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Adenosine modulates Mg^{2+} uptake in distal convoluted tubule cells via A_1 and A_2 purinoceptors

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Kang, Hyung Sub, Dirk Kerstan, Long-Jun Dai, Gordon Ritchie, and Gary A. Quamme. Adenosine modulates Mg^{2+} uptake in distal convoluted tubule cells via A_1 and A_2 purinoceptors. *Am J Physiol Renal Physiol* 281: F1141–F1147, 2001.—Adenosine plays a role in the control of water and electrolyte reabsorption in the distal tubule. As the distal convoluted tubule is important in the regulation of renal Mg^{2+} balance, we determined the effects of adenosine on cellular Mg^{2+} uptake in this segment. The effect of adenosine was studied on immortalized mouse distal convoluted tubule (MDCT) cells, a model of the intact distal convoluted tubule. The rate of Mg^{2+} uptake was measured with fluorescence techniques using mag-fura 2. To assess Mg^{2+} uptake, MDCT cells were first Mg^{2+} depleted to 0.22 ± 0.01 mM by being cultured in Mg^{2+} -free media for 16 h and then placed in 1.5 mM $MgCl_2$; next, changes in intracellular Mg^{2+} concentration ($[Mg^{2+}]_i$) were determined. $[Mg^{2+}]_i$ returned to basal levels, 0.53 ± 0.02 mM, with a mean refill rate, $d([Mg^{2+}]_i)/dt$, of 137 ± 16 nM/s. Adenosine stimulates basal Mg^{2+} uptake by $41 \pm 10\%$. The selective A_1 purinoceptor agonist N^6 -cyclopentyladenosine (CPA) increased intracellular Ca^{2+} and decreased parathyroid hormone (PTH)-stimulated cAMP formation and PTH-mediated Mg^{2+} uptake. On the other hand, the selective A_2 receptor agonist 2-[p-(2-carboxyl-ethyl)-phenylethylamino]-5'-N-ethyl-carboxamidoadenosine (CGS) stimulated Mg^{2+} entry in a concentration-dependent fashion. CGS increased cAMP formation and the protein kinase A inhibitor RpcAMPS inhibited CGS-stimulated Mg^{2+} uptake. Selective inhibition of phospholipase C, protein kinase C, or mitogen-activated protein kinase enzyme cascades with U-73122, Ro-31-8220, and PD-98059, respectively, diminished A_2 agonist-mediated Mg^{2+} entry. Aldosterone potentiated CGS-mediated Mg^{2+} entry, and elevation of extracellular Ca^{2+} diminished CGS-responsive cAMP formation and Mg^{2+} uptake. Accordingly, MDCT cells possess both A_1 and A_2 purinoceptor subtypes with intracellular signaling typical of these respective receptors. We conclude that adenosine has dual effects on Mg^{2+} uptake in MDCT cells through separate A_1 and A_2 purinoceptor pathways.

intracellular magnesium; fluorescence; intracellular calcium transients; intracellular adenosine 3',5'-cyclic monophosphate; immortalized mouse distal convoluted tubule cells

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ADENOSINE MODULATES A VARIETY of transport functions in the distal tubule. Adenosine increases transepithelial resistance and diminishes osmotic water absorption and Na^+ transport in inner medullary collecting duct (IMCD) cells in culture and Cl^- secretion in distal and collecting tubule cells (5, 15, 17, 18). On the other hand, adenosine stimulates Na^+ transport in amphibian kidney A6 cells, a model of the distal tubule (8, 10), and Cl^- conductance in rabbit distal convoluted tubule cells (16). Adenosine also stimulates Ca^{2+} reabsorption in a mixture of rabbit primary connecting tubule and cortical collecting duct cells (10). The diversity of responses might be due to expression of different purine receptors in cells comprising the distal tubule. Three receptor subtypes (A_1 , A_2 , A_3) have been identified, all of which are coupled to G proteins. The A_1 receptor is coupled to G_i , leading to inhibition of adenylate cyclase, and to G_q , resulting in activation of phospholipase C, intracellular Ca^{2+} release, and an increase in protein kinase C activity (15). A_2 receptors are coupled, through G_q , to stimulate adenylate cyclase (15). The A_3 receptors, like the A_1 receptor subtypes, are coupled to a G_i and G_q but are only found in the heart and nervous system (6). Thus adenosine may have diverse effects on electrolyte reabsorption in the distal tubule. The convoluted portion of the distal tubule provides the final control of urinary Mg^{2+} excretion, as there is no Mg^{2+} reabsorption beyond this segment (14). Accordingly, any influence of adenosine on distal magnesium transport would be expected to alter renal Mg^{2+} excretion.

In the present studies, we determined the effect of adenosine on Mg^{2+} uptake into immortalized mouse distal convoluted tubule (MDCT) cells, a model we have extensively used to study Mg^{2+} handling in the distal convoluted tubule (4). The distal convoluted tubule has not been extensively studied because performing *in vivo* or *in vitro* perfusion experiments is difficult. Our studies using MDCT cells suggest that the rate of Mg^{2+} entry reflects overall transepithelial reabsorption (4). The MDCT cell line possesses many of the properties of the intact distal convoluted tubule, including many hormone receptors and extracellular di-

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valent cation-sensing receptors (CaSR). We have reported that hormones such as parathyroid hormone (PTH), glucagon, and arginine vasopressin (AVP) stimulate Mg^{2+} uptake (2, 4). Aldosterone potentiates hormone-mediated Mg^{2+} entry (3) and high extracellular Ca^{2+} and Mg^{2+} levels inhibit hormone-responsive uptake (1). Accordingly, MDCT cells are a useful model to study controls of Mg^{2+} transport. In the present study, we show that adenosine may stimulate Mg^{2+} entry or inhibit hormone-mediated Mg^{2+} uptake in MDCT cells via A_2 and A_1 receptors, respectively. We infer from these studies that adenosine might modulate Mg^{2+} transport in the intact distal convoluted tubule.

