Adenosine stimulates phosphate and glucose transport in opossum kidney epithelial cells

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COULSON, RICHARD, ROGER A. JOHNSON, RAY A. OLSSON, DENISE R. COOPER, AND STEVEN J. SCHEINMAN. *Adenosine stimulates phosphate and glucose transport in opossum kidney epithelial cells.* Am. J. Physiol. 260 (Renal Fluid Electrolyte Physiol. 29): F921–F928, 1991.—We have examined the effects of adenosine on sodium-coupled phosphate and glucose transport in cultured opossum kidney (OK) cells, a continuous cell line that resembles proximal tubule. Adenosine analogues *R*-(−)-N^6-phenylisopropyladenosine (PIA) and 2′,5′-dideoxyadenosine (DDA) were employed as adenosine A_1 receptor and P site-selective agonists, respectively. Sodium-dependent phosphate uptake activity (Na-P_i symport) increased by ~25% above both basal and parathyroid hormone (PTH)-inhibited levels in cells treated with PIA (0.1, 1 μM) but not in cells treated with DDA (100 μM). Adenosine (PIA) also stimulated sodium-coupled 3-*O*-methylglucose transport by ~40%. Intracellular adenosine 3′,5′-cyclic monophosphate (cAMP) content was inversely related to Na-P_i symport activity in cells treated with PIA and PTH. However, changes in Na-P_i symport activity did not consistently relate to changes in intracellular cAMP. Protein kinase C was activated 15 s after treatment of OK cells with 1 μM PIA. Preincubation of cells with 3 μM staurosporine attenuated the effect of 1 μM PIA on phosphate uptake. These data suggest that Na-P_i and Na-glucose symport activities are stimulated by adenosine acting at a receptor coupled to more than one intracellular signal. It is likely that both protein kinases A and C are involved in these actions of adenosine.

opossum kidney cells; adenosine 3′,5′-cyclic monophosphate; adenylate cyclase; caffeine; protein kinase C

ADENOSINE IS AN AUTACOID that regulates a variety of renal functions. Adenosine has been demonstrated to lower glomerular filtration rate, redistribute blood flow, and lower renin release (30) probably by interaction with A_1 and A_2 adenosine receptors located in the afferent and efferent arterioles (25). Attempts to demonstrate direct effects of adenosine on tubular functions are complicated by the potent effects of adenosine on renal hemodynamics (8, 24). Evidence for adenosine receptors on renal epithelial cells has been gathered from a variety of tubule preparations. Adenosine, probably by its action on A_2 receptors, has been shown to stimulate adenosine 3′,5′-cyclic monophosphate (cAMP) production and sodium transport in the toad kidney epithelial cell (19), hydraulic conductivity in rabbit cortical collecting tubule (13), and chloride transport in cortical thick ascending limb (5), cortical collecting tubule (29), and the shark rectal gland, a model of the thick ascending limb of the mammalian renal tubule (14). The adenosine analogue 2′,5′-dideoxyadenosine (DDA), which is selective for the P site, inhibits hydraulic conductivity in cortical collecting tubules presumably by its direct inhibitory action at the catalytic site of adenylate cyclase (13). Adenosine A_2 receptors coupled through the G protein to stimulation of adenylate cyclase have been identified in dog kidney collecting ducts (1), rabbit cortical collecting tubule cells (4) and tubules (15), and rat renal papillae (33). Attenuation by adenosine of hormone-activated renal adenylate cyclase has been demonstrated for vasopressin (4, 28, 31) and parathyroid hormone (11). This attenuation probably occurs through an A_1 adenosine receptor subtype, A_{1b}, which is coupled through a G protein to inhibition of adenylate cyclase activity. Adenosine, presumably binding to another subtype of the A_1 receptor, A_{1a}, has also been shown to elevate intracellular calcium (2) and phosphoinositide turnover (3) in cortical collecting tubule cells. This adenosine receptor subtype is presumed to be coupled to stimulation of phospholipase C activity. The adenosine receptor antagonist 1,3-dimethyl-8-phenylxanthine inhibits the Na-P_i symporter in opossum kidney (OK) cells (12), suggesting that endogenous adenosine stimulates Na-dependent phosphate uptake by these epithelial cells. Few studies have attempted to correlate a transport function with intracellular signaling in response to adenosine.

We have exposed rat kidneys and cultured renal epithelial cells (OK cells) to an A_1 receptor-selective adenosine analogue, *R*(−)N^6-phenylisopropyladenosine (PIA), and a P site-selective adenosine analogue, DDA. The OK cell is a continuous renal epithelial cell line that resembles proximal tubule in a number of respects, including the presence of sodium-coupled phosphate, glucose, and amino acid transporters on the apical membrane (21). We have studied the effects of adenosine on sodium-dependent phosphate uptake (Na-P_i symport), glucose uptake, aminoisobutyrate uptake, protein kinase C activation, and cAMP production. Although DDA was more effective than PIA in blocking parathyroid hormone (PTH)-dependent stimulation of adenylate cy-

clase, only PIA stimulated Na-P_i symport, suggesting that PIA signal transduction involves intracellular signals in addition to cAMP. Since PIA also stimulated glucose transport and activated protein kinase C, the effects of adenosine may not be limited to sodium-coupled phosphate uptake, and both protein kinases A and C may be involved in its physiological action.

