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ATP inhibits Mg²⁺ uptake in MDCT cells via P2X purinoceptors

L.-J. Dai, H. S. Kang, D. Kerstan, G. Ritchie and G. A. Quamme

Am J Physiol Renal Physiol, November 1, 2001; 281 (5): F833-F840.

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B. L. Jensen, J. Stubbe, P. B. Hansen, D. Andreasen and O. Skott

Am J Physiol Renal Physiol, June 1, 2001; 280 (6): F1001-F1009.

[Abstract] [Full Text] [PDF]

Magnesium Transport in the Renal Distal Convoluted Tubule

L.-J. Dai, G. Ritchie, D. Kerstan, H. S. Kang, D. E. C. Cole and G. A. Quamme

Physiol Rev, January 1, 2001; 81 (1): 51-84.

[Abstract] [Full Text] [PDF]

beta -Adrenergic agonists stimulate Mg²⁺ uptake in mouse distal convoluted tubule cells

H. S. Kang, D. Kerstan, L.-J. Dai, G. Ritchie and G. A. Quamme

Am J Physiol Renal Physiol, December 1, 2000; 279 (6): F1116-F1123.

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PGE₂ stimulates Mg²⁺ uptake in mouse distal convoluted tubule cells

LONG-JUN DAI, BRIAN BAPTY, GORDON RITCHIE, AND GARY A. QUAMME

Department of Medicine, University of British Columbia, Vancouver Hospital and Health Sciences Centre, Koerner Pavilion, Vancouver, British Columbia, Canada V6T 1Z3

Dai, Long-Jun, Brian Bapty, Gordon Ritchie, and Gary A. Quamme. PGE₂ stimulates Mg²⁺ uptake in mouse distal convoluted tubule cells. *Am. J. Physiol.* 275 (Renal Physiol. 44): F833–F839, 1998.—Prostaglandins have diverse effects on renal electrolyte reabsorption, inhibiting NaCl absorption in the thick ascending limb and modulating sodium and calcium transport in cortical collecting cells. It is unclear what effect, if any, prostaglandins have on tubular magnesium handling. The effects of prostaglandin E₂ (PGE₂) were studied on immortalized mouse distal convoluted tubule (MDCT) cells by measuring cellular cAMP formation with radioimmunoassays and Mg²⁺ uptake with fluorescence techniques. Intracellular free Mg²⁺ concentration ([Mg²⁺]_i) was measured on single MDCT cells using microfluorescence with mag-fura 2. To assess Mg²⁺ uptake, MDCT cells were first Mg²⁺ depleted to 0.22 ± 0.01 mM by culturing in Mg²⁺-free media for 16 h and then placed in 1.5 mM MgCl₂, and the changes in [Mg²⁺]_i were determined. [Mg²⁺]_i returned to basal levels, 0.53 ± 0.02 mM, with a mean refill rate, d([Mg²⁺]_i)/dt, of 173 ± 8 nM/s. Indomethacin, 5 μM, diminished basal Mg²⁺ uptake, suggesting that endogenous prostaglandins may stimulate Mg²⁺ entry in control cells. PGE₂ stimulated Mg²⁺ entry in a concentration-dependent manner with maximal response of 311 ± 12 nM/s, at a concentration of 10⁻⁷ M, which represented an 80 ± 3% increase in uptake rate above control values. This was associated with a sixfold increase in intracellular cAMP generation. PGE₂-stimulated Mg²⁺ uptake was completely inhibited with the Rp diastereoisomer of adenosine 3',5'-cyclic monophosphothionate (Rp-cAMPS), a protein kinase A inhibitor, and U-73122, a phospholipase C inhibitor, and partially by chelerythrine, a protein kinase C inhibitor. Accordingly, PGE₂-mediated Mg²⁺ entry rates involve multiple intracellular signaling pathways. These studies demonstrate that PGE₂ stimulates Mg²⁺ uptake in a cell line of MDCT.

intracellular magnesium; fluorescence; intracellular adenosine 3',5'-cyclic monophosphate; prostaglandin E₂; indomethacin

PROSTAGLANDIN E₂ (PGE₂), the major cyclooxygenase metabolite of renal arachidonic acid, has a number of diverse actions on the kidney (18). In addition to its ability to influence renal hemodynamics, PGE₂ inhibits NaCl absorption within the thick ascending limb (27) and modulates sodium and water transport in the cortical collecting duct (CCD) (14, 15). These functions are mediated by four different prostaglandin receptors (EP₁, EP₂, EP₃, and EP₄) that are selectively located to the apical and/or basolateral epithelial membranes (6,

15, 22, 28, 29). The influence of prostaglandins on renal divalent cation handling is unclear. Using clearance studies, a number of investigators have reported that prostaglandins increase urinary calcium and magnesium excretion (10, 20, 23). As PGE₂ inhibits NaCl absorption in the thick ascending limb, it may be expected that prostaglandins would increase calcium and magnesium excretion through diminished reabsorption in the loop (17, 27). However, van Baal and colleagues (29) have shown that PGE₂ stimulated calcium reabsorption in the rabbit CCD segment of the distal tubule. Like the CCD, the distal convoluted tubule synthesizes prostaglandins, principally PGE₂ (9). Accordingly, PGE₂ may have important actions on transport within the distal convoluted tubule.

