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β -Adrenergic agonists stimulate Mg^{2+} uptake in mouse distal convoluted tubule cells

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Kang, Hyung Sub, Dirk Kerstan, Long-Jun Dai, Gordon Ritchie, and Gary A. Quamme. β -Adrenergic agonists stimulate Mg^{2+} uptake in mouse distal convoluted tubule cells. *Am J Physiol Renal Physiol* 279: F1116–F1123, 2000.— β -Adrenergic agonists influence electrolyte reabsorption in the proximal tubule, loop of Henle, and distal tubule. Although isoproterenol enhances magnesium absorption in the thick ascending limb, it is unclear what effect, if any, β -adrenergic agonists have on tubular magnesium handling. The effects of isoproterenol were studied in immortalized mouse distal convoluted tubule (MDCT) cells by measuring cellular cAMP formation with radioimmunoassays and Mg^{2+} uptake with fluorescence techniques. Intracellular free Mg^{2+} concentration ($[Mg^{2+}]_i$) was measured in single MDCT cells by using microfluorescence with mag-fura-2. To assess Mg^{2+} uptake, MDCT cells were first Mg^{2+} depleted to 0.22 ± 0.01 mM by culturing in Mg^{2+} -free media for 16 h and then placed in 1.5 mM $MgCl_2$, and the changes in $[Mg^{2+}]_i$ 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 168 ± 11 nM/s. Isoproterenol stimulated Mg^{2+} entry in a concentration-dependent manner, with a maximal response of 252 ± 11 nM/s, at a concentration of 10^{-7} M, that represented a 50 \pm 7% increase in uptake rate above control values. This was associated with a sixfold increase in intracellular cAMP generation. Isoproterenol-stimulated Mg^{2+} uptake was completely inhibited with RpcAMPS, a protein kinase A inhibitor, and U-73122, a phospholipase C inhibitor, and partially blocked by RO 31-822, a protein kinase C inhibitor. Accordingly, isoproterenol-mediated Mg^{2+} entry rates involve multiple intracellular signaling pathways. Aldosterone potentiated isoproterenol-stimulated Mg^{2+} uptake (326 ± 31 nM/s), whereas elevation of extracellular Ca^{2+} inhibited isoproterenol-mediated cAMP accumulation and Mg^{2+} uptake, 117 ± 37 nM/s. These studies demonstrate that isoproterenol stimulates Mg^{2+} uptake in a cell line of mouse distal convoluted tubules that is modulated by hormonal and extracellular influences.

isoproterenol; propranolol; intracellular magnesium; fluorescence; intracellular 3',5'-cyclic adenosine monophosphate; protein kinase A; phospholipase C; protein kinase C; phorbol ester; aldosterone; extracellular calcium

RENAL NERVES INFLUENCE ELECTROLYTE reabsorption in the proximal tubule, the loop of Henle, and distal tubule

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(25). The proximal actions of β -adrenergic agonists have been extensively characterized; however, they also directly influence electrolyte transport in the distal tubule, but these effects have not been fully investigated. Among the many direct tubular actions, β -adrenergic agonists, such as isoproterenol, increase magnesium transport in the cortical segment of the thick ascending limb (cTAL) (2). The cTAL reabsorbs a significant portion of the filtered magnesium and as such plays an important role in controlling renal magnesium balance (35). Using RT-PCR, Elalouf et al. (14) showed that the β_1 -receptor was principally the cTAL receptor subtype but that some β_2 -receptor was also present (14). Both receptor subtypes were shown to be associated with increases in intracellular cAMP accumulation, suggesting that receptor-mediated signaling pathways, involving protein kinase A activity, may play a role in influencing electrolyte transport (8). The distal tubule, comprising the distal convoluted tubule, connecting tubule, and collecting tubule collecting ducts, also possess β -adrenergic-receptors (1, 7, 21, 29, 33, 36, 37). Isoproterenol influences distal tubular electrolyte handling by stimulating chloride absorption and K^+ , H^+ , and bicarbonate secretion in the collecting tubule (20, 23, 24, 28). Again, the receptor subtypes appear to encompass both β_1 - and β_2 -receptors that are associated with changes in intracellular cAMP and Ca^{2+} release (22, 36). Gesek and White (17) demonstrated, with RT-PCR and receptor-binding studies, that the immortalized mouse distal convoluted tubule cell line (MDCT) possesses both β_1 - and β_2 -receptor subtypes. In their study, isoproterenol stimulated cAMP formation and $^{22}Na^+$ uptake but not $^{45}Ca^{2+}$ entry into MDCT cells (17). Although β -adrenergic agents have been shown to increase magnesium absorption in the thick ascending limb, no experiments have been directed at the distal convoluted tubule. The distal convoluted tubule reabsorbs $\sim 10\%$ of the filtered magnesium and determines the final urinary excretion as there is no transport beyond in the collecting ducts (32).