METHODS

Kidney perfusion. Kidneys were isolated, perfused for 100 min, and used to determine urinary cAMP and phosphate clearance by a previously described technique (12). Unlike the OK cells, it was not necessary to include phosphodiesterase inhibitors such as Ro 20–1724 to demonstrate large cAMP responses to PTH. PIA, when present, was added at zero perfusion time; PTH was added to all kidneys after 40 min of perfusion. PIA was used at a high concentration in these experiments [(500 μM total, ~50 μM free, i.e., not bound to albumin (32)]. Lower concentrations of PIA were less effective (data not shown).

Cultured OK cells. OK cells were supplied by Dr. B. Sacktor at *passage 61* and studied between *passages 65* and *92*. Cells were grown to confluence in 10 days on 9.5-cm^2 wells on six-well plates (Falcon). Growth medium was Dulbecco's minimum essential medium (DMEM, which contains 1 mM phosphate and 5.6 mM glucose) supplemented with 10% newborn calf serum, 4 mM glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin. Medium was changed on the 1st day after seeding and every 48 h thereafter. In some experiments, 1 mM caffeine was added to the growth medium from *days 3* to *10*. In these experiments caffeine was not included in the final preincubations and incubations for assay of phosphate uptake or cAMP generation.

Phosphate transport. Phosphate transport into OK cells was assayed on *day 10* after seeding, as previously described (12). Wells containing cell monolayer and DMEM growth medium were incubated for 120 min at 37°C with adenosine analogue, adenosine deaminase (1.5 U/ml), 10 pM PTH, or hormone solvent. When we used staurosporine, it was added to the growth medium for 10 min before 120-min incubation with adenosine or PTH. Cells were rinsed three times with transport medium containing (in mM) 137 Na, 5.4 K, 2.8 Ca, 1.2 Mg, 148 Cl, 0.1 PO_4, 1.2 SO_4, and 14 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), pH 7.4, and uptake was initiated by adding 1 ml of this medium containing adenosine analogue, adenosine deaminase, PTH or hormone solvent, and 1 μCi of $^{32}PO_4$ (Amersham). Hormones (adenosine, PTH) were included in both 120-min preincubation and 5-min uptake assay media to maximize effectiveness. Plates were shaken at 100 cycles/min for 5 min at 25°C, and uptake was stopped by adding chilled medium without sodium and phosphate for three rinses. Cells were solubilized in 0.5% Triton X-100. Glucose and aminoisobutyrate uptakes were performed in the same experimental protocol except *1*) the transport medium contained 1.25 mM phosphate, *2*) uptake of 600 μM 3-*O*-[methyl-^{14}C]glucose or 100 μM [^{3}H]aminoisobutyrate was for 30 s, and *3*) the stop solution was ice-cold 137 mM choline chloride and 14 mM HEPES (pH 7.4). Rates of uptake of phosphate, glucose, or aminoisobutyrate were minimal and not routinely determined when sodium was replaced by *N*-methylglucamine or choline. Phosphate, glucose, and aminoisobutyrate uptakes were expressed as nanomoles substrate transported per milligram protein per assay time.

Intracellular cAMP and adenylate cyclase. Experiments were performed in the presence of the cAMP-phosphodiesterase inhibitor Ro 20–1724 unless otherwise indicated. cAMP experiments were performed 10 days after seeding. Growth medium was replaced with protein-free Krebs-Henseleit bicarbonate medium (pH 7.45 at 5% CO_2-95% O_2) containing (in mM) 143 Na, 5.9 K, 2.5 Ca, 1.2 Mg, 128 Cl, 25 HCO_3, 1.2 PO_4, 1.2 SO_4, 5.6 glucose, 1 pyruvate, and 0.1 Ro 20–1724, as well as adenosine analogues, adenosine deaminase (1.5 U/ml), or appropriate solvents. After incubating the cells for 10 min in this medium, 10 nM PTH was added, and the plates were gently swirled for 2 min at room temperature (25°C). The cells were then washed with isotonic saline and extracted with 1 ml 2 M perchloric acid. cAMP was measured by radioimmunoassay of alumina-extracted samples (27) and expressed as picomoles per milligram protein. Adenylate cyclase was measured in OK cell membranes. OK cells were grown in 175-cm^2 flasks and harvested by trypsin-EDTA treatment for 10–15 min at 37°C. After arresting the trypsin with serum-supplemented DMEM, the cell suspension was centrifuged at 50 *g* for 2 min, the cell pellet was washed twice with phosphate-buffered saline, and the cells were frozen at −80°C until ready for assay. OK cells were disrupted at 4°C in 1 mM $NaHCO_3$ and 5 mM EDTA (pH 7.5) by sequential treatments with a Dounce (glass/glass) homogenizer, a nitrogen bomb, and a Dounce homogenizer. After centrifugation at 20,000 rpm for 60 min (Beckman Rotor JA20–1), the pellet was resuspended in 1 mM $NaHCO_3$; this was repeated for a total of three washes. Adenylate cyclase was assayed for 15 min at 37°C in a reaction volume of 100 μl containing 50 mM glycylglycine (pH 7.5), 100 μM Ro 20–1724, 100 μM and 0.5 μCi [α-^{32}P]ATP (ICN), 10 μM guanosine 5'-triphosphate (GTP), 4 mM $MgCl_2$, 100 μg/ml myokinase, 5 U/ml adenosine deaminase, 5 mM creatine phosphate, 100 μg/ml creatine phosphokinase, 70 μg membrane protein, and indicated amounts of hormone or adenosine analogue. Hormones were diluted in 10 mM sodium acetate buffer-0.1% albumin (pH 6); PIA, Ro 20–1724, and forskolin were first dissolved in dimethylsulfoxide and DDA in water. Reactions were terminated by precipitation with $ZnCO_3$, and [^{32}P]cAMP was purified as previously described (18). Activity was expressed as picomoles cAMP per milligram protein per 5 min.