In the present studies, we determined the effect of PGE₂ on Mg²⁺ uptake into immortalized mouse distal convoluted tubule (MDCT) cells (11). The MDCT cell line possesses many of the properties of the intact distal convoluted tubule. The MDCT cells exhibit amiloride-inhibitable sodium transport and chlorothiazide-sensitive NaCl cotransport (11). Amiloride and chlorothiazide also stimulate Ca²⁺ and Mg²⁺ entry into these cells (8, 11, 19). Furthermore, parathyroid hormone (PTH) and calcitonin stimulate calcium uptake while glucagon and arginine vasopressin (AVP) increase Mg²⁺ entry in MDCT cells (7, 12). Accordingly, we used this cell line to investigate the actions of PGE₂ on Mg²⁺ uptake in the distal convoluted tubule. The distal convoluted tubule has not been extensively studied because it is difficult to perform in vitro perfusion experiments. As there is not an available isotope for magnesium, we determined Mg²⁺ entry into MDCT cells in the present studies by first depleting the cells of intracellular Mg²⁺ by culturing in Mg²⁺-free media for 16 h. The Mg²⁺-depleted MDCT cells were then placed in medium containing 1.5 mM magnesium, and the refill rate, d([Mg²⁺]_i)/dt, was measured with microfluorescence studies using mag-fura 2 (8). Mg²⁺ uptake rate is concentration dependent and selective for magnesium (8). Moreover, the influx rate is rapid and reproducible so that it is possible to determine the effects of extracellular influences on transport rates. In the present study, we show that PGE₂ stimulates Mg²⁺ entry in MDCT cells possibly through cAMP-dependent mechanisms.

METHODS

Materials. Basal DMEM and Ham's F-12 media (DMEM-F12) were purchased from GIBCO. Customized Mg²⁺-free media were purchased from Stem Cell Technologies (Vancouver, BC). Fetal calf serum was from Flow Laboratories (McLean, VA). Mag-fura 2-AM was obtained from Molecular

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Probes (Eugene, OR). The protein kinase A (PKA) inhibitor, Rp-cAMPS (the Rp diastereoisomer of adenosine 3',5'-cyclic monophosphothionate), and phospholipase C (PLC) inhibitor, U-73122, were purchased from Calbiochem (San Diego, CA). PGE₂, PTH, indomethacin, and other materials were from Sigma (St. Louis, MO).

Cell culture. Distal convoluted tubule cells were isolated from mice, immortalized, and functionally characterized as previously described by Friedman and Gesek and their colleagues (11). The MDCT cell line was grown on 60-mm plastic culture dishes (Corning Glass Works, Corning Medical and Scientific, Corning, NY) in DMEM-F12, 1:1, media supplemented with 10% fetal calf serum, 1 mM glucose, 5 mM L-glutamine, 50 U/ml penicillin, and 50 µg/ml streptomycin in a humidified environment of 5% CO₂-95% 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 magnesium and 1.0 mM calcium. In the experiments indicated, MDCT cells were cultured in Mg²⁺-free media (<0.01 mM) where indicated for 16–24 h prior to study. Other constituents of the Mg²⁺-free culture media were similar to the complete media. These media contained 0.2% BSA rather than the fetal calf serum.

Determination of cAMP concentration. cAMP was determined in confluent MDCT cell monolayers cultured in 24-well plates in DMEM-F12 media without serum but with 0.1% BSA. The media contained 0.6 mM magnesium or zero magnesium where indicated. After addition of either glucagon or AVP, MDCT cells were incubated at 37°C for 5 min in the presence of 0.1 mM IBMX. The cAMP was extracted with 5% trichloroacetic acid which was removed with ether and the extract acidified with 0.1 N HCl. The aqueous phase was dried, then dissolved in Tris-EDTA buffer, and cAMP was measured with a radioimmunoassay kit (Diagnostic Products, Los Angeles, CA).

