

# The Effect of Caffeine on Renal Epithelial Cells from Patients with Autosomal Dominant Polycystic Kidney Disease

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**Abstract.** Autosomal dominant polycystic kidney disease (ADPKD) is a hereditary disorder characterized by the progressive enlargement of cysts derived from tubules. Tubule cell proliferation and chloride-dependent fluid accumulation, mechanisms underlying cyst expansion, are accelerated by adenosine 3':5'-cyclic monophosphate (cAMP). This study examined the extent to which caffeine may stimulate the production of cAMP by cyst epithelial cells, thereby adversely increasing proliferation and fluid secretion. Mural epithelial cells from ADPKD cysts and normal human kidney cortex cells (HKC) were cultured, and cAMP levels were determined in response to caffeine and receptor-mediated agonists linked to adenylyl cyclase. Caffeine, a methylxanthine, slightly increased basal levels of cAMP, as did other nonselective phosphodiesterase (PDE) inhibitors, 1-methyl-3-isobutyl xanthine and theophylline and rolipram, a specific PDE IV inhibitor. More importantly, clinically relevant concentrations of caffeine (10 to 50  $\mu$ M) potentiated the effects of desmopressin (DDAVP), pros-

taglandin E<sub>2</sub> (PGE<sub>2</sub>), and isoproterenol to increase cAMP levels in both ADPKD and HKC cells. By contrast, at concentrations that augmented the DDAVP response, caffeine attenuated cAMP accumulation by adenosine, implicating an action apart from the inhibition of PDE. Caffeine enhanced the effect of DDAVP to stimulate transepithelial short-circuit current of polarized ADPKD monolayers, reflecting an increase in chloride secretion. Caffeine potentiated the effect of DDAVP and PGE<sub>2</sub> to increase the levels of phosphorylated extracellular signal-regulated kinase (P-ERK). By contrast, P-ERK levels in HKC cells were not raised by increased intracellular concentrations of cAMP. It is concluded that PDE inhibition by caffeine increases the accumulation of cAMP, and through this mechanism activates the ERK pathway to cellular proliferation and increases transepithelial fluid secretion in ADPKD cystic epithelium. Caffeine is, therefore, a risk factor for the promotion of cyst enlargement in patients with ADPKD.

Autosomal dominant polycystic kidney disease (ADPKD) is the most common potentially lethal hereditary renal disorder in adults (1). The intrarenal production of adenosine 3':5'-cyclic monophosphate (cAMP) has a major role in the pathogenesis of the disease because this second messenger stimulates transepithelial secretion and accumulation of cyst fluid as well as cell proliferation (2–4). Receptor-mediated agents that increase the activity of adenylyl cyclase (arginine vasopressin [AVP] prostaglandin E<sub>2</sub> [PGE<sub>2</sub>],  $\beta$ -adrenergic agonists) and inhibitors of phosphodiesterase (caffeine, theophylline) consequently have the potential to accelerate the progression of ADPKD (4–7). However, no direct studies of renal cells derived from human ADPKD cysts have examined the hypothesis that caffeine in concentrations that are clinically relevant has

the potential to adversely affect renal cyst enlargement by promoting increased intracellular levels of cAMP.

In the current study, we examined cells cultured from mural cysts of patients with ADPKD and cells cultured from normal human renal cortex (HKC) for the effect on the accumulation of cAMP of caffeine alone and in combination with adenylyl cyclase agonists of physiologic interest. The results are interpreted to support the view that caffeine has the potential to enhance the progression of ADPKD by increasing the production of cAMP in renal epithelial cells.

## Materials and Methods

### Cell Culture

Cells were retrieved from the renal cysts of three patients with ADPKD who underwent elective nephrectomy and from the normal renal cortex (HKC) of a nephrectomy specimen removed for adenocarcinoma. The cells were placed in primary culture as described previously (4,8,9). The protocols were approved by the Human Subjects Committee at the University of Kansas Medical Center. Cells maintained in liquid nitrogen were thawed and directly seeded onto collagen-coated Transwell plates (Costar, Cambridge, MA) containing a 1:1 mixture of Dulbecco's modified Eagle's medium and Hams F12 (DMEM/F12) supplemented with 5% fetal bovine serum (FBS; Hyclone, Logan, UT), 100 U/ml penicillin, 0.1 mg/ml streptomycin, and 5  $\mu$ g/ml insulin, 5  $\mu$ g/ml transferin, and 5 ng/ml sodium selenite (ITS; Collaborative Biomedical Products, Bedford, MA). Media were replaced every 2 d.