Protein kinase C. OK cells grown to confluence in 175 or 225-cm^2 flasks were trypsinized for 1 min followed by soybean trypsin inhibitor (0.1 mg/ml) and bovine serum albumin (1 mg/ml) in Dulbecco's phosphate-buffered saline (DPBS). The cells were washed and suspended in DMEM, aliquoted into 15-ml tubes, and then treated with adenosine (PIA) for one of three time periods, *time zero*, 15 s, and 5 min. The reaction was terminated by adding ice-cold DMEM and centrifuging at 2,000 *g* for 5

min. The cell pellets were extracted, separated into cytosol and membrane components (relative protein contents, 1 cytosol:1.5 membrane), and finally fractionated by applying 3 mg protein to a Mono-Q (Pharmacia) column and collecting twelve consecutive eluate fractions (10). The latter procedure also frees the sample of endogenous activators and inhibitors of protein kinase C activity. All twelve fractions off the Mono-Q were assayed for protein kinase C activity. Assays contained in 250 μl: 5 μmol tris(hydroxymethyl)aminomethane (Tris)·HCl (pH 7.5), 1.25 μmol Mg acetate, 2.5 nmol [γ-^{32}P]ATP (0.2 μCi, Amersham), 50 μg histone H-1 (type III-S, Sigma), 125 nmol $CaCl_2$ in excess of chelator concentrations, and 10 μg phosphatidylserine with 0.2 μg diolein. Basal activity was measured in the presence of 125 nmol ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA) instead of Ca and phospholipid. The reaction was initiated by addition of [^{32}P]ATP mixture and incubated at 30°C for 5 min before addition of 1 ml 25% (vol/vol) trichloracetic acid. Precipitated protein was collected by filtration on nitrocellulose membranes, which were counted for radioactivity. Activity was expressed as picomoles [^{32}P]ATP per min per fraction eluting off the Mono-Q; specific activity was the sum of activity in each fraction and was expressed as picomoles [^{32}P]ATP per min per milligram protein applied to the Mono-Q (see Fig. 7).

PTH. PTH was employed at different concentrations chosen on the basis of pilot experiments to be submaximal and yet elicit a significant detectable response. For phosphate uptake, the dose was 10 pM (1 nM perfused kidney), for cAMP the dose was 10 nM (1 nM perfused kidney), and for adenylate cyclase the dose was 100 nM.

Protein. Protein was determined by a protein assay kit (Pierce Chemical, Rockford, IL).

Materials. Ro 20–1724 was a gift from Dr. W. E. Scott, Hoffmann-LaRoche, NJ, or was purchased from Biomol Research Labs. Adenosine deaminase was from Sigma or Calbiochem, and caffeine was from Research Biochemicals. In all studies using adenosine deaminase, ammonium sulfate, when present, was removed before using the enzyme by resuspending the enzyme pellet in phosphate-buffered saline after centrifugation at 12,000 *g* for 25 min. DDA was synthesized from 2′-deoxyadenosine (Sigma) by Dr. Siu-Mei Helena Yeung. Bovine PTH-(1—34) was obtained from Bachem.

Statistical analyses. Unpaired Students's *t* test and two-way analyses of variance with repeated measures (Kirk's SPF-PQ design) were employed where appropriate. Data are means ± SE.

RESULTS

Adenosine effects on phosphate uptake and cAMP generation. In the isolated rat kidney perfused with 500 μM PIA the fractional excretion of phosphate was significantly lower than in control kidneys. However, the phosphaturia after 1 nM PTH was not attenuated by PIA (Fig. 1). Urinary cAMP excretion exhibited the opposite pattern. That is, adenosine (PIA) did not alter the early (20–40 min) rise in cAMP but markedly attenuated the later (40–100 min) rise in cAMP after a 1 nM bolus of PTH.