Cytoplasmic Mg²⁺ measurements. Coverslips were mounted into a perfusion chamber, and intracellular free Mg²⁺ concentration ([Mg²⁺]_i) was determined with the use of the Mg²⁺-sensitive fluorescent dye, mag-fura 2. The cell-permeant acetoxymethyl ester (AM) form of the dye was dissolved in DMSO with Pluronic acid F-127 (0.125%, Molecular Probes) to a stock concentration of 5 mM and then diluted to 5 µM mag-fura 2-AM in media for 20 min at 23°C. Cells were subsequently washed three times with buffered salt solution containing (in mM) 145 NaCl, 4.0 KCl, 0.8 K₂HPO₄, 0.2 KH₂PO₄, 1.0 CaCl₂, 5.0 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 with this buffer solution before measurement of fluorescence.

Epifluorescence microscopy was used to monitor changes in mag-fura 2 fluorescence within single MDCT cells. The chamber (0.5 ml) was mounted on an inverted Nikon Diaphot-TMD microscope, with a Fluor ×100 objective, and fluorescence was monitored under oil immersion within a single cell over the course of 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 (chopper speed set at 100 Hz) and emission at 505 nm. All experiments were performed at 23°C with continuous change of bathing solution (1 ml/min). Media changes were made without interruption in recording.

The [Mg²⁺]_i was calculated from the ratio of the fluorescence at the two excitation wavelengths as previously described using a dissociation constant (*K*_d) of 1.4 mM for the mag-fura 2·Mg²⁺ complex (8). The minimum (*R*_{min}) and maximum (*R*_{max}) ratios were determined for the cells at the end of each experiment using 20 µM digitonin. *R*_{max} for mag-fura 2 was found by the addition of 50 mM MgCl₂ in the absence of Ca²⁺, and *R*_{min} was obtained by removal of Mg²⁺ and addition of 100 mM EDTA, pH 7.2. The change in [Mg²⁺]_i with time, d([Mg²⁺]_i)/dt, was determined by linear regression analysis of the fluorescence tracing over the initial 500 s.

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. A probability of *P* < 0.05 was taken to be statistically significant. All results are means ± SE where indicated.

RESULTS

PGE₂ stimulates cAMP formation in MDCT cells. Of the four prostaglandin receptor subtypes, EP₂ and EP₄ receptors are coupled to adenylate cyclase, which upon stimulation increases intracellular cAMP concentration (6, 29). As cAMP increases Mg²⁺ entry into MDCT cells, we determined the effects of PGE₂ on cAMP release in these cells (7). PGE₂, 10⁻⁷ M, stimulated intracellular cAMP formation by about sixfold in MDCT cells (Fig. 1). Next, we determined whether indomethacin, a cyclooxygenase inhibitor, modulates basal PGE₂-mediated cAMP syntheses. Indomethacin, 5 µM, was added to the serum-free culture media 16 h prior to experimentation to ensure complete inhibition of cyclooxygenase. In control cells, indomethacin modestly reduced basal cAMP levels from 22 ± 2 to 17 ± 4 pmol·mg protein⁻¹·5 min⁻¹, which was not significantly different from control cells (Fig. 1). Addition of exogenous PGE₂ to the MDCT cells stimulated cAMP formation in control and indomethacin-treated cells (Fig. 1). These studies indicate that MDCT cells have prostaglandin receptor E₂ or E₄ subtypes that are

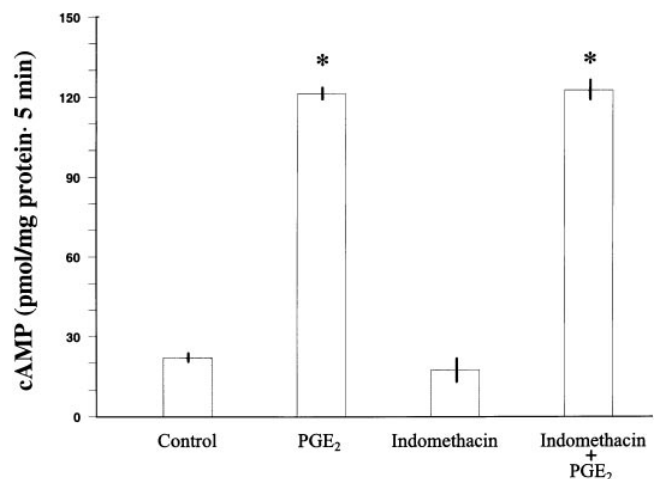
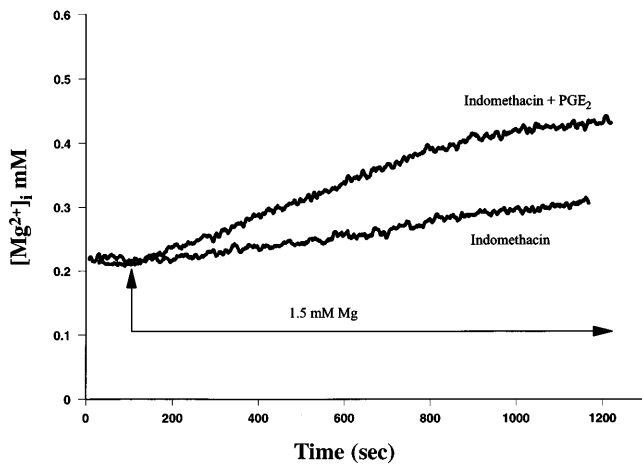