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### Measurements of Intracellular cAMP

$3 \times 10^4$  cells were seeded into individual chambers of collagen-coated Transwell plates in DMEM/F12 containing 5% FBS, ITS, and penicillin/streptomycin. Near confluence, the medium was changed to 1% FBS to reduce the rate of growth. Twenty-four hours later, cells were rinsed in fresh DMEM/F12 containing 1% FBS for 15 min and in an isotonic Ringer's solution for 15 min. The rinsing procedure ensured the removal of autocrine cAMP agonists that may have accumulated in the conditioned medium. Stock solutions of Desmopressin (DDAVP), PGE<sub>2</sub>, isoproterenol, and adenosine were added directly to the incubation medium. Stock solutions of the PDE inhibitors caffeine, rolipram, 1-methyl-3-isobutyl xanthine (IBMX), and theophylline were made in DMSO, and 1:100 to 1:1000 dilutions of this were added directly to the incubation medium. Equivalent concentrations of DMSO were added to control medium. For the highest concentration (1000  $\mu$ M), caffeine was not completely soluble in the stock solution, so a well-mixed slurry was added directly to the medium. PGE<sub>2</sub>, isoproterenol, adenosine, caffeine, IBMX, and theophylline were purchased from Sigma (St. Louis, MO). DDAVP was purchased from Rhone Poulenc Rorer Pharmaceuticals (Collegeville, PA). Rolipram was purchased from Calbiochem (La Jolla, CA).

Intracellular cAMP was extracted into 80% methanol, reconstituted in 0.05 mol/L sodium acetate, and quantified by an enzyme-immunoassay system (Amersham Pharmacia Biotech, Buckinghamshire, UK). In parallel experiments, cell protein content was measured with a BCA protein assay kit (Pierce, Rockford, IL). cAMP content was initially expressed in pmol/cm<sup>2</sup> and converted to pmol/mg protein using a conversion factor determined in the parallel experiments.

### Bioelectric Measurements

Monolayers of ADPKD cells, grown on Snapwell membranes (Costar), were mounted in modified Ussing chambers as described previously (8,10). The chambers contained 5 ml of medium and were gassed and stirred with a slow stream of 5% CO<sub>2</sub>/95% O<sub>2</sub>. The medium used in these experiments was an isotonic Ringer's solution that contained no serum or serum components. The transepithelial electrical potential difference (V<sub>te</sub>), the short-circuit current (I<sub>sc</sub>), and monolayer resistance (R<sub>te</sub>) were determined. The monolayers were maintained in the short-circuited state, and the open-circuit voltage was determined at 1- to 5-min intervals. After a period of equilibration, measurements were made in sequential 5-min periods. I<sub>sc</sub> was recorded 15 min after the addition of agonists or a combination of agonists with caffeine to both basolateral and apical solutions.

### Antibodies

Anti-ERK1 (C-16), ERK2 (C-14), and phospho-ERK (E-4) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-rabbit, -mouse, or -rat IgG-conjugated horseradish peroxidase (HRP) antibodies (Santa Cruz Biotechnology) were used as secondary antibody.

### Western Blot Analyses

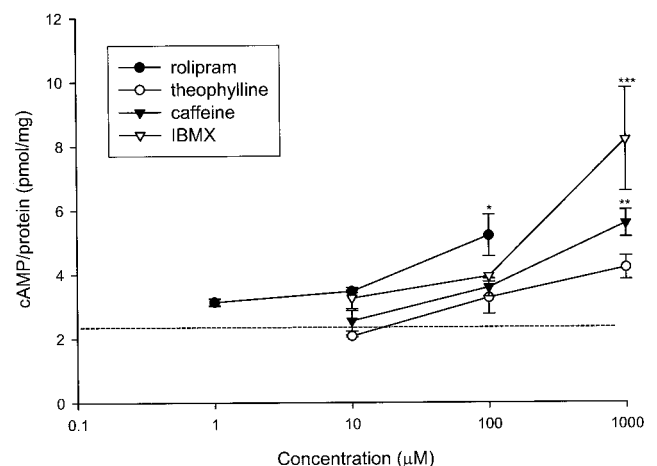
$10^5$  cells were seeded in 100-mm-diameter plastic dishes in DMEM/F12 with 5% FBS, ITS, and penicillin/streptomycin. Near confluence, FBS was reduced to 1% for 24 h. Before incubation with agonists, cells were washed in 1% FBS for 15 min and in Ringer's medium for 15 min. Caffeine, DDAVP, and PGE<sub>2</sub> were added in Ringer's medium and incubated for 15 min. Cells were lysed in 500  $\mu$ l of ice-cold lysis buffer (TLB; 20 mmol/L Tris [pH 7.4], 137 mmol/L NaCl, 25 mmol/L  $\beta$ -glycerophosphate, 2 mmol/L ethylenediamine tetraacetic acid [EDTA], 1 mmol/L sodium orthovanadate, 2 mmol/L NaHPO<sub>4</sub>, 1% Triton X-100,