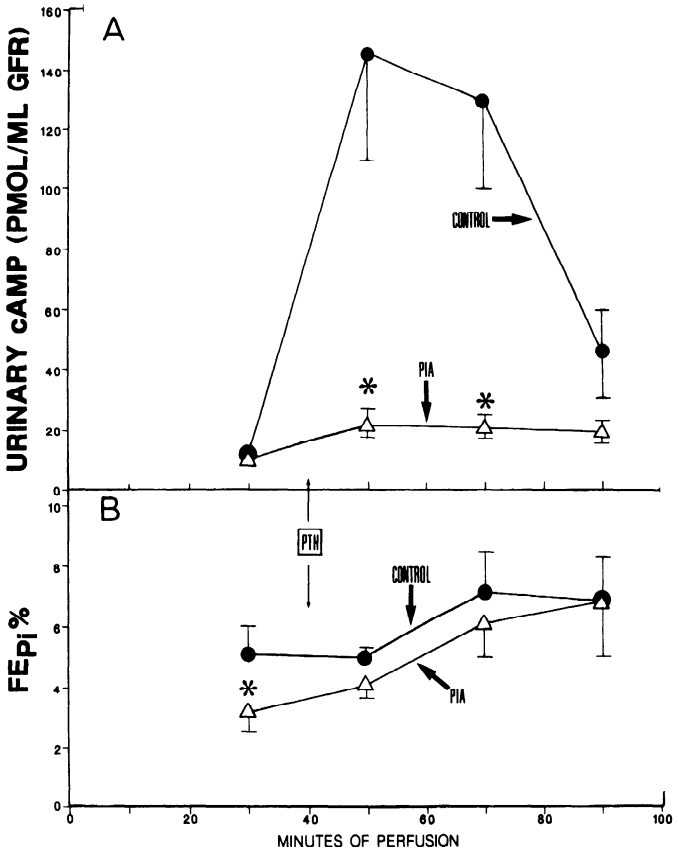

FIG. 1. Effect of *R*(−)-phenylisopropyladenosine (PIA) on urinary adenosine cAMP excretion (*A*) and fractional excretion of phosphate (FE_{P_i}%) (*B*) by isolated perfused rat kidney. PIA (500 μM) or vehicle (control) were present in perfusion medium from *time zero*. Bovine parathyroid hormone [PTH-(1—34)] was added to perfusion medium of all kidneys after 40 min of perfusion to final concentration of 1 nM. Four consecutive 20-min urine samples were collected after 20-min equilibration period; perfusate was sampled at midpoint of each urine collection. PIA significantly lowered FE_{P_i} before PTH was added; $^*P < 0.05$, Student's *t* test. PIA significantly lowered urinary cAMP after PTH addition ($P < 0.01$, analysis of variance). Data are means ± SE for 10 kidneys in each group.

TABLE 1. *Effect of adenosine deaminase on Na-P_i uptake and intracellular cAMP levels in opossum kidney cells*

Additions	Na-P_i Uptake, nmol·mg protein^{-1}·5 min^{-1}	*n*	cAMP, pmol/mg protein	*n*
No additions	4.36±0.17	10	10±1	23
Adenosine deaminase (1.5 U/ml)	2.63±0.16†	10	8±1	23

Values are means ± SE; *n* = no. of wells. Na-P_i, sodium-dependent phosphate. * $P < 0.001$ by unpaired Student's *t* test.

Subsequent studies on the regulation of phosphate uptake by adenosine were performed with OK cells in culture. To determine whether endogenous adenosine was stimulating phosphate uptake, cells were pretreated with adenosine deaminase (1.5 U/ml) to remove endogenous adenosine. Adenosine deaminase treatment lowered the Na-P_i symport activity by ~40% (Table 1). Adenosine deaminase treatment did not alter intracellular cAMP content (Table 1).

PIA (0.1 and 1 μM) significantly stimulated the phosphate uptake (by 35 and 12%, respectively; Table 2).

TABLE 2. *Effect of purine nucleosides PIA and DDA on Na-P_i uptake and cAMP generation in cultured opossum kidney cells*

Additions, μM	Na-P_i Uptake, nmol·5 min^{-1}·mg $protein^{-1}$	n	cAMP, pmol/mg protein	n	Adenylate Cyclase, pmol·5 min^{-1}·mg $protein^{-1}$	n
Control	4.53±0.09	34	12.2±1.0	7	≤2	5
0.01 PIA	4.60±0.32	10	10.4±1.0	5	NM	
0.1 PIA	6.12±0.27*	10	8.5±0.1*	3	NM	
1 PIA	5.06±0.14*	15	11.9±1.6	8	≤2	3
100 DDA	4.39±0.13	10	10.5±1.1	12	≤2	3
PTH	2.86±0.08†	34	586±33†	7	20±1†	4
PTH + 0.01 PIA	2.98±0.12	10	680±64	5	NM	
PTH + 0.1 PIA	3.21±0.21	10	605±92	3	NM	
PTH + 1 PIA	3.20±0.14*	15	604±49	8	11±1*	3
PTH + 100 DDA	2.70±0.10	10	107±16*	12	2±1*	4

Values are means ± SE, n = no. of wells or adenylate cyclase assays. PTH (parathyroid hormone) concentration was 10 pM for P_i uptake, 10 nM for cAMP, and 100 nM for adenylate cyclase. Assays for cAMP and adenylate cyclase were performed in presence of 100 μM Ro 20-1724. Control and PTH values for Na-P_i symport were pooled. Statistical comparisons were made between equal number of control and test values generated during same experiment. DDA, 2′,5′-dideoxyadenosine; NM, not measured. * $P < 0.05$ by unpaired Student's t test, significant effect of purine nucleoside vs. appropriate control level. † $P < 0.05$ by unpaired Student's t test, significant effect of PTH vs. appropriate control value.

PTH (10 pM) inhibited Na-P_i symport activity by ~40%. PIA (0.1 and 1 μM) attenuated by 12% the inhibition of Na-P_i symport by PTH (Table 2). PIA had a dose-dependent effect on the cAMP content of OK cells; low concentrations of PIA (0.01–1 μM) had no effect or slightly lowered cAMP (Table 2); high concentrations (10 and 100 μM) elevated cAMP content (Fig. 2). This suggests that an A_1 adenosine receptor-adenylate cyclase coupled effect predominated at a PIA concentration <1 μM, whereas an A_2 adenosine receptor-adenylate cyclase coupled effect predominated at PIA >1 μM. PIA at 0.1 μM, a concentration that should saturate A_1 but not A_2 receptors (4), caused a significant lowering (by 30%) of basal cAMP levels and a significant stimulation (by 35%) of basal phosphate uptake. PIA at 1 μM had no effect on PTH-elevated cAMP content, although it attenuated (by 45%) the stimulation of adenylate cyclase activity and the inhibition of phosphate uptake (by 12%) (Table 2).