Fig. 1. Prostaglandin E₂ (PGE₂) stimulates cAMP production in mouse distal convoluted tubule (MDCT) cells. PGE₂, 10⁻⁷ M, was added 5 min prior to cAMP determinations according to techniques given in the METHODS. In those cells indicated, indomethacin, 5 µM, was added to the culture media 16 h prior to the cAMP measurements. Values are means ± SE for 4–5 confluent cell plates. **P* < 0.001, significantly different from the respective control values.

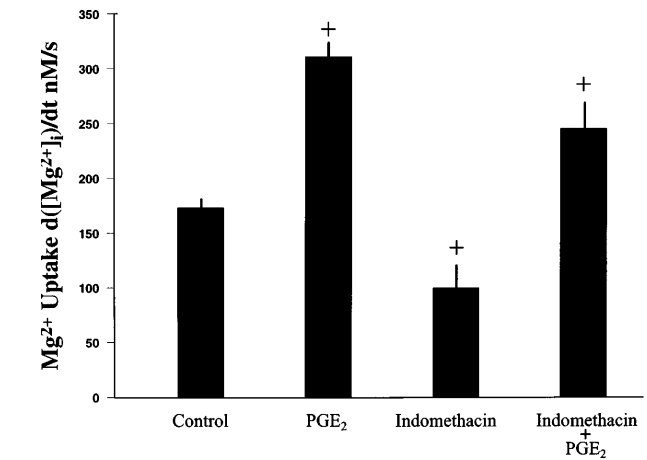
coupled to adenylate cyclase. Accordingly, PGE₂ may affect Mg²⁺ uptake into MDCT cells by stimulating cellular cAMP formation (7).

PGE₂ stimulates Mg²⁺ uptake in MDCT cells. To determine Mg²⁺ uptake, subconfluent MDCT monolayers were cultured in Mg²⁺-free medium for 16 h. These cells possessed a significantly lower [Mg²⁺]_i, 0.22 ± 0.01 mM, than cells cultured in normal media, 0.51 ± 0.02 mM. When the Mg²⁺-depleted MDCT cells were placed in a bathing solution containing 1.5 mM MgCl₂, intracellular Mg²⁺ concentration increased with time and leveled at a [Mg²⁺]_i value of 0.48 ± 0.07 mM (*n* = 9), which was similar to basal levels observed in normal cells. The average rate of refill, d([Mg²⁺]_i)/dt, measured as the change in [Mg²⁺]_i with time, was 173 ± 8 nM/s (*n* = 9 cells), as determined over the first 500 s following addition of 1.5 mM MgCl₂ (8). Mg²⁺ uptake is inhibited by a number of inorganic cations such as La³⁺ and Mn²⁺, but not Ca²⁺, and by organic channel blocks such as nifedipine (8). We used this approach to determine the effects of PGE₂ on Mg²⁺ uptake into MDCT cells. PGE₂, 10⁻⁷ M, stimulated Mg²⁺ entry by 80%, from 173 ± 8 nM/s to 311 ± 12 (*n* = 4, *P* < 0.001).

Distal tubule cells produce endogenous prostaglandins that may modulate basal Mg²⁺ uptake (4, 9). We used indomethacin to determine whether endogenous prostaglandin affects basal Mg²⁺ entry rate. MDCT cells were treated for 16 h with indomethacin, 5 μM, prior to determining d([Mg²⁺]_i)/dt, the Mg²⁺ uptake rate (Fig. 2). Basal Mg²⁺ entry was significantly diminished in those cells treated with indomethacin (100 ± 20 nM/s, *n* = 4) compared with control cells (173 ± 8 nM/s) (Fig. 3). These studies suggest that endogenous prostaglandins are formed in MDCT cells, which stimu-