In the present studies, we determined the effect of β -adrenergic agonists on Mg^{2+} uptake into immortal-

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ized MDCT cells. The MDCT cell line possesses many of the properties of the intact distal convoluted tubule. MDCT cells exhibit amiloride-inhibitable Na^+ transport and chlorothiazide-sensitive $NaCl$ cotransport (16). Amiloride and chlorothiazide also stimulate Mg^{2+} entry into these cells (11). Furthermore, parathyroid hormone (PTH), glucagon, and arginine vasopressin (AVP) increase Mg^{2+} entry in MDCT cells (9, 10, 12). Accordingly, we used this cell line to investigate the actions of isoproterenol on Mg^{2+} uptake in the distal convoluted tubule. The distal convoluted tubule has not been extensively studied because it is difficult to localize specific effects in intact superficial tubules or to perform in vitro perfusion experiments. As there is not an available isotope for magnesium to perform flux measurements, we determined Mg^{2+} entry into Mg^{2+} -depleted MDCT cells. The cells were depleted of intracellular Mg^{2+} by culturing in magnesium-free media for 16 h; then, the Mg^{2+} -depleted MDCT cells were placed in a medium containing 1.5 mM magnesium and the refill rate, $d([Mg^{2+}]_i)/dt$, was measured with microfluorescent studies by using mag-fura 2 (11). Mg^{2+} uptake rate is concentration dependent and selective for magnesium (11). 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 isoproterenol stimulates Mg^{2+} entry in MDCT cells, in part, through cAMP-dependent mechanisms.

METHODS

Materials. Basal DMEM and Ham's F-12 media were purchased from GIBCO (Gaithersburg, MD). Customized magnesium-free media were purchased from Stem Cell Technologies (Vancouver, BC). Fetal calf serum was from Flow Laboratories (McLean, VA). Mag-fura-2-acetoxymethyl ester (AM) was obtained from Molecular Probes (Eugene, OR). The protein kinase A inhibitor RpcAMPS, the phospholipase C inhibitor U-73122, and protein kinase C inhibitor RO 31-822 were purchased from Calbiochem, (San Diego, CA). Isoproterenol, propranolol, the adenylate cyclase inhibitor 2',5'-dideoxyadenosine (DDA), and phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA), and other materials were from Sigma (St. Louis, MO).

Cell culture. Distal convoluted tubule cells were isolated from mice, immortalized by Pizzonia (31), and functionally characterized as previously described by Friedman et al. (16). The MDCT cell line was grown on 60-mm plastic culture dishes (Corning Glass Works, Corning Medical and Scientific, Corning, NY) in DMEM-Ham's F-12, 1:1, media supplemented with 10% fetal calf serum, 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 fluorescent 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 nominally Mg^{2+} -free media (<0.01 mM) where indicated for 16–24 h before study. Other

constituents of the Mg^{2+} -free culture media were similar to the complete media. These media contained 0.1% BSA rather than the fetal calf serum.

Determination of cAMP concentration. Intracellular 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. After addition of isoproterenol, MDCT cells were incubated at 37°C for 5 min in the presence of 0.1 mM IBMX. 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 cAMP was measured with a radioimmunoassay kit (Diagnostic Products, Los Angeles, CA).