DDA, the adenosine P-site agonist, had no effect on Na-P_i symporter activity under any circumstance and had no effect on basal cAMP levels. However, DDA significantly attenuated (by 82%) the PTH-elevated cAMP and the PTH-stimulated adenylate cyclase activity (by 90%) (Table 2).

These data suggest a weak correlation between the ability of adenosine to lower cAMP content and its ability to stimulate sodium-dependent phosphate uptake.

Caffeine feeding and the effect of PIA on phosphate transport and cAMP generation. For a 7-day period cells were fed 1 mM caffeine, which was included in the growth medium. This procedure was believed to upregulate the adenosine A_1 receptor (17). Caffeine-fed OK cells exhibited a twofold elevation in Na-P_i symport activity (Fig. 3). Although caffeine feeding augmented basal Na-P_i symporter activity, it did not alter the magnitude of the response of the symporter to PIA or PTH. Caffeine feeding also inhibited the growth of the OK cells. The protein content of each 9.5-cm^2 well after 10 days of culture was reduced by caffeine feeding from 523 ± 8 to 465 ± 9 μg protein/well (n = 24 each group, $P < 0.001$ unpaired Student's t test). This small (11%) reduction in protein content could not account for the twofold increase in Na-P_i transport activity. In fact, transport

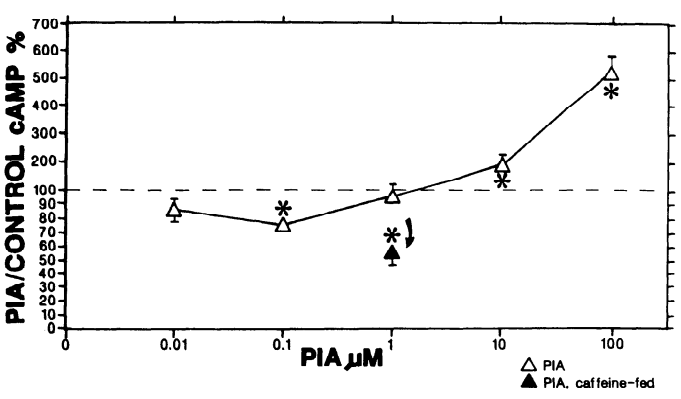

FIG. 2. Concentration-dependent effect of PIA on opossum kidney (OK) cell cAMP content. Data are presented as ratio relating to control (i.e., without PIA) value shown in Table 2. Each point is mean ± SE for 3–8 wells (PIA, open triangles) or 8 wells (PIA with caffeine feeding, solid triangle). *$P < 0.05$, significant effect of adenosine analogue vs. respective control value by Student's t test.

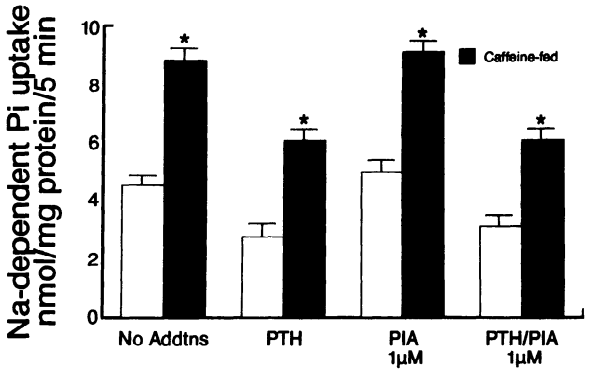

FIG. 3. Effect of caffeine feeding on Na-dependent uptake of phosphate by OK cells. PTH dose was 10 pM; PIA dose was 1 μM. Data values (in nmol·mg $protein^{-1}$·5 min^{-1}) from *left* to *right* are, respectively, control vs. caffeine fed: 4.53 ± 0.09 vs. 8.96 ± 0.58, 2.86 ± 0.08 vs. 6.09 ± 0.13, 5.06 ± 0.14 vs. 9.38 ± 0.28, 3.20 ± 0.14 vs. 6.31 ± 0.26. *$P < 0.001$, significant effect of caffeine feeding (solid bar) vs. non-caffeine-fed controls (open bar). For experimental details see METHODS. Caffeine fed, n = 5 wells; controls, n = 15–34 wells. Pair-fed controls (n = 5) vs. caffeine fed exhibited same magnitude of effect (data not shown) and were used for statistical comparisons.

activity, when expressed per well rather than per milligram protein, was still significantly (1.6-fold) higher in the caffeine-fed group (data not shown).

PIA (1 μM) lowered basal and PTH-elevated cAMP (Fig. 4) in caffeine-fed OK cells. The fact that the ability of PIA to stimulate phosphate uptake was augmented after caffeine feeding (Fig. 3) suggests a coupling between cAMP content and phosphate uptake. However, caffeine feeding alone did not alter the basal or PTH-elevated cAMP content, even though Na-P_i symporter activities were doubled in these caffeine-fed cells (Figs. 3 and 4). Therefore, caffeine feeding per se would appear to stimulate Na-P_i symporter activity by a mechanism that does not involve lowered cAMP synthesis.