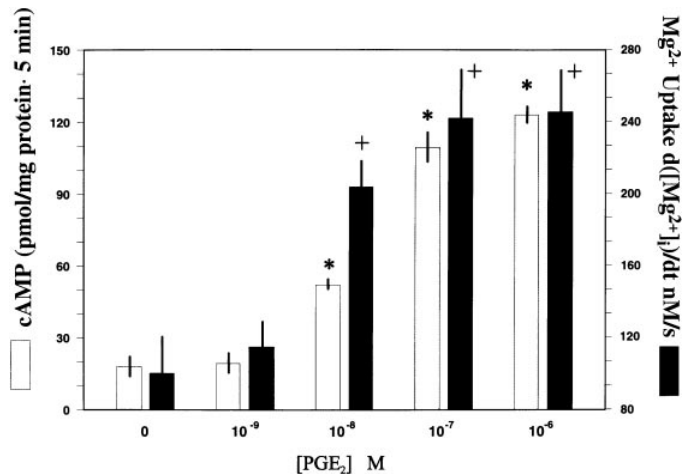
late basal Mg²⁺ uptake rate. PGE₂ stimulated Mg²⁺ uptake in indomethacin-treated cells by 56–73% (Fig. 3). *PGE₂ stimulates cAMP formation and Mg²⁺ uptake in a concentration-dependent manner.* In these experiments, we pretreated the MDCT cells with indomethacin for 20 min prior to cAMP determinations. PGE₂ increased cAMP syntheses in a concentration-dependent manner with a maximal stimulation at ~10⁻⁷ M (Fig. 4). PGE₂ added to the refill buffer solution also increased the rate of Mg²⁺ entry into Mg²⁺-depleted



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MDCT cells in a concentration-dependent manner. PGE₂, 10⁻⁷ M, increased the mean Mg²⁺ entry rate from 173 ± 8 to 241 ± 26 nM/s (*n* = 4), which represented a stimulation of 39 ± 4% above control values (Fig. 3). In all cases where measured, [Mg²⁺]_i returned to basal levels, 0.47 ± 0.05 mM, in PGE₂-treated cells, similar to control observations. The effect of PGE₂ on Mg²⁺ uptake was concentration dependent with maximal rate of stimulation at 10⁻⁶ M (248 ± 28 nM/s, *n* = 4) and half-maximal stimulation at a concentration ~10⁻⁸ M (Fig. 4). We have previously reported that dihydropyridines inhibit Mg²⁺ uptake into Mg²⁺-depleted MDCT cells (8). To determine whether PGE₂-induced Mg²⁺ entry is mediated through a dihydropyridine-sensitive pathway, we examined the effect of the channel blocker, nifedipine, on the changes in [Mg²⁺]_i following placement in the refill buffer solution containing 1.5 mM MgCl₂. The presence of 10⁻⁵ M nifedipine inhibited PGE₂-stimulated Mg²⁺ uptake, from 241 ± 26 to 24 ± 2 nM/s, indicating that this pathway is sensitive to channel blockers, supporting the notion that PGE₂-stimulated uptake is the same as the entry pathway observed in control cells (8).

PGE₂ stimulates Mg²⁺ uptake through multiple intracellular signaling pathways. Next, we determined the effect of PKA inhibition on PGE₂-stimulated Mg²⁺ uptake. Rp-cAMPS, a PKA inhibitor, was applied 5 min prior to performing Mg²⁺ uptake measurements (7). Rp-cAMPS inhibited basal Mg²⁺ entry rates (101 ± 8 nM/s, *n* = 4), as well as PGE₂-stimulated Mg²⁺ uptake (192 ± 15 nM/s, *n* = 4), suggesting that activation of PKA is involved with prostaglandin actions (Fig. 5). Pretreatment of MDCT cells with the PLC inhibitor, U-73122, inhibited Mg²⁺ uptake to 148 ± 4 nM/s (*n* = 4), whereas the PKC inhibitor, chelerythrine, diminished PGE₂-stimulated uptake by 51% (240 ± 9 nM/s, *n* = 4) (Fig. 6). The actions of these inhibitors are