10% glycerol, 1 mmol/L phenylmethylsulfonyl fluoride [PMSF], 5  $\mu$ g/ml aprotinin, 5  $\mu$ g/ml leupeptin, 2 mmol/L benzamidine, and 0.5 mmol/L dithiothreitol). Insoluble cell lysate was removed by centrifugation. Aliquots of soluble cellular protein were quantified. Cell lysate (20  $\mu$ g of protein) was then heated (95 to 100°C) in sodium dodecyl sulfate (SDS) sample buffer, separated by 10% SDS-polyacrylamide gel electrophoresis (PAGE), and transferred to nitrocellulose membranes (Hybond ECL, Amersham). After transfer, the membranes were blocked with 5% milk in TBS-T (20 mmol/L Tris-HCl, 137 mmol/L NaCl, and 0.05% Tween 20 [pH 8.0]) for 1 h at room temperature or overnight at 4°C. Blocked membranes were incubated with primary antibody in 5% milk in TBS-T for 2 h at room temperature or overnight at 4°C. Membranes were then washed three times with TBS-T and incubated with secondary antibody with 5% milk in TBS-T for 1 h. The membranes were washed three times with TBS-T, and proteins were visualized using an enhanced chemiluminescence system (ECL, Amersham Life Science, Arlington Heights, IL). Intensity of the blots was detected and quantified by a Fluor-S Max multi-imager system (Bio-Rad, Hercules, CA).

## Results

### Effects of Methylxanthines on cAMP Content of Cells from ADPKD Kidneys

In these experiments, cells were rinsed carefully before adding phosphodiesterase (PDE) inhibitors to reduce endogenous activation of adenylyl cyclase by autocrine or paracrine substances produced by the cells. We determined the relative potency of caffeine, theophylline, rolipram, and IBMX to increase cAMP accumulation in the cells in the absence of exogenous adenylyl cyclase agonists. The increase in intracellular cAMP caused by these PDE inhibitors was concentration-dependent (Figure 1). Theophylline, a weaker inhibitor, caused a small increase in cAMP levels, but the values did not reach statistical significance. This initial study suggests that, despite



**Figure 1.** Concentration-dependent effects of phosphodiesterase (PDE) inhibitors on basal adenosine 3':5'-cyclic monophosphate (cAMP) levels in autosomal dominant polycystic kidney disease (ADPKD) cells. Cells were extensively rinsed before addition of PDE inhibitors for 15 min. Horizontal dashed line indicates basal cAMP level ( $2.32 \pm 0.24$  pmol/mg). cAMP is expressed in relation to total protein content. Values are mean  $\pm$  SE;  $n = 6$ . ADPKD cells are from two different patients. \*  $P < 0.05$ , \*\*  $P < 0.01$ , and \*\*\*  $P < 0.001$  compared with the baseline value.

extensive washing before adding PDE, a small amount of endogenous activated adenylyl cyclase remained in these cells.

We next determined the effect on cAMP accumulation in renal cells after treatment with receptor-mediated agonists. DDAVP, a V2 receptor agonist, PGE<sub>2</sub>, a renal autocooid, and isoproterenol, a β-adrenergic receptor agonist caused concentration-dependent increases in cAMP (Figure 2). Each of these agonists increased cAMP to levels several times greater than seen with the highest concentration of caffeine alone (Figure 1; 1000 μM). To determine the extent to which caffeine may potentiate receptor-mediated increases in cAMP, sub-maximal concentrations of DDAVP (20 mU/ml), PGE<sub>2</sub> (12.5 ng/ml), and isoproterenol (1 nM) were evaluated in conjunction with the methylxanthine. Concentrations of caffeine that slightly increased cAMP levels when added alone strikingly potentiated the effects of DDAVP (Figure 3). This synergistic interaction was illustrated most clearly at the highest concentration of caffeine used in the study (1000 μM). DDAVP alone increased cAMP to 31.3 ± 13.6 pmol/mg (n = 12). By contrast, the addition of caffeine, which alone increased basal cAMP to 2.9 ± 0.5 pmol/mg of protein (n = 12), further increased cAMP levels to 81.6 ± 21.4 pmol/mg (n = 12). Thus, the caffeine effect was more than additive to that of DDAVP alone, indicating a synergistic mechanism of action.

