the biosynthesis of PG by CCCT cell monolayers indicate that $PGE_2$ is the major if not the exclusive PG product formed both under basal conditions and in response to hormonal stimuli. $PGE_2$ was found on both sides of the cell monolayer in similar amounts. This result cannot be ascribed to simple transcellular diffusion, since the flux of $PGE_2$ across the monolayer is quite slow. Thus, $PGE_2$ is released on both sides of the CCCT cell monolayer, which suggests, in turn, that $PGE_2$ is released on both the blood and urine sides of the canine collecting tubule in vivo. A previous report had indicated that the major site of entry of primary PG into urine occurs at the level of Henle's loop (36). While this may be true of rat kidneys perfused with angiotensin II, our results suggest that, at least in the dog, $PGE_2$ can enter the urine at the level of the collecting tubule under conditions where renal bradykinin

*Table IV. Inhibition by $PGE_2$ of AVP-induced cAMP Release from CCCT Cell Monolayers on Millipore Filters in the Presence of Inhibitors of PG Synthesis**

| | Extracellular cAMP (pmol/60 min/chamber) | | | |
|---|---|---|---|---|
| Treatment | No inhibitor | Ibuprofen | No inhibitor | Aspirin |
| 1 No effector | 6.5±0.1 | 6.8±1.1 | 7.1±0.4 | 6.3±1.3 |
| 2 AVP alone | 10.8±1.3‡ | 17.8±1.1‡ | 11.9±0.5‡ | 18.2±1.1‡ |
| 3 $PGE_2$ alone | 6.5±0.5 | 7.8±0.2 | 7.2±0.2 | 7.7±0.3 |
| 4 AVP + $PGE_2$ | 4.4±1.0§ | 7.7±0.3§ | 7.2±0.3§ | 7.5±0.4§ |

* CCCT cells on Millipore filters were preincubated for 60 min at 37°C with $10^{-11}$ *M* $PGE_2$ (treatments 3 and 4) or with buffer alone (treatments 1 and 2) on both sides of the monolayer. At the end of the preincubation period, the media was removed and the monolayers were incubated for an additional 60 min period at 37°C with no effector, $10^{-8}$ *M* AVP alone, $10^{-11}$ *M* $PGE_2$ alone, or $10^{-8}$ *M* AVP plus $10^{-11}$ *M* $PGE_2$. AVP was added only to the basolateral side. In the chambers where a PG synthesis inhibitor ($10^{-3}$ *M* aspirin or $10^{-4}$ *M* Ibuprofen) was used, it was present in the media 30 min before the preincubation period and then throughout preincubation and treatment periods (i.e., a total of 150 min). Buffer was used for the same time in chambers without such inhibitors. $10^{-4}$ IBMX was present in all chambers throughout the 150-min period. Each value represents the mean of duplicate chambers.
‡ Significantly different from control value (i.e., no effector).
§ Significantly different from value of treatment with AVP alone ($P < 0.05$).

*Table V. Effect of Preincubation Time on the Inhibition by $PGE_2$ of AVP-induced Formation of cAMP by CCCT Cells on Culture Dishes**

| | Preincubation | | | Intracellular cAMP (fmol/μg cell protein) | |
|---|---|---|---|---|---|
| | Time with buffer alone | Time with $PGE_2$ ($10^{-11}$ *M*) | Treatment | 2 min | 3 min |
| | *min* | *min* | | | |
| 1 | 60 | 0 | No effector | 2.4±0.2 | 1.7±0.2 |
| 2 | 60 | 0 | AVP alone | 8.4±0.2‡ | 5.0±0.1‡ |
| 3 | 60 | 0 | AVP + $PGE_2$ | 6.8±0.4‡ | 4.5±1.3‡ |
| 4 | 55 | 5 | AVP + $PGE_2$ | 9.1±0.5‡ | 5.3±0.6‡ |
| 5 | 50 | 10 | AVP + $PGE_2$ | 7.1±1.5‡ | 4.2±0.1‡ |
| 6 | 40 | 20 | AVP + $PGE_2$ | 2.6±0.6§ | 1.7±0.1§ |
| 7 | 20 | 40 | AVP + $PGE_2$ | 2.1±0.2§ | 2.2±0.3§ |
| 8 | 0 | 60 | AVP + $PGE_2$ | 2.3±0.5§ | 2.2±0.4§ |

* Nonconfluent CCCT cells seeded on culture dishes were preincubated for the indicated time periods at 37°C with $10^{-11}$ *M* $PGE_2$ (treatments 4–8) or with buffer alone (treatments 1–3). In treatments where the preincubation with $PGE_2$ was performed for <60 min (treatments 4–7), the cells were preincubated in buffer alone followed by the preincubation with $10^{-11}$ *M* $PGE_2$ (e.g., treatment 4 included 55 min of preincubation in buffer alone followed by 5 min of preincubation in the presence of $PGE_2$). At the end of the full preincubation period, the media was removed and the cells were incubated for 2 or 3 min with no effector, $10^{-8}$ *M* AVP alone, $10^{-11}$ $PGE_2$ alone, or $10^{-8}$ *M* AVP plus $10^{-11}$ *M* $PGE_2$. Preincubations and incubations were performed in the presence of $10^{-4}$ *M* IBMX. Each value represents the mean of triplicate wells. Intracellular cAMP was measured by radioimmunoassay as described in the text.
‡ Significantly different from control value (i.e., no effector).
§ Significantly different from value of treatment with AVP alone ($P < 0.05$).

or AVP concentrations are elevated (cf 37). Our results also indicate that if $PGE_2$ functions extracellularly to influence collecting tubule metabolism, $PGE_2$ presumable can affect events from both the apical and basolateral cell surfaces. As discussed in more detail below, this concept is consistent with the lack of sidedness to the effects of $PGE_2$ on cAMP metabolism in CCCT cells.