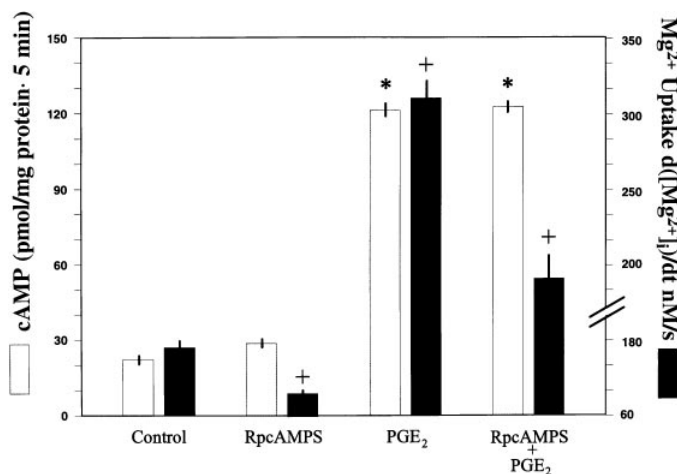


Fig. 5. PGE₂ stimulates Mg²⁺ uptake, in part, through cAMP-mediated pathways. Protein kinase A (PKA) inhibitor, Rp-cAMPS (Rp diastereoisomer of adenosine 3',5'-cyclic monophosphothionate), 0.5 μM, was added 5 min prior to the determination of Mg²⁺ uptake with and without PGE₂, 10⁻⁷ M. Values are means ± SE. **P* < 0.01, significance of cAMP formation rates with parathyroid hormone (PTH) vs. the respective control values. +*P* < 0.01, significance of for Mg²⁺ entry rates with PTH vs. the respective control values.

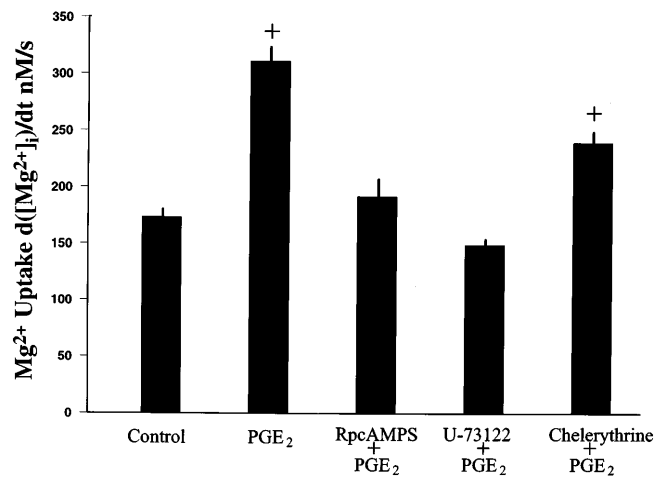


Fig. 6. PGE₂ stimulates Mg²⁺ uptake through multiple signaling pathways. Inhibitors for PKA (Rp-cAMPS), phospholipase C (U-73122), and PKC (chelerythrine), were added at concentrations of 0.5, 15, and 5 μM, respectively, 5 min prior to the addition of PGE₂. Values are means ± SE for 4–5 cells. +*P* < 0.01, significant difference from control uptake rates.

compared with the PKA inhibitor (Fig. 6). These results suggest that PGE₂ uses a number of intracellular signaling pathways to alter Mg²⁺ entry into MDCT cells.

Activation of the extracellular Mg²⁺/Ca²⁺-sensing mechanism inhibits PGE₂-stimulated cAMP generation and Mg²⁺ uptake. The MDCT possesses an extracellular Mg²⁺/Ca²⁺-sensing mechanism that upon activation with polyvalent cations such as Mg²⁺, Ca²⁺, or neomycin inhibits hormone-mediated cAMP generation and glucagon- and AVP-stimulated Mg²⁺ uptake (1, 2). To determine whether activation of Mg²⁺/Ca²⁺ sensing alters PGE₂ actions, we pretreated cells for 5 min with neomycin prior to the addition of PGE₂. Neomycin modestly inhibited PGE₂ stimulation of cAMP generation but completely inhibited PGE₂-stimulated Mg²⁺ uptake (Fig. 7). Extracellular Mg²⁺/Ca²⁺ sensing may modulate PGE₂-stimulated Mg²⁺ entry in distal tubule cells.

DISCUSSION

The distal tubule reabsorbs significant amounts of magnesium and plays an important role in determining the final urinary excretion rate (19). In contrast to more proximal segments of the nephron, distal magnesium transport processes are postulated to be active and transcellular in nature (18, 19). Hormonal control of magnesium transport in this segment provides the fine-tuning of renal conservation contributing to whole body magnesium balance. Micropuncture studies showed that Mg²⁺ reabsorption within the distal tubule is controlled by peptide hormones including PTH, glucagon, and calcitonin (3, 21). More recently, we have shown that glucagon and AVP stimulate Mg²⁺ entry in MDCT cells (7). The actions of these hormones are, in part, through cAMP-mediated pathways. In the present study, we show that PGE₂ stimulates Mg²⁺ uptake in MDCT cells, in part, through increases in cell cAMP