To determine if caffeine potentiation of DDAVP-induced cAMP accumulation was unique to ADPKD cells, we performed the same experiment using HKC lacking mutations in polycystins (Figure 4). Interestingly, 10 and 100 μM caffeine, diminished cAMP to 2.7 ± 0.8 and 1.7 ± 0.3 pmol/mg, respectively, below the baseline level of 4.6 ± 0.9 pmol/mg. On the other hand, the highest concentration of caffeine (1000 μM) increased the cAMP level to 9.6 ± 1.9 pmol/mg above the baseline level. In the HKC cells, DDAVP (20 mU/ml) increased cAMP from the baseline to 81.9 ± 7.2 pmol/mg, and this was increased further by incremental concentrations of caffeine (Figure 4). Thus, as with ADPKD cells, caffeine

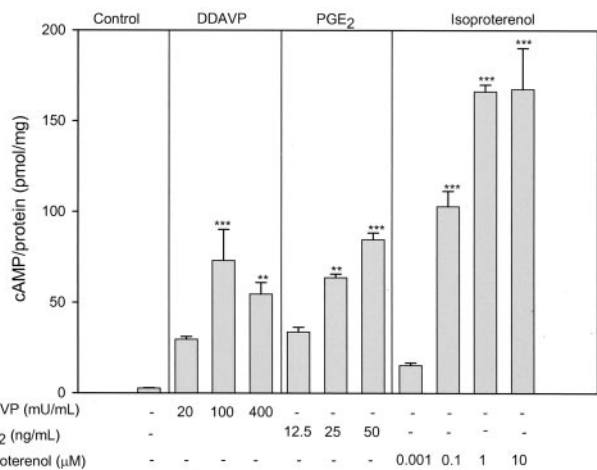


Figure 2. Effect of receptor-mediated agonists on cAMP levels in ADPKD cells. Cells were rinsed extensively before addition of agonists for 15 min. \*\* P < 0.01 and \*\*\* P < 0.001 indicate difference from the baseline value; n = 4.

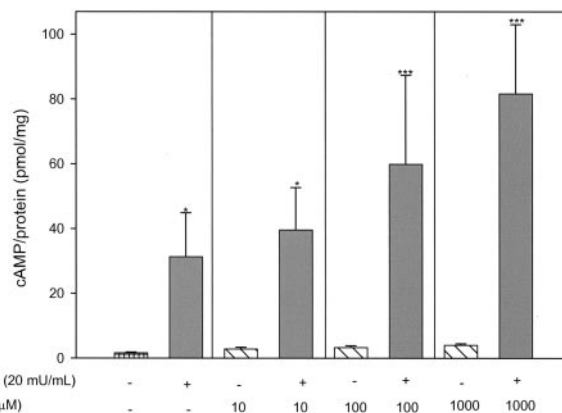


Figure 3. Potentiation of desmopressin (DDAVP)-induced cAMP accumulation by caffeine in ADPKD cells. Cells were rinsed extensively before adding agonists for 15 min. Values are mean ± SE (n = 12) from three different ADPKD patients. \* P < 0.05 and \*\*\* P < 0.001 compared with the baseline value.

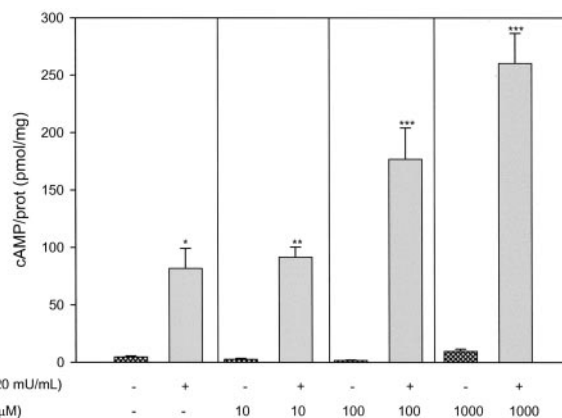
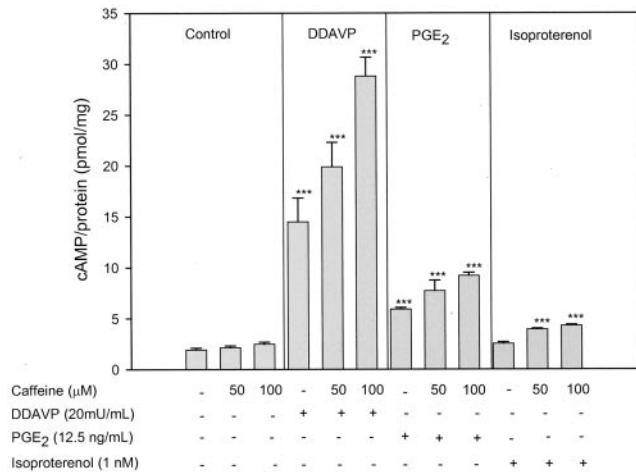


Figure 4. Potentiation of DDAVP-induced cAMP accumulation in human kidney cortex cells (HKC) cells by caffeine. Cells were rinsed extensively before adding agonists for 15 min. Values are mean ± SE; n = 4. \* P < 0.05, \*\* P < 0.01, and \*\*\* P < 0.001 compared with the baseline value.

potentiated the effect of DDAVP to stimulate the accumulation of cAMP, although in contrast to ADPKD cells, low concentrations of the methylxanthine alone appeared to inhibit the accumulation of the cyclic nucleotide.