MATERIALS AND METHODS

Cell culture. Distal convoluted tubule cells were isolated from mice and immortalized by Pizzonia et al. (13) and functionally characterized as described by Friedman and Gesek and their colleagues (7). The MDCT cell line was grown on 60-mm plastic culture dishes (Corning Glass Works, Corning Medical and Scientific, Corning, NY) in basal DMEM-Ham's F-12, 1:1, media (GIBCO) supplemented with 10% fetal calf serum (Flow Laboratories, McLean, VA), 1 mM glucose, 5 mM L-glutamine, 50 U/ml penicillin, and 50 μ g/ml streptomycin in a humidified environment of 5% CO_2 -95% air at 37°C. For the fluorescence studies, confluent cells were washed three times with PBS containing 5 mM EGTA, trypsinized, and seeded on glass coverslips. Aliquots of harvested cells were allowed to settle onto sterile glass coverslips in 100-mm Corning tissue culture dishes, and the cells were grown to subconfluence over 1–2 days in supplemented media as described above. The normal media contained 0.6 mM Mg^{2+} and 1.0 mM Ca^{2+} . In the experiments indicated, MDCT cells were cultured in Mg^{2+} -free media (<0.01 mM) for 16–24 h before study. Other constituents of the Mg^{2+} -free culture media were similar to the complete media. Customized Mg^{2+} -free media were purchased from Stem Cell Technologies (Vancouver, BC). These media contained 0.2% bovine serum albumin rather than fetal calf serum.

Cytoplasmic Mg^{2+} and Ca^{2+} measurements. Coverslips were mounted in a perfusion chamber, and intracellular Mg^{2+} and Ca^{2+} concentration ($[Mg^{2+}]_i$ and $[Ca^{2+}]_i$, respectively) were determined with the use of the Mg^{2+} - and Ca^{2+} -sensitive fluorescent dyes mag-fura 2 and fura 2, respectively (Molecular Probes, Eugene, OR). The cell-permeant acetoxymethyl ester (AM) form of the dye was dissolved in DMSO to a stock concentration of 5 mM and then diluted to 5 or 10 μ M fura 2-AM in media for 20 min at 37°C. Cells were subsequently washed three times with buffered salt solution containing (in mM) 145 NaCl, 4.0 KCl, 0.8 Na_2HPO_4 , 0.2 KH_2PO_4 , 1.0 $CaCl_2$, 5 glucose, and 20 HEPES/Tris, at pH 7.4. The MDCT cells were incubated for a further 20 min to allow for complete deesterification and washed once before measurement of fluorescence.

Epifluorescence microscopy was used to monitor changes in the mag-fura 2 or fura 2 fluorescence of single MDCT cells cultured in monolayers. The chamber was mounted on an inverted Nikon Diaphot-TMD microscope, with a Fluor $\times 100$ objective, and fluorescence within a single cell was monitored under oil immersion over the course of the study. Fluorescence was recorded at 1-s intervals using a dual-excitation wavelength spectrofluorometer (Delta-scan, Photon Technologies, Princeton, NJ) with excitation for mag-fura 2 at 335 and 385 nm, for fura 2 at 340 and 380 nm (chopper speed set at 100 Hz/s), and emission at 505 nm. All experiments were

performed at 21°C because the mag-fura 2 and fura 2 responses were found to be identical at room temperature and 37°C. Media changes were made without an interruption in recording.

Free $[Mg^{2+}]_i$ and $[Ca^{2+}]_i$ were calculated from the ratio of the fluorescence at the two excitation wavelengths as described using a dissociation constant (K_d) of 1.4 mM and 224 nM, respectively, for the mag-fura 2- Mg^{2+} and fura 2- Ca^{2+} complexes (2). The minimum (R_{min}) and maximum (R_{max}) ratios were determined for the cells at the end of each experiment using 20 μ M digitonin.

R_{max} for mag-fura 2 was found by the addition of 50 mM $MgCl_2$ in the absence of Ca^{2+} , and R_{min} was obtained by removal of Mg^{2+} and addition of 100 mM EDTA, pH 7.2. The excitation spectrum of the cellular mag-fura 2 under these conditions was similar to that of free mag-fura 2 in the same solutions. R_{max} and R_{min} for fura 2 were obtained with Ca^{2+} and EGTA by previously published techniques (2).

Determination of cAMP concentration. cAMP was determined in confluent MDCT cell monolayers cultured in 24-well plates in DMEM-Ham's F-12 media without serum but with 0.1% BSA. The media contained 0.6 mM or 0 Mg^{2+} where indicated. After addition of the agonist to be tested, MDCT cells were incubated at 37°C for 5 min. cAMP was extracted with 5% trichloroacetic acid, which was removed with ether, and the extract was acidified with 0.1 N HCl. The aqueous phase was dried, dissolved in Tris-EDTA buffer, and then cAMP was measured with a radioimmunoassay kit (Diagnostic Products, Los Angeles, CA).

Statistical analysis. Representative tracings of fluorescent intensities are given, and significance was determined by Student's *t*-test or Tukey's analysis of variance as appropriate. All results are expressed as means \pm SE where indicated.

RESULTS

Adenosine alters Mg^{2+} uptake into Mg^{2+} -depleted MDCT cells. Because there is not an appropriate radioisotope for Mg^{2+} to directly measure Mg^{2+} transport rates, we developed the following model to assess Mg^{2+} influx into single MDCT cells (4). Subconfluent MDCT monolayers were cultured in Mg^{2+} -free medium for 16 h. These cells possessed a significantly lower $[Mg^{2+}]_i$, 0.22 ± 0.01 mM, than that observed in normal MDCT cells, 0.53 ± 0.02 mM. When the Mg^{2+} -depleted MDCT cells were placed in a bathing solution containing 1.5 mM $MgCl_2$, $[Mg^{2+}]_i$ increased with time and plateaued at 0.50 ± 0.07 mM, $n = 7$, which was similar to that observed in normal cells (4). The mean rate of refill, $d([Mg^{2+}]_i)/dt$, measured as the change in $[Mg^{2+}]_i$ with time, was 137 ± 16 nM/s, $n = 7$, experiments, as determined over the first 500 s after addition of Mg^{2+} . We have previously reported data that indicate the Mg^{2+} uptake is concentration dependent and selective for Mg^{2+} (4).