Effects of adenosine on sodium-coupled glucose and aminoisobutyrate uptakes. Preincubation of OK cells with adenosine (0.1 or 1 μM PIA) for 120 min significantly elevated uptake of 3-*O*-methylglucose (by 29 or 53%, respectively). PIA did not alter aminoisobutyrate uptake (Fig. 5).

Adenosine and protein kinase C. Preincubation of OK cells with 3 μM staurosporine for 10 min before incubation with 1 μM PIA for 120 min abolished the stimulatory effect of PIA on phosphate uptake (Fig. 6). OK cells exposed to 1 μM PIA showed a transient activation of protein kinase C. Thus, after 15 s of exposure to PIA, cytosolic activity was diminished and membrane activity was augmented (Fig. 7). After 5 min of exposure the cytosolic activity remained suppressed, but membrane activity was no longer elevated.

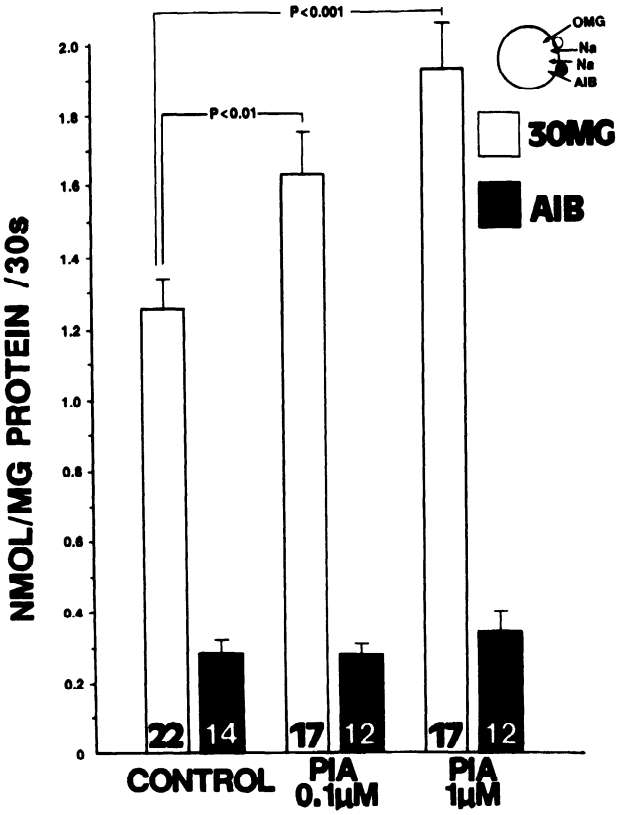

FIG. 5. Effect of adenosine (PIA) on sodium-coupled transport of 3-*O*-methylglucose (3OMG) and aminoisobutyric acid (AIB) into opossum kidney (OK) cells. Cells grown in 9.5-cm^2 wells were preincubated for 120 min with growth medium containing, where appropriate, 0.1 or 1.0 μM PIA. Uptakes of 600 μM [^{14}C]3OMG (open bars) or 100 μM [^{3}H]aminoisobutyrate (solid bars) into OK cells were measured after 30-s incubation in medium also containing, where appropriate, 0.1 or 1.0 μM PIA. $P < 0.01$ and $P < 0.001$, significant effects of PIA vs. control assessed by unpaired Student's t test. Data are means ± SE; no. of wells is shown at bottom of each bar.

DISCUSSION

These studies demonstrate a stimulation by adenosine of sodium-dependent phosphate and glucose transporters in renal epithelial cells of proximal tubular origin. It confirms and extends our previous observation, using a xanthine to antagonize adenosine receptors, that endogenous adenosine stimulated Na-P_i symport in OK cells and in isolated rat kidneys (12). The fact that adenosine also lowered the fractional excretion of phosphate and urinary excretion of cAMP in the isolated rat kidney suggests that the adenosine effects observed in the cultured OK cells have functional significance in the intact kidney. The stimulatory action of adenosine on these transporters was demonstrated by using PIA, which is a selective agonist for the adenosine A_1 receptor. Presumably, adenosine stimulates transporters located on the apical membrane by first binding to receptors believed to be located on the basolateral membrane. The signaling mechanism for this adenosine effect may involve more than one receptor, each coupled to a different second messenger system. The inhibition of Na-P_i symport by PTH is known to be associated with activation of both protein kinases A and C (20, 22). In a similar fashion, the stimulation of Na-P_i symport by adenosine might involve simultaneous or sequential inhibition of protein kinase A and activation of protein kinase C. Thus, under certain conditions, adenosine was shown to lower the cAMP content of OK cells and, by implication, lower the activity of cAMP-dependent protein kinase A. In other experiments phosphate uptake was altered with no accompanying change in cAMP content, for example, with adenosine deaminase treatment (Table 1). Even in these cases the change in transport may be associated with a change in protein kinase A activity since Martin et al. (22) demonstrated that measures of protein kinase A activity were more sensitive than corresponding meas-

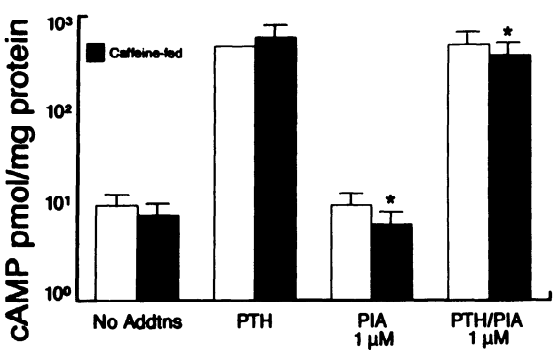

FIG. 4. Effect of caffeine feeding on basal and PTH-stimulated cAMP content of OK cells. PTH dose was 10 nM; PIA was 1 μM. Data values (in pmol/mg protein) from *left* to *right* are, respectively, control vs. caffeine fed: 12.2 ± 1 vs. 10.1 ± 0.8, 586 ± 33 vs. 638 ± 62, 11.9 ± 1.6 vs. 7.7 ± 0.9 ($P < 0.05$), 604 ± 49 vs. 397 ± 32 ($P < 0.005$). *$P < 0.05$, significant effect by Student's t test of caffeine feeding (solid bars) vs. control feeding (open bars). Data are means ± SE of 7–8 wells in each group.