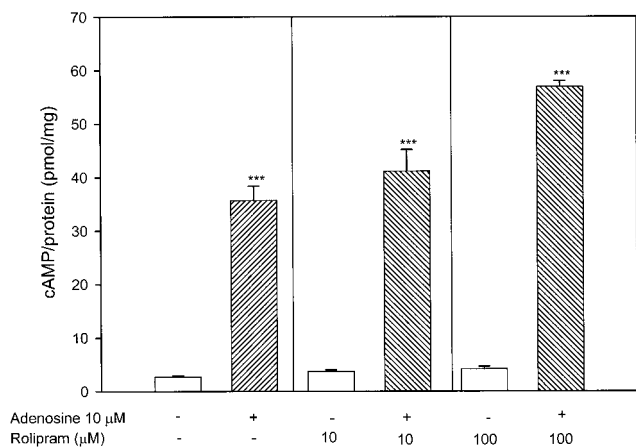
It may be expected that plasma levels of caffeine in the renal medulla may fall within the range of 10 to 100 μM in individuals ingesting moderate amounts of coffee (11–14). We determined, therefore, the extent to which representative adenylyl cyclase agonists may be potentiated by clinically relevant concentrations of caffeine (Figure 5). The modest concentrations of caffeine (50 and 100 μM) clearly augmented the effect of a submaximal concentration of DDAVP, but the increases in cAMP levels caused by the methylxanthine in conjunction with PGE<sub>2</sub> and isoproterenol appeared to be simply additive. This set of experiments demonstrates that caffeine has the capacity to potentiate the action of certain cAMP agonists in ADPKD cells.





**Figure 5.** Potentiation by caffeine of DDAVP-, prostaglandin E<sub>2</sub> (PGE<sub>2</sub>)-, and isoproterenol-induced cAMP accumulation in ADPKD cells. ADPKD cells were rinsed extensively before adding agonists for 15 min. Values are mean  $\pm$  SE;  $n = 4$ . \*\*\*  $P < 0.001$  compared with the baseline value.

The effects of caffeine, IBMX, and theophylline on the generation of cAMP are complicated by the fact that they also inhibit A<sub>1</sub> and A<sub>2</sub> adenosine receptors (11,15–17). Thus under conditions in which adenosine may be generated by renal epithelial cells, these methylxanthines may decrease (block A<sub>2</sub> receptors) or increase (block A<sub>1</sub> receptors) basal levels of cAMP. To explore the potential interference of adenosine receptor blockade in relation to the PDE inhibitor effect, we determined the effect of rolipram, a type IV PDE inhibitor that does not interact with adenosine receptors, on cAMP levels in ADPKD cells. Rolipram alone increased cAMP levels from  $2.7 \pm 0.2$  to  $4.1 \pm 0.5$  ( $n = 4$ ) pmol/mg of protein. Adenosine (10  $\mu$ M) alone strongly increased cAMP levels, and rolipram (100  $\mu$ M) potentiated this effect (Figure 6). These findings indicate



**Figure 6.** Potentiation of adenosine-induced cAMP stimulation in ADPKD cells by rolipram. ADPKD cells were rinsed extensively before adding agonists for 15 min. Values are mean  $\pm$  SE;  $n = 4$ . \*\*\*  $P < 0.001$  compared with the baseline value.

that in ADPKD cells an effect of methylxanthines on adenosine receptors is not a requirement for them to inhibit PDE.

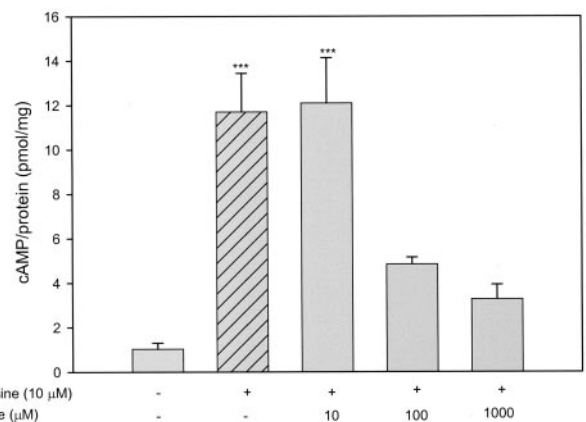
To evaluate caffeine as an adenosine antagonist, cAMP levels were measured in ADPKD cells after adding adenosine alone or in combination with increasing concentrations of the methylxanthine. In contrast to rolipram, caffeine did not potentiate the effect of adenosine. Rather, the methylxanthine at concentrations greater than 10  $\mu$ M diminished the effect of adenosine to stimulate cAMP accumulation (Figure 7). These results are interpreted to indicate that caffeine antagonized but did not completely block the effect of adenosine to stimulate cAMP production, probably through the inhibition of A<sub>2A</sub> receptors (15).