Adenosine stimulated Mg^{2+} uptake in Mg^{2+} -depleted MDCT cells by $41 \pm 10\%$ of control values (Fig. 1). The adenosine/ P_1 receptor family in epithelial cells comprises A_1 and A_2 adenosine receptors that have been identified by molecular and pharmacological studies (15). We used N^6 -cyclopentyladenosine (CPA), a selective A_1 agonist, and 2-[*p*-(2-carbonyl-ethyl)-phenylethylamino]-5'-*N*-ethylcarboxamidoadenosine (CGS),

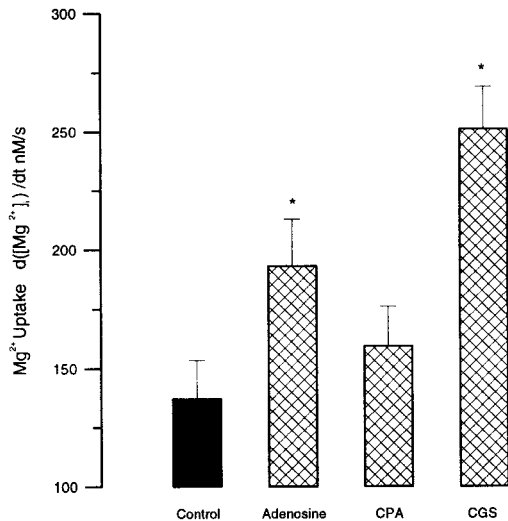


Fig. 1. Adenosine alters Mg^{2+} uptake in Mg^{2+} -depleted mouse distal convoluted tubule (MDCT) cells. MDCT cells were cultured in Mg^{2+} -free media (<0.01 mM) for 16 h. Fluorescence studies were performed in buffer solutions in absence of external magnesium, and where indicated, $MgCl_2$ (1.5 mM final concentration) was added to observe changes in intracellular Mg^{2+} concentration ($[Mg^{2+}]_i$). The buffer solutions contained (in mM) 145 NaCl, 4.0 KCl, 0.8 K_2HPO_4 , 0.2 KH_2PO_4 , 1.0 $CaCl_2$, 5.0 glucose, and 10 HEPES/Tris, pH 7.4, with and without 1.5 mM $MgCl_2$. N^6 -cyclopentyladenosine (CPA), a selective A_1 agonist, and 2-[*p*-(2-carbonyl-ethyl)-phenylethylamino]-5'-*N*-ethylcarboxamidoadenosine (CGS), an A_2 agonist, were added at concentrations of 10 μ M. Fluorescence was measured at 1 data point/s with 25-point signal averaging, and the tracing was smoothed according to methods previously described (2). Values are means \pm SE for 5–7 cells. * $P < 0.01$, Mg^{2+} entry rates vs. control values.

an A_2 agonist, to determine the P1 subtype by which adenosine alters Mg^{2+} entry. The agonists, CPA and CGS, were from RBI (Sigma, St. Louis, MO). The A_1 agonist, CPA (10 μ M) did not alter basal Mg^{2+} uptake, 159 ± 17 nM/s, $n = 5$, but the A_2 receptor agonist CGS nearly doubled the entry rate to 252 ± 18 nM/s, $n = 5$.

The relatively selective antagonist of A_1 receptors, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), and of A_2 receptors, 3,7-dimethyl-1-propargylxanthine (DMPX), were used to confirm that adenosine stimulated Mg^{2+} entry by A_2 purinoceptors. The antagonists were from RBI. The A_2 receptor antagonist DMPX inhibited adenosine-stimulated Mg^{2+} uptake from 252 ± 18 to 120 ± 15 nM/s, $n = 4$; the latter value was not different from basal values (Fig. 2). DPCPX did not alter adenosine responses (Fig. 2). These findings support the conclusion that adenosine increases Mg^{2+} entry via A_2 purinoceptors.

Next, we determined some of the receptor-mediated signaling mechanisms involved with the adenosine-mediated Mg^{2+} uptake. The most commonly recognized signal transduction mechanism for the A_2 receptor is activation of adenylate cyclase. This implies coupling with the G protein G_s , although other G proteins may also be involved (15). A_1 purinoceptors, on the other hand, mediate a broad range of signaling responses caused by its coupling to different G proteins within the $G_{i/o}$ family (15). This signaling pathway leads to diminished cAMP, activation of phospholipase

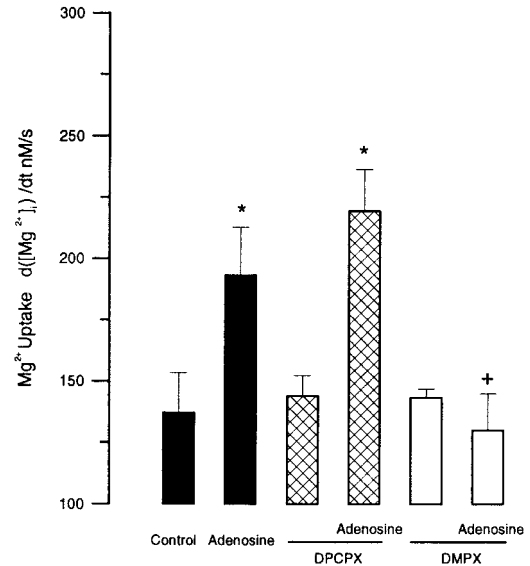


Fig. 2. Antagonists of A_2 purinoceptors inhibit adenosine-mediated Mg^{2+} uptake. Relatively selective antagonists of A_1 receptors, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), and A_2 receptors, 3,7-dimethyl-1-propargylxanthine (DMPX), were added where indicated 15 min before the addition of adenosine at concentrations of 10 μ M. *, +: $P < 0.01$, Mg^{2+} entry rates vs. control and adenosine values, respectively.

C, which, in turn, leads to membrane phosphoinositide metabolism and increased production of inositol triphosphate and Ca^{2+} mobilization. First, we determined receptor-mediated $[Ca^{2+}]_i$ with fluorescence. Adenosine resulted in a transient increase in Ca^{2+} concentration, from basal concentrations of 95 ± 7 to 516 ± 35 nM, which is typical of receptor-mediated intracellular Ca^{2+} release (Table 1). The A_1 agonist CPA also initiated a transient increase in intracellular Ca^{2+} of a magnitude similar to that for adenosine. However, the A_2 agonist CGS did not illicit large changes in Ca^{2+} signaling (129 ± 6 nM). The observation that CPA induced Ca^{2+} signaling supports the idea that there are A_1 purinoceptors present in MDCT cells. Second, we determined the effect of the A_1 and A_2 receptor agonists on intracellular cAMP formation. Adenosine and CGS increased intracellular cAMP pro-

Table 1. P_1 receptor agonists stimulate cytosolic Ca^{2+} transients in MDCT cells