Cytoplasmic Mg^{2+} measurements. The coverslips with MDCT cells were mounted into a perfusion chamber, and intracellular free Mg^{2+} concentration ($[Mg^{2+}]_i$) was determined with the use of the Mg^{2+} -sensitive fluorescent dye mag-fura-2. The cell-permeant 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 μ M 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 K_2HPO_4 , 0.2 KH_2PO_4 , 1.0 $CaCl_2$, 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 subconfluent MDCT cells. The chamber (0.5 ml) was mounted on an inverted Nikon Diaphot-TMD microscope, with a Fluor \times 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 by 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.

$[Mg^{2+}]_i$ was calculated from the ratio of the fluorescence at the two excitation wavelengths as previously described by using a dissociation constant of 1.4 mM for the mag-fura-2- Mg^{2+} complex (9). The minimum and maximum ratios were determined for the cells at the end of each experiment using 20 μ M digitonin. The maximum ratio for mag-fura-2 was found by the addition of 50 mM $MgCl_2$, in the absence of Ca^{2+} , and the minimum ratio was obtained by removal of Mg^{2+} and the addition of 100 mM EDTA, pH 7.2. The change in $[Mg^{2+}]_i$ with time $\{d([Mg^{2+}]_i)/dt\}$ 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 Students' *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 \pm SE where indicated.

RESULTS

Isoproterenol stimulates Mg^{2+} uptake in MDCT cells. To determine Mg^{2+} uptake, subconfluent MDCT monolayers were cultured in magnesium-free medium for 16 h. These cells possessed a significantly lower $[Mg^{2+}]_i$, 0.22 ± 0.01 mM, than did cells cultured in normal media, 0.53 ± 0.02 mM. When the Mg^{2+} -depleted MDCT cells were placed in a bathing solution

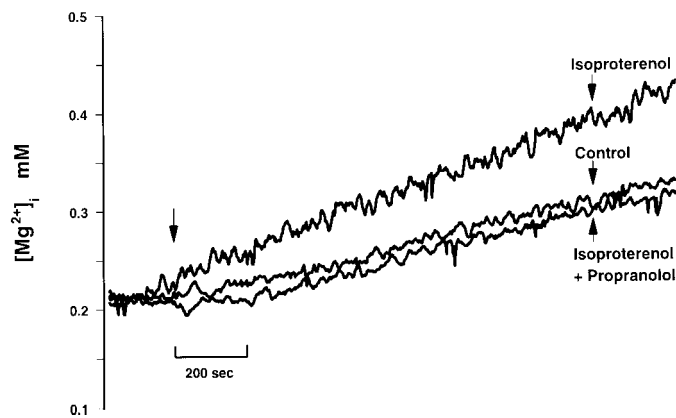


Fig. 1. Isoproterenol stimulates Mg^{2+} uptake in Mg^{2+} -depleted mouse distal convoluted tubule (MDCT) cells. Fluorescence studies were performed in buffer solutions in absence of external magnesium, and, where indicated, $MgCl_2$ (1.5 mM final concentration) was added to observe changes in intracellular 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 $MgCl_2$. Where indicated, 10^{-7} M isoproterenol was added to the buffer solution from a stock solution. Fluorescence was measured at 1 data point/s with 25-point signal averaging, and the tracing was smoothed according to methods previously described (11).