### Bioelectric Effect of Caffeine on ADPKD Cells

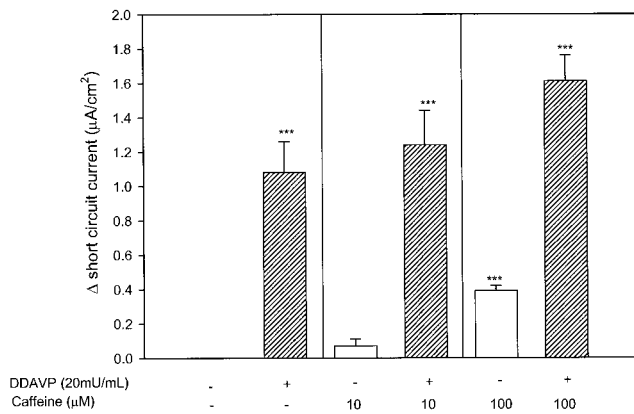
It has previously been shown in ADPKD that the increase in Isc in response to cAMP agonists reflects an increase in transepithelial chloride secretion (2,3). Twenty-five confluent ADPKD monolayers from three subjects developed stable baseline Vte ( $-1.6 \pm 0.1$  mV), Rte ( $472 \pm 44$  ohm/cm<sup>2</sup>), and Isc ( $2.6 \pm 0.2$   $\mu$ A/cm<sup>2</sup>). Fifteen minutes after adding 10 and 100  $\mu$ M caffeine, Isc increased  $0.1 \pm 0.04$   $\mu$ A/cm<sup>2</sup> and  $0.4 \pm 0.03$   $\mu$ A/cm<sup>2</sup>, respectively (Figure 8). DDAVP alone raised Isc by  $1.1 \pm 0.2$   $\mu$ A/cm<sup>2</sup>. Adding DDAVP and caffeine simultaneously increased the Isc to levels greater than when either agent was added alone.

### Effect of Caffeine, DDAVP, and PGE<sub>2</sub> on Phosphorylated ERK in ADPKD Cells

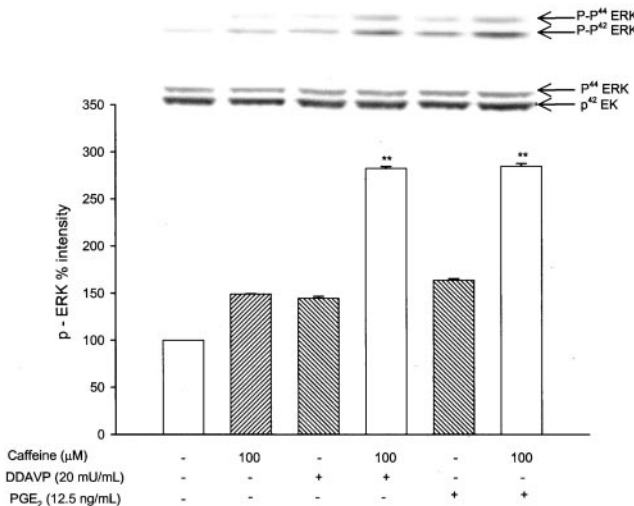
Previous studies have demonstrated that cAMP activates ERK, a signaling kinase that promotes increased cellular proliferation (4). Western blot studies were done to determine the extent to which caffeine increased the expression of P-ERK in ADPKD cells and potentiated the effects of cAMP agonists (Figure 9). Caffeine (100  $\mu$ M) alone increased P-ERK intensity by 49%; DDAVP and PGE<sub>2</sub> increased P-ERK intensity by 44.6% and 63.6%, respectively. In combination with caffeine, DDAVP and PGE<sub>2</sub> increased P-ERK intensity by an additional 180 and 185%, respectively. ERK expression was not changed by the



**Figure 7.** Attenuation of adenosine-induced cAMP stimulation in ADPKD cells by caffeine. ADPKD cells were rinsed extensively before adding caffeine and adenosine for 15 min. Values are mean  $\pm$  SE;  $n = 4$ . \*\*\*  $P < 0.001$  compared with the baseline value.