	Basal $[Ca^{2+}]_i$, nM	$\Delta[Ca^{2+}]_i$, nM
Adenosine	95 ± 7 (3)	$516 \pm 35^*$ (3)
CPA	113 ± 3 (4)	$568 \pm 24^*$ (4)
CGS	108 ± 4 (4)	$129 \pm 6^*$ (4)

Values are means \pm SE with no. of separate observations in parentheses. Normal mouse distal convoluted tubule (MDCT) cells were loaded with fura 2, and P1 receptor agonists were added to buffer solutions at a concentration of 10 μ M. N^6 -cyclopentyladenosine (CPA) is a selective A_1 agonist and 2-[*p*-(2-carbonyl-ethyl)-phenylethylamino]-5'-*N*-ethylcarboxamidoadenosine (CGS) an A_2 agonist. Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured with microfluorescence using fura 2. The change in intracellular Ca^{2+} concentration ($\Delta[Ca^{2+}]_i$) was the maximal change in cytosolic Ca^{2+} from basal $[Ca^{2+}]_i$. * $P < 0.001$ vs. basal $[Ca^{2+}]_i$.

duction by about threefold, from 20 ± 2 to 54 ± 9 and 65 ± 10 $\text{pmol} \cdot \text{mg protein}^{-1} \cdot 5 \text{ min}^{-1}$, respectively, whereas CPA had no effect (25 ± 2 $\text{pmol} \cdot \text{mg protein}^{-1} \cdot 5 \text{ min}^{-1}$) (Table 2). PTH stimulated cAMP in control cells (42 ± 6 $\text{pmol} \cdot \text{mg protein}^{-1} \cdot 5 \text{ min}^{-1}$). CPA diminished PTH-stimulated cAMP (35 ± 2 $\text{pmol} \cdot \text{mg protein}^{-1} \cdot 5 \text{ min}^{-1}$), whereas the A_2 receptor agonist CGS increased PTH-stimulated cAMP formation (69 ± 9 $\text{pmol} \cdot \text{mg protein}^{-1} \cdot 5 \text{ min}^{-1}$), which was similar to the results for CGS alone; i.e., no additive effect. These findings indicate that MDCT cells possess both A_1 and A_2 purinoceptors that have the classic signaling pathways of each of the respective receptor families.

A_1 purinoceptor agonists inhibit receptor-stimulated Mg^{2+} uptake. As the A_1 purinoceptor agonists inhibit PTH-stimulated cAMP formation (Table 2), we tested whether CPA might inhibit hormone-mediated Mg^{2+} uptake. Pretreatment of MDCT cells with CPA diminished PTH-stimulated Mg^{2+} entry rate by $27 \pm 9\%$ (Fig. 3). The pretreatment of MDCT cells with CPA also inhibited CGS-stimulated Mg^{2+} uptake from 252 ± 18 , $n = 4$, to 196 ± 14 , $n = 3$, nM/s (Fig. 3). These observations indicate that A_1 purinoceptor agonists inhibit hormone-stimulated Mg^{2+} entry and modulate the actions of A_2 purinoceptor agonists in MDCT cells.

Characterization of A_2 purinoceptor agonist-stimulation of Mg^{2+} uptake in MDCT cells. CGS increased Mg^{2+} uptake in a concentration-dependent fashion with the maximal dose of $\sim 10 \mu\text{M}$ (Fig. 4). These data for selective P_1 receptor agonists indicate that adenosine stimulates Mg^{2+} uptake by the A_2 purinoceptor.

To characterize some of the A_2 -mediated signaling pathways that adenosine uses to stimulate Mg^{2+} uptake, we pretreated the MDCT cells with a number of well-known inhibitors of kinases involved in G protein transduction. RpcAMPS, an inhibitor of protein kinase A, inhibited CGS agonist-stimulated Mg^{2+} entry rates (143 ± 16 nmol/s , $n = 3$), supporting the above observation that receptor-mediated cAMP formation is involved in A_2 -receptor agonist-mediated actions (Fig. 5). Pretreatment of MDCT cells with the phospholipase C inhibitor U-73122 inhibited A_2 -adrenergic agonist-stimulated Mg^{2+} uptake (124 ± 25 nmol/s , $n = 5$), and the protein kinase C inhibitor Ro-31-822 diminished

Table 2. P_1 receptor agonists alter intracellular cAMP accumulation

	Control	PTH
Control	20 ± 2 (4)	$42 \pm 6^*$ (4)
Adenosine	$54 \pm 9^\dagger$ (4)	
CPA	25 ± 2 (4)	$35 \pm 2^{*\dagger}$ (4)
CGS	$65 \pm 10^\dagger$ (4)	$69 \pm 9^\dagger$ (3)

Values are means \pm SE of cAMP accumulation, expressed as $\text{pmol} \cdot \text{mg protein}^{-1} \cdot 5 \text{ min}^{-1}$, with no. of separate observations in parentheses. P_1 receptor agonists ($10 \mu\text{M}$) were added 5 min before the addition of parathyroid hormone (PTH; 10^{-7} M), and cAMP was measured 5 min later. * , † : $P < 0.01$, mean values with PTH vs. control or P_1 receptor agonist vs. control values and P_1 receptor agonist or P_1 receptor agonist plus vs. control or PTH alone, respectively.