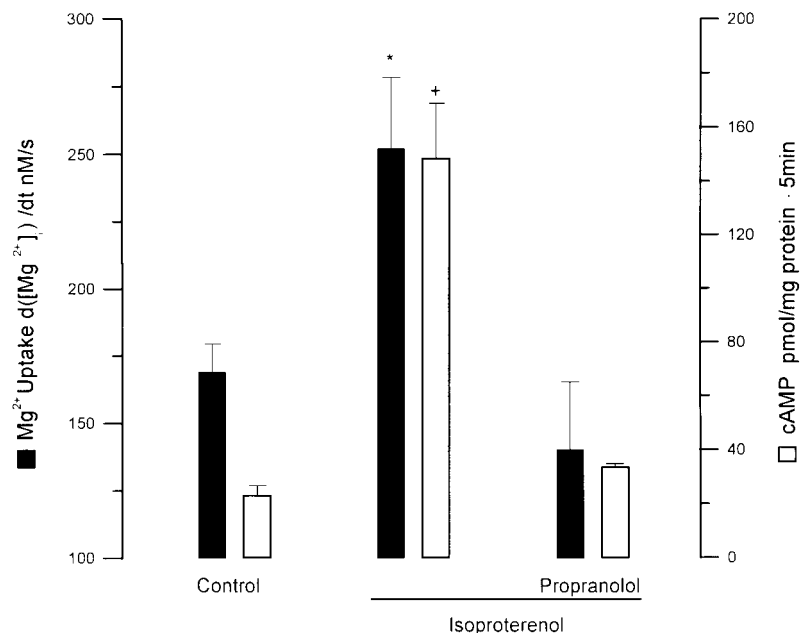
containing 1.5 mM $MgCl_2$, intracellular Mg^{2+} concentration increased with time and leveled at a $[Mg^{2+}]_i$ of 0.54 ± 0.03 mM, $n = 9$, which was similar to basal levels observed in normal cells (Fig. 1). The average rate of refill, $d([Mg^{2+}]_i)/dt$, measured as the change in $[Mg^{2+}]_i$ with time, was 169 ± 11 nM/s, $n = 9$ cells, as determined over the first 500 s after addition of 1.5 mM $MgCl_2$ (Fig. 2). We used this approach to determine the effects of β -adrenergic agonists on Mg^{2+} uptake into MDCT cells.

Isoproterenol added to the refill buffer solution increased the rate of Mg^{2+} entry into Mg^{2+} -depleted

MDCT cells (Fig. 1). Isoproterenol (10^{-7} M) increased the mean Mg^{2+} entry rate from 169 ± 11 to 252 ± 26 nM/s, $n = 6$, measured at $23^\circ C$, which represented a stimulation of $49 \pm 7\%$ above control values measured after 500 s (Fig. 2). Isoproterenol increased Mg^{2+} uptake so that $d([Mg^{2+}]_i)/dt$ was not linear, in some cases, over the 500-s interval, but this time frame provided a relative measurement for comparisons. In the cases when it was measured, $[Mg^{2+}]_i$ in isoproterenol-treated cells returned to basal levels, 0.48 ± 0.12 mM, similar to that in control observations. These experiments were repeated at $37^\circ C$: control, 163 ± 13 nM/s, $n = 4$ and isoproterenol, 272 ± 38 nM/s, $n = 3$. As these values were not significantly different from those measured at $23^\circ C$, the remaining studies were performed at room temperature. The effect of isoproterenol on Mg^{2+} uptake was concentration dependent, with the maximal rate of stimulation at 10^{-6} M, 264 ± 28 nM/s, $n = 4$ and half-maximal stimulation at a concentration of $\sim 10^{-8}$ M (Fig. 3). We have previously reported that dihydropyridines inhibit Mg^{2+} uptake into Mg^{2+} -depleted MDCT cells (11). To determine whether isoproterenol-induced Mg^{2+} entry is mediated through a dihydropyridine-sensitive pathway, we examined the effect on the changes in $[Mg^{2+}]_i$ of the channel blocker nifedipine after placement in the refill buffer solution containing 1.5 mM $MgCl_2$. The presence of 10^{-5} M nifedipine inhibited isoproterenol-stimulated Mg^{2+} uptake, 25 ± 12 nM/s, which was similar to that observed in control cells (11). These findings support the notion that isoproterenol-stimulated Mg^{2+} uptake is the same as the entry pathway observed in control cells.