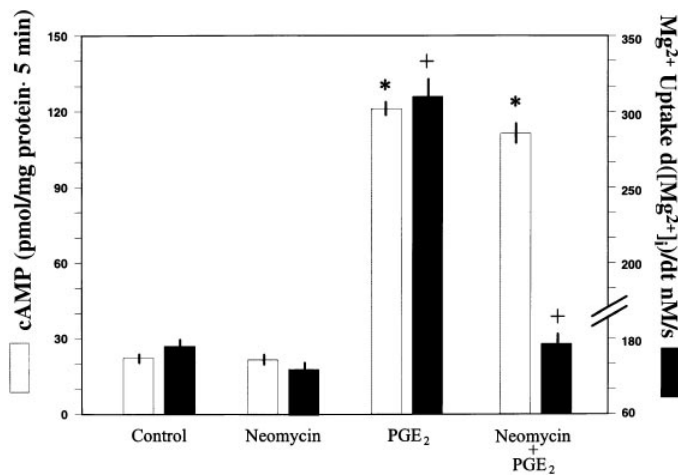


Fig. 7. Summary of the effects of Mg²⁺/Ca²⁺-sensing mechanism activation on PGE₂-stimulated cAMP formation and Mg²⁺ uptake. cAMP was measured by radioimmunoassay, and Mg²⁺ uptake, d([Mg²⁺]_i)/dt, was determined with 1.5 mM extracellular Mg²⁺ in absence and presence of neomycin, 50 μM, as indicated. Neomycin was added 5 min prior to addition of PGE₂, 10⁻⁷ M, and MgCl₂, 1.5 mM. Mg²⁺ uptake rate was determined over the initial 500 s following addition of PGE₂. Values are means ± SE for 3–5 cells. * *P* < 0.001, significant difference of cAMP determinations from the respective control values. + *P* < 0.001, significant difference of Mg²⁺ uptake determinations from the respective control values.

levels. We infer from these results that prostaglandins may modulate distal tubule magnesium transport and, together with peptide hormones, orchestrate renal magnesium conservation.

PGE₂ is the major arachidonate metabolite synthesized by cyclooxygenase in the mammalian kidney. It is synthesized along the length of the nephron including the convoluted segment of the distal tubule (4, 9). PGE₂ exerts a number of diverse physiological functions in the nephron, in part, through different receptor subtypes (5, 6). EP₁ and EP₃ subtypes mediate intracellular Ca²⁺ signaling and inhibition of adenylate cyclase, respectively, that result in inhibition of NaCl absorption within the thick ascending limb (27) and CCD (17) and AVP-stimulated water transport in the CCD (14, 22). EP₂ and EP₄ subtypes are coupled to adenylate cyclase, which upon stimulation, enhances transepithelial calcium transport in the rabbit CCD (29). Moreover, these receptors may be colocalized to the same cell type but polarized to apical or basolateral membranes (15, 22, 29). Van Baal et al. (29) have shown that apical and basolateral PGE₂ stimulate calcium absorption through EP₂ and/or EP₄ receptors, whereas activation of basolateral EP₃ receptors inhibits basal and hormone-stimulated calcium transport. In the present studies, we show that PGE₂ stimulates Mg²⁺ uptake, in part, through cAMP-mediated mechanisms, but we were unable to determine the polarization of receptors because the immortalized MDCT cells used here do not form tight junctions and are unlikely to be polarized (11). Accordingly, it is not known whether the PGE₂ effects in the MDCT cell line are due to luminal or basolateral prostaglandin.

On balance, prostaglandins are thought to have natriuretic actions by way of their actions on the thick

ascending limb and CCD (15, 27). Three clearance studies concluded that arachidonic acid metabolites inhibit tubular reabsorption of calcium and magnesium resulting in increased urinary excretion (10, 20, 23). Schneider et al. (23) infused PGE₂ into dog renal arteries and showed that calcium and magnesium excretion increased in association with a rise in urinary sodium excretion. Roman et al. (20) and Friedlander and Amiel (10) reported that meclofenamate or indomethacin infusion in rats decreased fractional magnesium excretion by ~40%. Again, the changes in urinary magnesium and calcium were associated with similar changes in sodium excretion. These observations are difficult to compare with the present ones because of associated changes in hemodynamics and filtration rates in the clearance studies. More recent identification of receptor subtypes may also explain the discrepancies of our results with those of earlier clearance studies. The present results are similar to those of van Baal et al. (29) performed in primary rabbit CCD cells. They reported that PGE₂ stimulated net apical-to-basolateral calcium transport in CCD cells grown to confluence on permeable supports. PGE₂ also stimulated cAMP formation in these cells, suggesting that PKA-dependent pathways were involved (29). However, in a preliminary report, these investigators reported that the changes in PGE₂-stimulated calcium transport were not directly associated with cAMP formation so that other signaling pathways may be present in rabbit CCD cells (16). Finally, van Baal et al. (29) have shown that primary CCD cells produce endogenous prostaglandins that affect basal calcium transport. Our studies indicate that PGE₂ may have important effects on Mg²⁺ entry within the immortalized mouse distal tubule cell line. The signaling pathways remain to be determined, but the evidence is that cAMP-mediated pathways are involved. However, our evidence also suggests that other signaling pathways may influence PGE₂ and peptide hormone responses. This notion is based on the observations that PLC and PKC inhibitors diminish Mg²⁺ uptake but also on the data where Mg²⁺ uptake is not directly associated with changes in intracellular cAMP accumulation (Fig. 7). Further studies are required to determine the intracellular signaling pathways of PGE₂ and the interactions of prostaglandins with hormone-mediated responses.