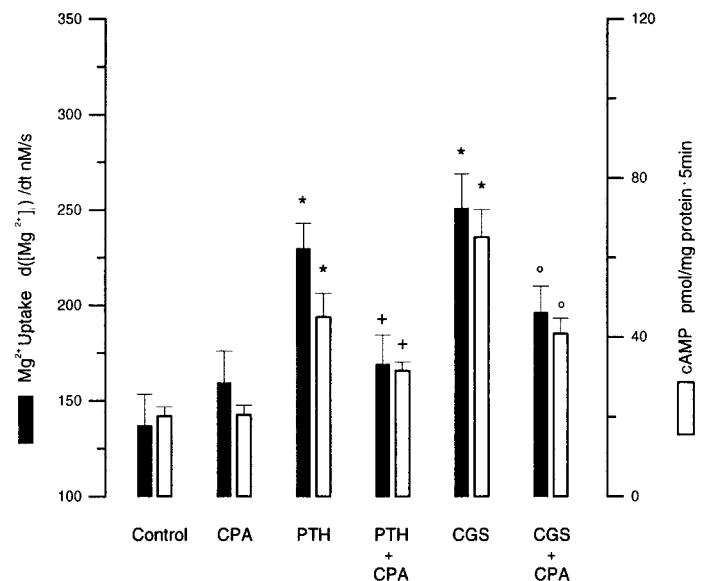


Fig. 3. A_1 purinoceptors inhibit parathyroid hormone (PTH)- and A_2 agonist-mediated cAMP formation and Mg^{2+} uptake in MDCT cells. Cells were pretreated with the A_1 agonist CPA ($10 \mu\text{M}$, 10^{-7} M) or the A_2 agonist CGS ($10 \mu\text{M}$) 5 min before the addition of PTH. Values are means \pm SE for 3–5 observations. * , † , $^\circ$: $P < 0.01$, mean Mg^{2+} entry rates and cAMP determinations of either PTH or CGS vs. respective control values, of PTH+CPA vs. PTH alone, and of CGS+CPA vs. CGS alone, respectively.

CGS agonist-stimulated uptake, from 252 ± 18 , $n = 5$, to 178 ± 18 nmol/s , $n = 4$ (Fig. 5). Inhibition of the MAP kinase cascade (extracellular-signal-regulated kinase, c-jun NH₂-terminal kinase, p38) with PD-98059 also decreased Mg^{2+} uptake (125 ± 22 nmol/s , $n = 4$). Accordingly, A_2 receptors act through a number of

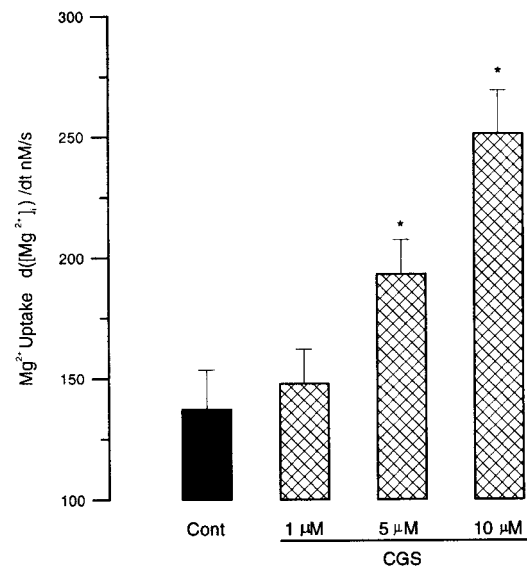


Fig. 4. Concentration dependence of CGS-stimulation of Mg^{2+} entry in MDCT cells. The rate of Mg^{2+} influx as determined by uptake rate $\{d([Mg^{2+}]_i)/dt\}$ was measured with the CGS concentrations shown, using fluorescence techniques performed according to procedures given in legend to Fig. 1. $d([Mg^{2+}]_i)/dt$ values were determined over the first 500 s of fluorescence measurements. Values are means \pm SE for 3–6 cells. Cont, control. * $P < 0.01$ for Mg^{2+} entry rates.

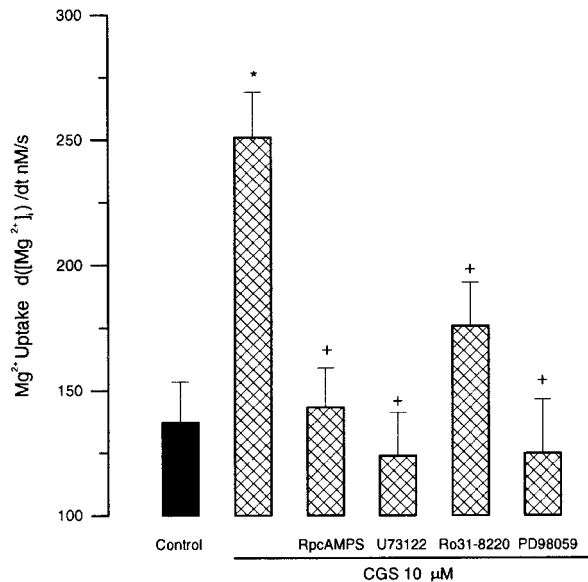


Fig. 5. CGS stimulates Mg^{2+} uptake through A_2 receptor-mediated signaling pathways. Inhibitors for protein kinase A [Rp-cAMPS (0.5 μ M)], phospholipase C [U-73122 (15 μ M)], and protein kinase C [Ro-31-822 (0.1 μ M)] were added to Mg^{2+} -depleted MDCT cells 5 min before the addition of CGS (10 μ M). The MAP kinase inhibitor PD-98059 (1.0 μ M) was added 10 min before CGS. Values are means \pm SE for 3–5 cells. * $P < 0.001$, CGS vs. control uptake rates. † $P < 0.01$, inhibitor + CGS vs. CGS alone.

receptor-mediated signaling pathways to affect changes in Mg^{2+} transport. These findings are similar to those we have reported for hormone (PTH, glucagon, AVP) receptor-mediated Mg^{2+} transport in MDCT cells (4). Accordingly, we would expect that aldosterone and high extracellular Ca^{2+} concentration might alter CGS-stimulated Mg^{2+} uptake.

Aldosterone potentiates A_2 receptor agonist-stimulated Mg^{2+} uptake in MDCT cells. We have previously shown that aldosterone, applied 16 h before experi-

mentation, increases PTH-, glucagon-, and AVP-mediated cAMP generation and potentiates hormone-mediated Mg^{2+} uptake (4). Although the cellular mechanisms are not known, it has been speculated that aldosterone-induced proteins modulate receptor signaling in epithelial cells (11). In the present study, we determined whether pretreatment of MDCT cells with aldosterone for 16 h potentiated the actions of the A_2 receptor agonist CGS. Treatment of cells with aldosterone, for 16 h before the study, did not significantly affect basal Mg^{2+} uptake (142 ± 11 nM/s, $n = 3$) but potentiated CGS-stimulated Mg^{2+} entry, from 251 ± 18 nM/s, $n = 4$, to 305 ± 17 nM/s, $n = 6$ (Fig. 6). Interestingly, aldosterone did not potentiate CGS-responsive cAMP production (62 ± 16 pmol·mg protein⁻¹·5 min⁻¹), suggesting that the actions are downstream of the generation of this second message (Fig. 6).