Next, we determined the effect of propranolol, a specific β -adrenergic receptor antagonist, on isoproterenol-stimulated Mg^{2+} uptake (Fig. 2). Propranolol inhibited isoproterenol-stimulated uptake, 140 ± 25

Fig. 2. Isoproterenol stimulates intracellular cAMP accumulation and Mg^{2+} uptake in MDCT cells. Mg^{2+} uptake was determined by techniques outlined in Fig. 1. The rate of Mg^{2+} uptake $\{d([Mg^{2+}]_i)/dt\}$ was measured over the first 500 s after addition of 10^{-7} M isoproterenol and 1.5 mM $MgCl_2$. Cellular cAMP determinations were performed 5 min after the addition of isoproterenol. In these studies, 10^{-7} M propranolol, the β -adrenergic antagonist, was added 5 min before 10^{-7} M isoproterenol. Values are means \pm SE for 3–6 cells. *, +: $P < 0.01$ for Mg^{2+} entry rates and cAMP concentrations, respectively, vs. respective control values.



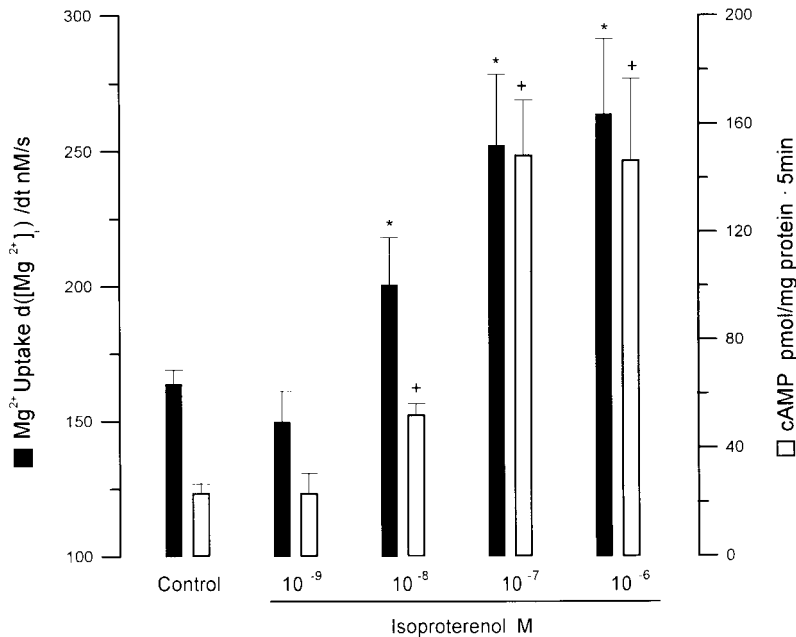


Fig. 3. Concentration dependence of isoproterenol stimulation of cAMP formation and Mg²⁺ entry in MDCT cells. The rate of Mg²⁺ influx, as determined by d([Mg²⁺]_i)/dt, was measured with the given isoproterenol concentrations by using fluorescence techniques performed according to that in Fig. 1. Values of d([Mg²⁺]_i)/dt were determined over the first 500 s of fluorescence measurements. Isoproterenol was added, at the concentrations indicated, 5 min before the measurement of cAMP in the presence of 3-isobutyl-1-methylxanthine. Values are means ± SE for 3–6 cells. *, +: P < 0.01 for Mg²⁺ entry rates and cAMP concentrations, respectively, vs. respective control values.

nM/s, n = 3, confirming that isoproterenol acts through β-adrenergic receptor-mediated mechanisms.

Isoproterenol stimulates Mg²⁺ uptake by cAMP-dependent protein kinase A- and phospholipase C-mediated intracellular signaling pathways. Gesek and White (17) reported that isoproterenol increased cAMP formation in MDCT cells but not intracellular Ca²⁺ concentration, suggesting that β-adrenergic receptors mediate transport functions, in part, by activating protein kinase A (17). As exogenous cAMP increases Mg²⁺ entry into MDCT cells, we determined the effects of isoproterenol on cAMP release in the MDCT cells used here (9). Isoproterenol (10⁻⁷ M) stimulated intracellular cAMP formation by about sixfold in MDCT cells (Fig. 3). Propranolol inhibited isoproterenol-mediated cAMP formation (Fig. 2). The concentration-dependent manner of isoproterenol-stimulated Mg²⁺ entry was associated with increases in intracellular cAMP formation (Fig. 3). Accordingly, isoproterenol may stimulate Mg²⁺ transport through cAMP-mediated signaling pathways.