**Figure 8.** Effect of caffeine on DDAVP-induced Cl<sup>-</sup> secretion in ADPKD cells. ADPKD monolayers were rinsed and incubated in Ringer's for 15 to 30 min before adding DDAVP, caffeine, or DDAVP plus caffeine. In some monolayers (white columns), caffeine was added alone. Values are mean ± SE; n = 5. \*\*\* P < 0.001 compared with the baseline value.



**Figure 9.** Potentiation of DDAVP- and PGE<sub>2</sub>-induced ERK activation by caffeine in ADPKD cells. ADPKD cells were rinsed extensively before adding agonists for 15 min. The Western blot is representative of experiments using cells from three different ADPKD kidneys. In the graph, phosphorylated p<sup>44</sup>/p<sup>42</sup> ERK is expressed as % of control intensity. Quantification of Western blot relative intensities, mean ± SE, n = 3. \*\* P < 0.01 compared with the baseline value of 100.

increased levels of intracellular cAMP, indicating that the increased levels of P-ERK were due to the activation of ERK. By contrast, in HKC cells P-ERK levels were not stimulated by DDAVP or caffeine or a combination of these agents (data not shown), confirming previous observations that cAMP does not activate ERK in renal cells lacking polycystin mutations.

**Discussion**

cAMP has an important role in determining the rate at which renal cysts enlarge in ADPKD (1–3). Perhaps the most important

effect of this second messenger is the stimulation of mural cell proliferation, the process that increases the surface area of cysts and increases their volume capacity (4,18). In contrast to normal renal tubules in which cell growth is inhibited by cAMP (6,19), the nucleotide stimulates the proliferation of ADPKD cells by activating the MAP kinase pathway (4). Thus, mutations in either PKD1 or PKD2 may radically alter the phenotypic response of renal epithelial cells to the growth effects of cAMP (1). Evidence derived from other cellular systems indicates that the mitogenic action of cAMP may be cell-specific (20,21).

cAMP also stimulates the transepithelial secretion of NaCl and water, thereby adding fluid to the cavity created by the proliferating mural cells (2,3). Thus, common agonists that promote physiologic actions by regulating the adenylyl cyclase pathway, e.g., AVP, PGE<sub>2</sub>, and β-adrenergic compounds, have the potential to accelerate the growth of cysts in polycystic kidney disorders.

In classic studies, caffeine was used to inhibit PDE and thereby raise intracellular levels of cAMP (22–24). Caffeine was used for many years as a reagent to block PDE in biochemical studies until more potent (1-methyl-3-isobutyl xanthine, theophylline) and specific (rolipram) inhibitors became available. In the current study, we found that caffeine, IBMX, and rolipram, and to a lesser extent, theophylline, increased basal intracellular levels of cAMP in renal epithelial cells derived from patients with ADPKD (Figure 1). This finding indicates that PDE inhibition prevents the metabolism of cAMP and suggests that there is a low level of adenylyl cyclase activity in ADPKD cells in the absence of exogenous agonists. More importantly, the PDE inhibitors, including caffeine, potentiated the effects of submaximal concentrations of receptor-mediated agonists of physiologic importance to elevate intracellular levels of cAMP (Figures 2, 3, and 6). Thus, by inhibiting PDE and reducing the metabolism of cAMP, amounts of caffeine too low to affect cAMP levels alone in resting renal cells can potentiate the actions of common adenylyl cyclase agonists to stimulate the growth of cysts in ADPKD.

In a recent study, levels of extracellular adenosine triphosphate (ATP) in the sub-micromolar range were found to activate specific purinergic receptors and stimulate chloride secretion in human ADPKD cells through an intracellular calcium signaling mechanism (25). In the current study, we determined that adenosine, the backbone of ATP and a major renal autotoxin, strikingly increased the accumulation of cAMP by ADPKD cells (Figure 6). This effect on cAMP levels was potentiated by rolipram, a selective inhibitor of PDE (5), supporting the view that adenosine signaling in ADPKD cells involves the activation of adenylyl cyclase.

The interaction of methylxanthines with adenosine is complicated by the fact that, in addition to inhibiting PDE, they may block A<sub>1</sub> and A<sub>2</sub> receptors, thereby influencing the generation of cAMP (5). Binding of adenosine to A<sub>1</sub> receptors blocks adenylyl cyclase through the activation of a Gi inhibitory mechanism. By contrast, binding to the A<sub>2</sub> subtype activates adenylyl cyclase through a Gs stimulatory protein (26–28). Rolipram, which lacks adenosine receptor affinity and primarily blocks PDE, elevated basal levels of cAMP (Figure 1) in ADPKD cells and strongly potentiated the effect of

adenosine to increase intracellular levels of the nucleotide (Figure 6). By contrast, caffeine did not potentiate the action of adenosine. Rather, this methylxanthine decreased the levels of intracellular cAMP generated by adenosine in a concentration-dependent manner (Figure 7). These experiments are most economically interpreted to indicate that caffeine inhibition of A<sub>2</sub> receptor activation in ADPKD cells obscures any effect of the methylxanthine to block A<sub>1</sub> receptors.