Elevation of extracellular Ca^{2+} inhibits A_2 receptor agonist-stimulated cAMP generation and Mg^{2+} uptake. MDCT cells possess an extracellular CaSR that, on activation with polyvalent cations such as Ca^{2+} , Mg^{2+} , or neomycin, inhibits PTH-, glucagon-, and AVP-mediated cAMP generation and glucagon- and AVP-stimulated Mg^{2+} uptake (1). To determine whether activation of the CaSR alters A_2 receptor agonist actions, we pretreated cells for 5 min with 5.0 mM $CaCl_2$ before the addition of CGS. Elevation of extracellular Ca^{2+} did not have any effects on basal Mg^{2+} entry (147 ± 10 nM/s, $n = 4$) but abolished CGS stimulation of cAMP generation (21 ± 2 pmol·mg protein⁻¹·5 min⁻¹, $n = 4$) and Mg^{2+} uptake (128 ± 13 nM/s, $n = 5$) (Fig. 7). The mechanisms by which the CaSR inhibits CGS actions remain unclear, but the receptor is coupled to $G\alpha_i$ proteins, which is consistent with the conclusion that CGS responses in MDCT cells are dependent, in part, on cAMP-mediated signaling pathways.

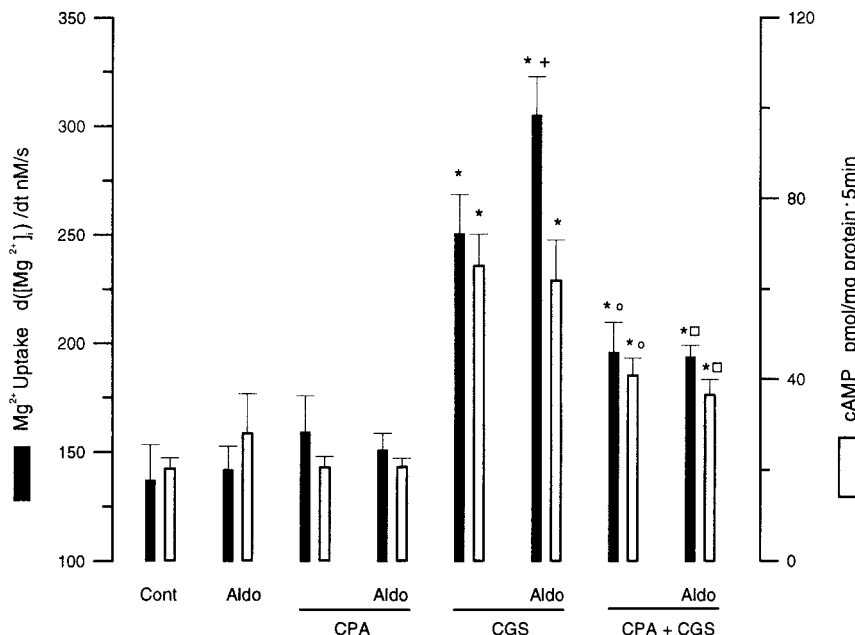


Fig. 6. Aldosterone (Aldo) potentiates CGS-mediated Mg^{2+} uptake. MDCT cells were incubated for 16 h in magnesium-free buffer solution containing aldosterone (10^{-7} M). CGS (10 μ M) was added where indicated, and Mg^{2+} uptake was determined after 500 s in 1.5 mM $MgCl_2$ or cAMP was measured after 5 min. Values are means \pm SE for 4–7 observations. *, †, ○, □: $P < 0.01$, mean Mg^{2+} entry rates and cAMP determinations vs. respective control values, CGS+aldosterone vs. CGS, CPA+CGS vs. CGS, and aldosterone+CPA+CGS vs. aldosterone+CGS, respectively.

ATP inhibits A_2 receptor agonist-stimulated Mg^{2+} uptake in MDCT cells. The relationship between the purines ATP and adenosine is complex. We have recently determined that ATP inhibits basal and hormone-stimulated Mg^{2+} transport by 21% in MDCT cells (2a). Our studies showed that this inhibition was via P2X purinoceptors as the selective P2X agonist β,γ -methylene-ATP (β,γ -Me-ATP) inhibited Mg^{2+} uptake, but the more P2Y selective agonists UTP, ADP, and 2-methylthio ATP were without effect. Accordingly, it was of interest to see whether ATP would have any effect on Mg^{2+} -conserving actions of adenosine. Pretreatment of MDCT cells with β,γ -Me-ATP prevented the stimulation of Mg^{2+} uptake by the A_2 receptor agonist (160 ± 9 nM/s, $n = 4$) (Fig. 8). These observations suggest that the purines may modulate Mg^{2+} uptake in MDCT cells by diverse receptor-mediated mechanisms.

DISCUSSION

The findings in this study indicate that adenosine modulates Mg^{2+} uptake in MDCT cells, a model for the intact distal convoluted tubule. Our data show that both A_1 and A_2 purinoceptor subtypes are present in MDCT cells. Adenosine and the selective A_2 agonist CPA elicited receptor-mediated intracellular Ca^{2+} signaling, whereas adenosine and the A_1 receptor agonist CGS increase cAMP formation (Tables 1 and 2). CPA inhibited PTH- and CGS-stimulated cAMP formation typical of A_1 purinoceptor-induced signaling involving G_i -coupled proteins (Fig. 3). This was associated with diminished Mg^{2+} uptake (Fig. 3). On the other hand, CGS stimulated cAMP formation in a manner charac-