To sort out some of the receptor-mediated signaling pathways, we used a number of selective enzyme inhibitors to determine the effect on isoproterenol-responsive Mg²⁺ uptake in MDCT cells. RpcAMPS or DDA, two different protein kinase A inhibitors, were applied 5 min before Mg²⁺ uptake measurements were performed (9). RpcAMPS and DDA inhibited the effects of isoproterenol on Mg²⁺ entry rates, 171 ± 25 nM/s, n = 3 and 148 ± 12 nM/s, n = 3, respectively, suggesting that activation of protein kinase A is involved in the actions of isoproterenol (Fig. 4). Pretreatment of MDCT cells with the phospholipase C inhibitor U-73122 also diminished isoproterenol-stimulated Mg²⁺ uptake, 143 ± 11 nM/s, n = 3, whereas the protein kinase C inhibitor RO 31-822 diminished isoproterenol-stimulated uptake by 36%, 208 ± 14 nM/s,

n = 3 (Fig. 4). These results suggest that isoproterenol alters Mg²⁺ entry into MDCT cells through cAMP-dependent protein kinase A- and phospholipase C-mediated signaling pathways; the results with U-73122 were equivocal.

As isoproterenol increases Mg²⁺ uptake in MDCT cells, in part, by RO 31-822-sensitive mechanisms, we

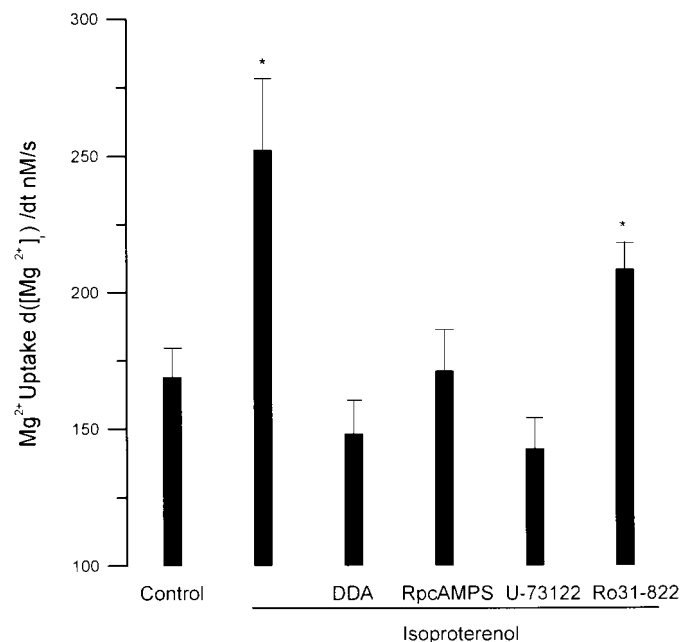


Fig. 4. Isoproterenol stimulates Mg²⁺ uptake through cAMP-protein kinase A- and phospholipase C-mediated signaling pathways. Inhibitors for protein kinase A (RpcAMPS, 0.5 μM) and 2'5'-dideoxyadenosine (DDA; 10 μM), phospholipase C (U-73122, 15 μM), and protein kinase C (RO 31-822, 0.1 μM) were added to Mg²⁺-depleted MDCT cells 5 min before the addition of 10⁻⁷ M isoproterenol. Values are means ± SE for 3–5 cells. *P < 0.01 vs. control uptake rates.