If prostaglandins stimulate magnesium absorption in the distal tubule, then what roles do they play in overall renal magnesium handling? We can speculate that an increase in PGE₂ results in diminished magnesium absorption within the thick ascending limb, increasing magnesium delivery to the distal tubule. From the present data, we infer that elevated PGE₂ levels would increase Mg²⁺ reabsorption within the distal convoluted tubule, limiting the urinary magnesium wasting that might otherwise occur. An example of this notion may be Bartter's disease. Bartter's syndrome is characterized by hypokalemia, metabolic alkalosis, hyperprostaglandin production, hyperreninemia, second-

ary hyperaldosteronism, and normal blood pressure (25). The evidence from clinical studies implicates defective salt transport in the thick ascending limb of the loop (25). Simon and colleagues (24, 25) have recently shown with linkage and mutational analysis that Na-2Cl-K cotransport, apical K⁺ conductance, or basolateral Cl⁻ conductance is defective. These alterations would be expected to decrease transepithelial voltage and passive Mg²⁺ reabsorption within the loop (17). It is surprising that Bartter's syndrome, a defect in loop absorption where the majority of filtered magnesium is reclaimed, is not more frequently associated with renal magnesium wasting. About one-fifth of Bartter's patients have abnormal magnesium concentrations, whereas patients with Gitelman's syndrome, due to a distal defect, uniformly demonstrate hypomagnesemia (19). Despite the high incidence of hypercalciuria in Bartter's patients, there is little effect on renal magnesium handling. Aberrant salt cotransport in the thick ascending limb would lead to defective magnesium and calcium absorption and increase delivery to the distal convoluted tubule. Although it remains to be determined why magnesium absorption in the distal convoluted tubule proceeds normally in most of these patients while calcium is excreted in the urine, elevated prostaglandin concentrations may stimulate distal Mg²⁺ reabsorption in Bartter's patients minimizing urinary magnesium excretion and the incidence of hypomagnesemia. The concerted actions of prostaglandins in the loop and distal tubule remain to be fully explored.

Extracellular Mg²⁺/Ca²⁺ sensing affects PGE₂-stimulated Mg²⁺ uptake in MDCT cells. Extracellular Mg²⁺/Ca²⁺ sensing within the distal tubule is important in renal electrolyte handling (19). We have reported that elevation of extracellular magnesium or calcium or the addition of the polyvalent cation, neomycin, completely inhibits peptide hormone-stimulated cAMP formation in MDCT cells (1, 2). Activation of Mg²⁺/Ca²⁺ sensing marginally inhibited PGE₂-mediated cAMP but completely inhibited PGE₂ stimulation of Mg²⁺ uptake increases in MDCT cells (Fig. 7). Hartle et al. (13) have reported that polyvalent cations inhibit PGE₁-stimulated cAMP production in MC3T3-E1 osteoblasts. Accordingly, elevation of extracellular Mg²⁺ and Ca²⁺ may have important effects on prostaglandin actions in many cell types including the renal epithelium.

In summary, PGE₂ stimulates Mg²⁺ entry into MDCT cells. The evidence indicates that these actions are, in part, dependent on cAMP-mediated intracellular signaling processes. However, as inhibitors of PLC and PKC also diminish PGE₂-stimulated Mg²⁺ entry, other pathways are likely involved in control of transport. Further studies are required to fully elucidate PGE₂-mediated signaling pathways and the interactions with other hormone responses. Although these studies determined Mg²⁺ entry into an established cell line, we infer from this data that prostaglandins may modulate renal magnesium handling by its actions within the distal convoluted tubule.

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Address for reprint requests: G. A. Quamme, Dept. of Medicine, Vancouver Hospital and Health Sciences Centre, Koerner Pavilion, 2211 Wesbrook Mall, Vancouver, British Columbia, Canada V6T 1Z3.

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