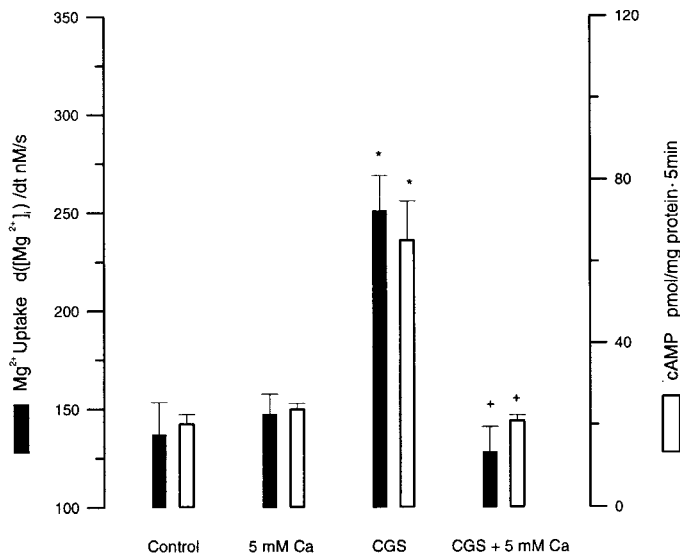


Fig. 7. Summary of the effects of extracellular Ca^{2+} on CGS-stimulated cAMP formation and Mg^{2+} uptake. cAMP was measured by radioimmunoassay, and $d([Mg^{2+}]_i)/dt$ was determined with 1.5 mM extracellular Mg^{2+} in the absence and presence of 5.0 mM $CaCl_2$ as indicated. $CaCl_2$ was added 5 min before the addition of 10 μ M CGS. $d([Mg^{2+}]_i)/dt$ was determined over the initial 500 s after addition of CGS. Values are means \pm SE for 4–7 cells. *, +: $P < 0.01$, mean Mg^{2+} entry rates and cAMP determinations vs. respective control values and CGS vs. CGS+5.0 mM extracellular Ca^{2+} , respectively.

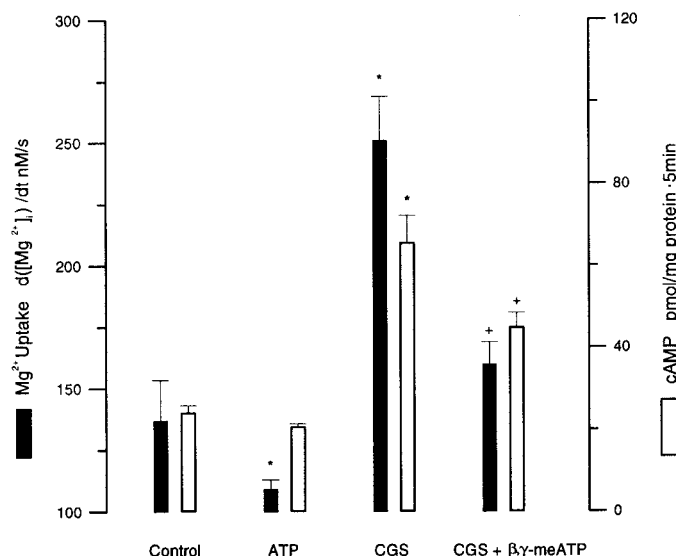


Fig. 8. Extracellular ATP inhibits A_2 receptor agonist-stimulated Mg^{2+} uptake in MDCT cells. P2X purinoceptor agonists, such as β,γ -methylene-ATP (β,γ -Me-ATP) inhibit hormone-mediated Mg^{2+} uptake (unpublished observations). The more selective P2X agonist, β,γ -Me-ATP (10^{-4} M) was added 5 min before 10 μ M CGS where indicated. Values are means \pm SE for 4–7 cells. *, +: $P < 0.01$, mean Mg^{2+} entry rates and cAMP determinations vs. respective control values and CGS vs. CGS+ β,γ -Me-ATP, respectively.

teristic of A_2 purinoceptor signal transduction. CGS increased Mg^{2+} entry (Fig. 3). Moreover, CGS-stimulated Mg^{2+} uptake was decreased by protein kinase A inhibition, supporting the notion that A_2 receptors modulate Mg^{2+} entry in MDCT cells via the G_s -coupled proteins (Fig. 6). A_1 and A_2 receptors are often polarized to either the apical or basolateral membrane so that adenosine may have divergent effects depending on the concentration at these two sides (15). Unfortunately, we are not able to determine the polarity of A_1 and A_2 receptors in MDCT cells; nevertheless, these observations demonstrate the multipotency of the effect of adenosine on Mg^{2+} transport. Polarization of the purinoceptors remains to be determined in the intact distal convoluted tubule.

Aldosterone modulates adenosine-stimulated Mg^{2+} entry in MDCT cells. We have shown that aldosterone potentiates hormone-responsive Mg^{2+} transport in MDCT cells (3). The prominent mechanism of steroids, which operate through nuclear receptors, is to control transcriptional regulation, expression, and posttranslational targeting of heterotrimeric G proteins such as G_{α_s} , G_{α_i} , G_{β} , G_{γ} , and phospholipase C (11). Pretreatment of MDCT cells with aldosterone potentiated CGS-stimulated Mg^{2+} entry (Fig. 6). Aldosterone may increase any of the above pathways or others that ultimately lead to increased adenosine-stimulated Mg^{2+} entry in MDCT cells. In support of this notion, aldosterone potentiates hormone-stimulated Mg^{2+} uptake without increasing cAMP formation so that other processes downstream of cAMP generation are involved.

These studies indicate that MDCT cell Mg^{2+} uptake is regulated at two levels: first by membrane-receptor

(adenosine) signaling and, second, by nuclear transcription-dependent receptor (aldosterone) signaling.

Extracellular Ca^{2+} affects A_2 agonist-mediated Mg^{2+} uptake in MDCT cells. The CaSR within the distal tubule is important in controlling Mg^{2+} entry in MDCT cells (1, 4). The extracellular Ca^{2+} - and Mg^{2+} -sensing mechanisms provide a negative-feedback loop to diminish the renal conserving actions of the circulating hormones like PTH, glucagon, and AVP (4). We have reported that elevation of extracellular Ca^{2+} or Mg^{2+} , or the addition of the polyvalent cation neomycin, inhibits peptide hormone-stimulated cAMP formation and hormone-responsive Mg^{2+} uptake in MDCT cells (1). Activation of CaSR inhibits A_2 agonist stimulation of Mg^{2+} uptake in MDCT cells (Fig. 7). The responses likely involve diminished A_2 agonist-mediated cAMP formation, phospholipase C, protein kinase C, or MAP kinase cascades (Fig. 5). These findings show that adenosine-mediated effects may be modulated by extracellular Ca^{2+} and Mg^{2+} concentration.

ATP inhibits adenosine-stimulated Mg^{2+} uptake. We have shown that ATP inhibits hormone-stimulated Mg^{2+} via P2X purinoceptors (2a). These receptors are coupled to ATP-gated channels that activate nonselective cation channels. In these studies, the selective P2X receptor agonist β, γ -Me-ATP inhibited basal and hormone-stimulated Mg^{2+} uptake by 32%. In the present study, β, γ -Me-ATP inhibited adenosine-stimulated Mg^{2+} uptake in MDCT cells (Fig. 8). The pathophysiological implications of these interactions are unclear, but autocrine or paracrine secretion or tissue damage leading to cellular ATP release and its degradation to adenosine may be sufficient to alter Mg^{2+} handling in the distal tubule.