We examined the extent to which cAMP generated by caffeine's potentiation of receptor-linked physiologic agonists affected mechanisms that have been shown to be important in the promotion of cyst enlargement. Net transepithelial chloride secretion coupled to the movement of sodium chloride and fluid into the cyst cavity are stimulated by a host of receptor-mediated agonists including vasopressin, one of the most important. The V<sub>2</sub> receptor agonist, DDAVP, increased intracellular levels of cAMP in ADPKD cells (Figures 2 through 5), and this effect was increased further by concentrations of caffeine in a clinically relevant range (Figure 8). Although the combination of caffeine and DDAVP was not synergistic, the additive effects of these agents to increase cAMP levels would promote the secretion of NaCl and fluid into renal cysts. It is interesting to note that caffeine has also been shown to stimulate chloride transport directly, independently of cAMP (29). The extent to which this mode of chloride transport may be operative in ADPKD cells was not explored in the current study.

cAMP is mitogenic in mural cells from ADPKD cysts but not in cells cultured from normal renal cortex (4,18). In ADPKD, the nucleotide activates the classic MAP kinase pathway distal to Ras in a way that complements the action of receptor-tyrosine-kinase agonists such as epidermal growth factor (EGF) (4,20,21). In the current study, we evaluated the effect of caffeine on the activation of ERK, an important intermediary in the MAP kinase pathway. Caffeine alone slightly increased P-ERK levels in ADPKD cells and greatly potentiated the effect of DDAVP and PGE<sub>2</sub> on P-ERK expression (Figure 9). P-ERK is thought to be a key intermediate in the pathway leading to cell proliferation in ADPKD cells (4,20,21). The evidence in this study is therefore consistent with the view that caffeine, by inhibiting PDE, promotes the accumulation of cAMP in response to endogenous adenylyl cyclase agonists, thereby increasing cellular proliferation and transepithelial fluid secretion in renal cysts.

The clinical relevance of the current studies depends on the extent to which cultured renal epithelial cells reflect the function of the *in situ* precursor cells. It is important to note in this regard that the stimulation of transepithelial fluid secretion by cAMP agonists has been demonstrated in intact renal cysts *in situ* and *in vitro* in studies of cells cultured from cysts (8,30,31). Experiments utilizing a murine model of polycystic kidney disease have also shown that renal and urine levels of cAMP are abnormally increased (32). Previous studies have shown that cAMP activates ERK in cultured ADPKD cells but not cells from normal kidneys (4). In a rat model of ADPKD, activation of ERK has been demonstrated in whole kidney extracts by Western blot and in individual cysts by immunocytochemistry (33). Activated ERK has also been detected in

the epithelial cells lining ADPKD cysts (34). Thus, the data from cell culture studies, though indirect, support a role for cAMP in fluid secretion and cell proliferation in ADPKD.

The oral administration of caffeine to Han:SPRD rats with ADPKD for 5 mo did not alter renal morphology or size appreciably; however, the methylxanthine elevated mean arterial BP significantly (7). GFR was 33% lower in the treated than in the untreated cystic animals, although this reduced level did not reach statistical significance, probably owing to the small number of animals in the treated group. An examination of the effect of caffeine on GFR and fibrosis markers in larger numbers of Han:SPRD animals would be interesting in this regard.

The extent to which caffeine may elevate renal tissue levels of cAMP depends on the amount that is ingested and the rate of metabolism by the liver (35–37). Pharmacokinetic studies indicate that the ingestion of 1 to 3 cups of coffee leads to plasma caffeine levels between 10 to 50  $\mu$ M in 30 to 90 min. Correspondingly higher levels of caffeine would be expected with greater ingested amounts of caffeine. Given the availability of coffee shops and huge containers of carbonated beverages containing caffeine, it would not be unexpected to find usage to exceed 1000 mg per day in the more avid drinkers.

The peak levels of caffeine that may be achieved in renal tissue after dietary ingestion are unknown. The extent to which caffeine may be secreted into the urine by the organic anion transport mechanism is unknown as is the extent to which the methylxanthine may be accumulated in the interstitium of the renal medulla by the counter-current multiplier mechanism. It is interesting to note that the Olympic Committee has established a urine caffeine eligibility limit of 62  $\mu$ M.

In view of the knowledge that cAMP increases cyst growth and that caffeine is used widely by coffee, tea, and carbonated beverage drinkers, the Polycystic Kidney Foundation has recommended that patients with PKD reduce or eliminate the use of these substances. The evidence presented in the current study supports that recommendation.

## Acknowledgments

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