Although there was no asymmetry to $PGE_2$ release, there was a sidedness to the effect of bradykinin on PG synthesis. Bradykinin acted only from the apical surface to cause $PGE_2$ release (Fig. 11). This sidedness of bradykinin action on the PG biosynthetic system was proposed by McGiff et al. (38) and is consistent with studies showing that kallikrein may be located on the apical side of the distal tubule (39, 40). It should be noted, however, that bradykinin appears to act only from the basolateral surface of rabbit collecting tubules to antagonize the hydroosmotic effect of AVP, and that this antagonism appears to be PGE mediated (41).

The lack of a sidedness to the effect of AVP on $PGE_2$ formation was unexpected (Figs. 6 and 11). AVP caused the release of $PGE_2$ when it was added to either side of CCCT cells even though AVP elicited cAMP release only when added to the basolateral side of the cells. These data suggest that AVP interacts with different receptor systems in the cases of PG and cAMP formation.

*$PGE_2$-AVP interactions.* The hypothesis that PG inhibit AVP-induced cAMP formation in the collecting tubule evolved from the classic study of Grantham and Orloff (12). Clear evidence supporting this concept in the collecting tubule system itself has been difficult to obtain (33, 42–46). However, there have been two recent reports that, in the absence of phosphodiesterase inhibitors, $PGE_2$ causes partial but significant inhibition of AVP-induced cAMP accumulation in rabbit collecting tubule segments (23, 46). We have found that $PGE_2$ inhibits both the release of cAMP that normally occurs in response to treatment of confluent CCCT cell monolayers with AVP and the accumulation of intracellular cAMP in nonconfluent CCCT cell monolayers treated with AVP. The effects observed in the CCCT cell system have slightly different characteristics than those reported by Torikai and Kurokawa (23) and Edwards et al. (46), in that inhibition by $PGE_2$ of AVP-induced cAMP release in the CCCT cell system is quantitative, is time-dependent, and involves concentrations of $PGE_2$ that are 5–7 orders of magnitude lower than those reported for the rabbit collecting tubule.

The biochemical mechanism by which $PGE_2$ inhibits AVP-induced cAMP formation is not yet clear. It appears that the effect occurs only with intact cells. For example, the phenomenon is apparent in slices (45), in intact collecting tubules (23, 46), and in CCCT cells, but $PGE_2$ fails either to activate cAMP phosphodiesterase (46) or to inhibit AVP-independent adenylate cyclase in permeabilized collecting tubule segments (44). This

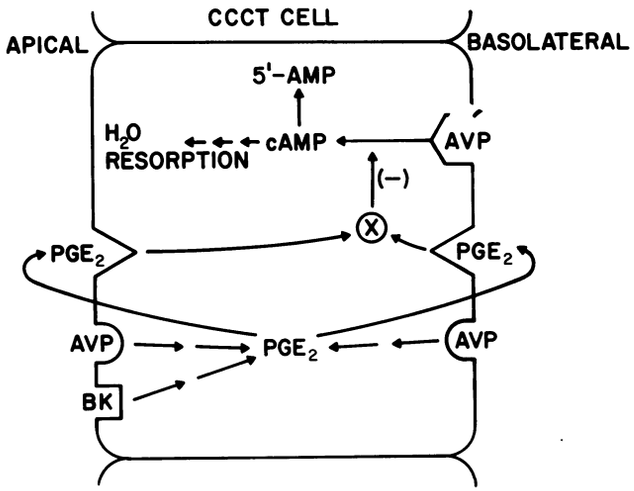


*Figure 11.* Model for AVP, bradykinin (BK), and $PGE_2$ interrelationships in CCCT cells.

suggests that $PGE_2$ must be causing production, mobilization, or sequestration of an intermediate factor(s) (denoted by an X in Fig. 11) which, in turn, modulates cAMP levels in the collecting tubule. Based on the 20-min time requirement (Table V), this intermediate could be a protein (47). Locher et al. (48) have demonstrated that in human phagocytes there is also a time dependence to the inhibition of AVP-induced cAMP synthesis by $PGE_2$.

It seems unlikely that $PGE_2$ is causing its inhibitory effect at the level of cAMP phosphodiesterase because $PGE_2$ does not decrease basal levels of intracellular cAMP in CCCT cells. A more reasonable view is that $PGE_2$ has an inhibitory effect on the AVP-inducible adenylate cyclase system. $PGE_2$ does not affect the affinity of binding of AVP to human phagocytes under conditions in which $PGE_2$ inhibits AVP-induced cAMP formation (48). Thus, the effect of $PGE_2$ is probably expressed at a post receptor step. The most applicable precedent for inhibition by $PGE_2$ of AVP-induced cAMP formation is seen in the heterologous desensitization of adenylate cyclase in human fibroblasts (49, 50). In this situation, $PGE_2$ attenuates the coupling of the hormone-receptor complex to the catalytic subunit of adenylate cyclase probably by modifying a guanosine triphosphate-binding subunit. Hopefully, the availability of large numbers of CCCT cells in culture will permit us to examine this hypothesis at the biochemical level.

## Acknowledgments

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