Role of adenosine in distal tubular Mg^{2+} handling. We infer from our data that adenosine modulates magnesium transport in the distal convoluted tubule of the nephron. Adenosine via A_1 receptors may inhibit hormone-stimulated Mg^{2+} uptake or, via A_2 , may stimulate Mg^{2+} entry. Accordingly, hormones such as PTH, vasopressin, and calcitonin stimulate distal Mg^{2+} , in part, through intracellular generation of cAMP that may be metabolized to 5'-AMP and adenosine within the cell (17). Adenosine is transported out of the cell by a nucleoside transporter. In addition, intracellular 5'-AMP may be transported out of the cell by a nucleotide transporter and further metabolized to adenosine by a membrane ecto-5'-nucleotidase, as summarized by Schwiebert et al. (17). Extracellular adenosine may act at cell-surface A_1 receptors to diminish hormone-mediated cAMP formation, leading to termination of the hormone stimulus. Alternatively, extracellular adenosine may act at cell-surface A_2 receptors to further increase cAMP, leading to a propagation of the hormone stimulus so that adenosine may act as an autocoid to stimulate Mg^{2+} reabsorption in conjunction with the known Mg^{2+} -conserving circulating hormones (4). Further studies are required to identify and locate nucleotide transporters and cell-surface purinoceptors in the distal convoluted tubule.

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REFERENCES

1. **Bapty BW, Dai LJ, Ritchie G, Canaff L, Hendy GN, and Quamme GA.** Activation of Mg^{2+}/Ca^{2+} -sensing inhibits hormone-stimulated Mg^{2+} uptake in mouse distal convoluted tubule cells. *Am J Physiol Renal Physiol* 275: F353–F360, 1998.
2. **Dai L-J, Bapty BW, Ritchie G, and Quamme GA.** Glucagon and arginine vasopressin stimulates Mg^{2+} uptake in mouse distal convoluted tubule cells. *Am J Physiol Renal Physiol* 274: F328–F335, 1998.
- 2a. **Dai L-J, Kang HS, Kerstan D, Ritchie G, and Quamme GA.** ATP inhibits Mg^{2+} uptake in MDCT cells via P2X purinoceptors. *Am J Physiol Renal Physiol* 281: F833–F840, 2001.
3. **Dai L-J, Ritchie G, Bapty B, and Quamme GA.** Aldosterone potentiates hormone-stimulated Mg^{2+} uptake in distal convoluted tubule cells. *Am J Physiol Renal Physiol* 274: F336–F341, 1998.
4. **Dai L-J, Ritchie G, Kerstan D, Kang HS, Cole DEC, and Quamme GA.** Magnesium transport in the renal distal convoluted tubule. *Physiol Rev* 81: 51–84, 2001.
5. **Edwards RM and Spielman WS.** Adenosine A_1 receptor-mediated inhibition of vasopressin action in inner medullary collecting duct. *Am J Physiol Renal Fluid Electrolyte Physiol* 266: F791–F796, 1994.
6. **Fredholm BB.** Adenosine receptors in the central nervous system. *News Physiol Sci* 10: 122–128, 1995.
7. **Friedman PA and Gesek FA.** Calcium transport in renal epithelial cells. *Am J Physiol Renal Fluid Electrolyte Physiol* 264: F181–F198, 1993.
8. **Hayslett JP, Macala LJ, Smallwood JL, Kalghatgi L, Galsalla-Herraiz J, and Isales C.** Adenosine stimulation of Na^+ transport is mediated by an A_1 receptor and a $[Ca^{2+}]_i$ -dependent mechanism. *Kidney Int* 47: 1576–1584, 1995.
9. **Hoenderop JGJ, Hartog A, Willems PHGM, and Bindels RJM.** Adenosine-simulated Ca^{2+} reabsorption is mediated by apical A_1 receptors in rabbit cortical collecting system. *Am J Physiol Renal Physiol* 274: F736–F743, 1998.
10. **Lang MA, Preston AS, and Handler JS.** Adenosine stimulates sodium transport in kidney A6 epithelia in culture. *Am J Physiol Cell Physiol* 249: C330–C336, 1985.
11. **Morris AJ and Malbon CC.** Physiological regulation of G protein-linked signaling. *Physiol Rev* 79: 1373–1430, 1999.
12. **Moyer BD, McCoy DE, Lee B, Kizer N, and Stanton BA.** Adenosine inhibits arginine vasopressin-stimulated chloride secretion in a mouse IMCD cell line (mIMCD-K2). *Am J Physiol Renal Fluid Electrolyte Physiol* 269: F884–F891, 1995.
13. **Pizzonia JH, Gesek FA, Kennedy SM, Coutermarsh BA, Bacskai BJ, and Friedman PA.** Immunomagnetic separation, primary culture, and characterization of cortical thick ascending limb plus distal convoluted tubule cells from mouse kidney. *In Vitro Cell Dev Biol* 27A: 409–416, 1991.
14. **Quamme GA.** Renal magnesium handling: new insights in understanding old problems. *Kidney Int* 52: 1180–1195, 1997.
15. **Ralevic V and Burnstock G.** Receptors for purines and pyrimidines. *Pharmacol Rev* 50: 413–492, 1998.
16. **Rubera I, Barrière H, Tauc M, Bidet M, Verheecke-Mauze C, Poujeol C, Cuiller B, and Poujeol P.** Extracellular adenosine modulates a volume-sensitive-like chloride conductance in immortalized rabbit DC1 cells. *Am J Physiol Renal Physiol* 280: F126–F145, 2001.
17. **Schwiebert EM, Karlson KH, Friedman PA, Dietl P, Spielman WS, and Stanton BA.** Adenosine regulates a chloride channel via protein kinase C and a G protein in a rabbit cortical collecting duct cell line. *J Clin Invest* 89: 834–841, 1992.
18. **Yagil C, Katni G, and Yagil Y.** The effect of adenosine on transepithelial resistance and sodium uptake in the inner medullary collecting duct. *Pflügers Arch* 427: 225–232, 1994.