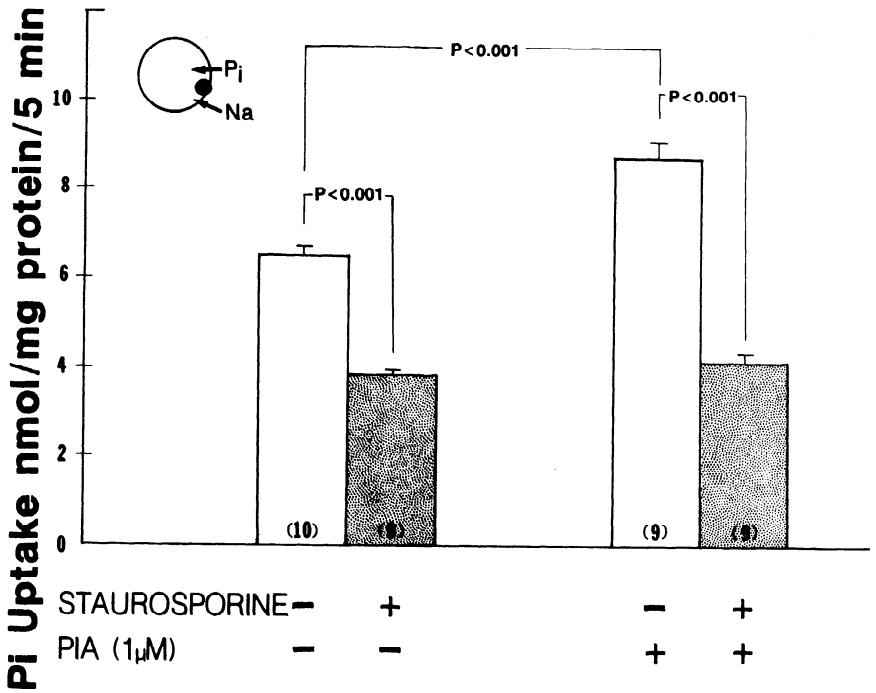

FIG. 6. Effect of 3 μM staurosporine on sodium-dependent uptake of phosphate by OK cells. Cells grown in 9.5-cm^2 wells were incubated for 10 min with staurosporine (stippled bar), 120 min with 1 μM PIA, and finally 5 min in transport medium containing 100 μM $^{32}PO_4$ and 1 μM PIA. Corresponding controls received only drug solvents. $P <$ 0.001, significant effects of PIA or staurosporine as indicated by unpaired Student's t test analysis. Data are means ± SE; no. of wells is shown at bottom of each bar.

ures of cellular cAMP content. Staurosporine, a protein kinase C inhibitor, abolished the stimulatory action of adenosine on phosphate uptake. Moreover, activation of protein kinase C, determined as the translocation of activity from cytosol to membrane, was found to occur after exposing OK cells to adenosine. In epithelial cells from the distal tubule, adenosine has been shown to increase intracellular calcium content and phosphatidylinositol turnover through an A_{1a} receptor, and to inhibit adenylate cyclase through an A_{1b} receptor (2, 3). It is possible that the Na-P_i and Na-glucose symporters of proximal epithelia are similarly regulated through the two adenosine receptor subtypes: *1*) A_{1a} positively coupled to phospholipase C and protein kinase C activities and *2*) A_{1b} negatively coupled to adenylate cyclase and protein kinase A activities. In fact activation of protein kinase C by a variety of means that bypass a receptor-coupled mechanism was shown to inhibit sodium-dependent phosphate and hexose uptakes but not amino acid uptake in proximal tubular cells (16). Our observation that DDA, the P-site directed adenosine analogue, which bypasses the receptor and directly inhibits adenylate cyclase activity, did not affect phosphate uptake further supports the hypothesis that adenosine affects these transporters by binding to a receptor. Moreover, this receptor would seem to be coupled to intracellular signals that affect both protein kinases A and C activities.

Stimulation of Na^+-K^+-ATPase activity by adenosine could account for the observed increases in sodium-dependent transport of phosphate and glucose, since this would increase the electrochemical gradient for sodium across the apical membrane. This is probably not the mechanism for adenosine's stimulation of these sodium-dependent transporters, since sodium-coupled amino acid transport was not stimulated. It is also possible that renal epithelial cells metabolize PIA to an ATP analogue, which might activate Na^+-K^+-ATPase. This is not likely to be the case since, after recirculation of 0.5 mM PIA through the rat kidney for 100 min, we found no metabolism of PIA (32) and no change in renal content of ATP or GTP (unpublished observation).

How this adenosine effect observed in vitro relates to transport function in the kidney in vivo can, as yet, only be surmised. Adenosine has been reported to lower renin release and glomerular filtration rate and to shunt blood from superficial to deep cortex (30). Adenosine has also been shown to stimulate transport in distal segments (13, 14, 19, 29) and now in proximal segments. Because sodium-coupled glucose cotransport contributes 25% to net sodium reabsorption by the kidney and sodium-phosphate cotransport contributes another 5%, it follows that increases in these transporters might contribute to the known antidiuretic action of adenosine (33). Adenosine, formed by ATP degradation, may play a role in the augmented sodium and phosphate reabsorption observed in the early phases of renal hypoperfusion (26). Later phases of hypoperfusion are associated with decreases in renal blood flow and glomerular filtration rate that, incidentally, are also products of adenosine action (30). Drugs, such as the xanthine theophylline, that antagonize adenosine at the receptor level have been demonstrated to increase urinary phosphate excretion independently of changes in glomerular filtration (9). We observed that caffeine inhibited the growth of OK cells, and this could be due to caffeine's inhibition of cAMP-phosphodiesterase activity or, more likely, to antagonism of endogenous adenosine at the receptor level (17). Adenosine is known to stimulate proliferation of renal (6) and endothelial (23) cells. It is possible that adenosine acts as a growth factor for surviving proximal epithelia after an ischemic insult, such as cortical hypoperfusion. Like adenosine, another growth factor insulin-like growth factor I (IGF-I) has been found to increase Na-P_i symporter activity in OK cells (7).

In conclusion, we have demonstrated in cultured OK

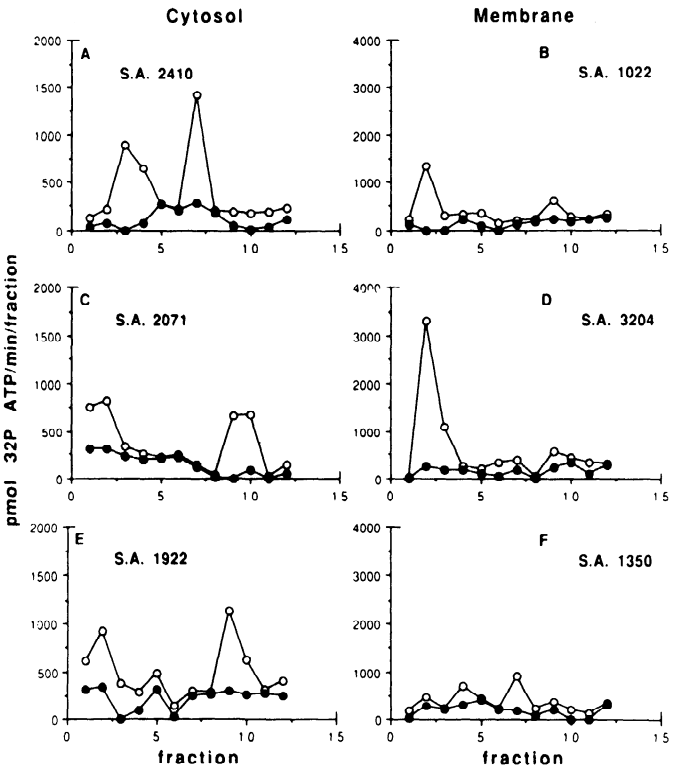

FIG. 7. Effect of 1 μM PIA on translocation of protein kinase C from cytosolic to membrane compartments of OK cells. Cells were treated for 15 s or 5 min with PIA; controls received PIA for zero time exposure. Data from 1 representative experiment are shown; data (not shown) from 6 experiments produced same qualitative changes. Protein kinase C activity is defined as histone phosphorylated in presence of calcium plus diolein plus phosphatidylserine (open circles) minus histone phosphorylated in presence of EGTA (closed circles). Protein kinase specific activity (S.A.) data are plotted against fraction number (*1–12*) eluting off Mono-Q column. Total protein kinase C activity (S.A.), i.e., sum of activity in each *fraction 1–12*, is shown and is expressed as pmol [^{32}P]ATP·min^{-1}·mg protein^{-1} applied to Mono-Q. *A*, *C*, and *E* show change in cytosolic protein kinase C activity from zero time (*A*), 15 s (*C*), and 5 min (*E*). *B*, *D*, and *F* show change in membrane protein kinase C activity from zero time (*B*), 15 s (*D*), and 5 min (*F*).

cells that adenosine stimulates the activity of the sodium-coupled phosphate and glucose transporters by a receptor-coupled mechanism that probably involves both activation of protein kinase C and inhibition of protein kinase A.

We thank Asheena Khalakdina, Robert Reid, Dorothee Stuebner, and Fern Warner for excellent technical assistance and Dr. I. M. Weiner for critical advice.

This research was supported by United States Veterans Administration Research Funds and the American Heart Association (New York State Affiliate, Mid-Hudson Chapter) to R. Coulson and National Institute of Diabetes and Digestive and Kidney Diseases Clinical Investigator Award DK-01495 to S. Scheinman.

Parts of this study were presented at the FASEB 72nd Annual Meeting, Las Vegas, NV, 1988, and the 22nd Annual Meeting of the American Society of Nephrology, Washington, DC, 1989, and were published as abstracts (*FASEB J.* 2: A1066, 1988, and *Kidney Int.* 37: 367, 1990).

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Received 26 February 1990; accepted in final form 15 January 1991.

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