tested whether phorbol ester-induced protein kinase C activation changes basal Mg^{2+} entry or isoproterenol-stimulated uptake. The phorbol ester TPA had no detectable effect on basal Mg^{2+} uptake, 144 ± 16 nM/s, $n = 3$, but potentiated the effects of isoproterenol, 410 ± 38 nM/s, $n = 3$ (Fig. 5). Isoproterenol-stimulated intracellular cAMP accumulation was not altered with phorbol esters (Fig. 5). We infer from these studies that β -adrenergic agonists stimulated Mg^{2+} transport, in part, through protein kinase A-mediated pathways and these effects may be modulated by other intracellular signaling processes responsive to phorbol esters. In support of this speculation, RpcAMPS, an inhibitor of protein kinase A, abolished TPA-mediated potentiation of isoproterenol-stimulated Mg^{2+} entry, indicating that phorbol esters interact with the protein kinase A-signaling pathway beyond adenylate cyclase (Fig. 5).

Aldosterone potentiates isoproterenol-stimulated Mg^{2+} uptake in MDCT cells. We have previously shown that aldosterone, applied 16 h before experimentation, increases PTH-, glucagon- and AVP-mediated cAMP generation that, in turn, potentiates hormone-mediated Mg^{2+} uptake (12, 13). Short-term (10–15 min) aldosterone administration changes neither Mg^{2+} uptake (13) nor intracellular Ca^{2+} signaling: control, 104 ± 5 nM and aldosterone, 104 ± 6 nM ($n = 4$). Although the cellular mechanisms are not known, it has been speculated that aldosterone-induced proteins modulate receptor signaling in epithelial cells (30). In the present study, we determined whether pretreatment of MDCT cells with aldosterone for 16 h potentiates the acute actions of isoproterenol. Treatment of cells with aldosterone, for 16 h before study, did not significantly affect basal Mg^{2+} uptake but potentiated isoproterenol-stimulated Mg^{2+} entry. (Fig. 6). Aldosterone did not change the level of isoproterenol-mediated cAMP formation, suggesting that the actions are down-

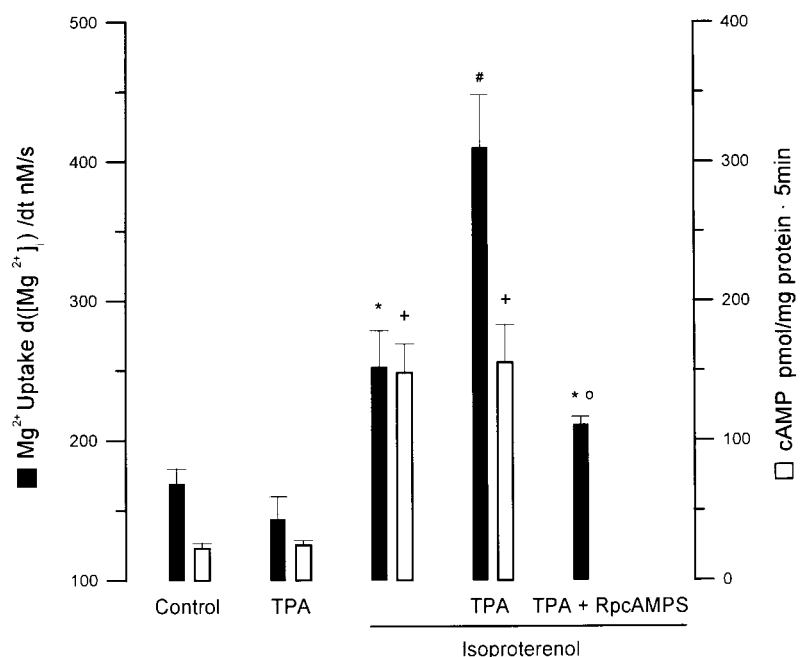
stream of the generation of this second message (Fig. 6).

Elevation of extracellular Ca^{2+} inhibits isoproterenol-stimulated cAMP generation and Mg^{2+} uptake. MDCT cells possess an extracellular Ca^{2+}/Mg^{2+} -sensing receptor, Ca_{sr} , 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 (3, 4). To determine whether activation of Ca_{sr} alters isoproterenol actions, we pretreated cells for 5 min with 5.0 mM $CaCl_2$ before the addition of isoproterenol. Elevation of extracellular Ca^{2+} did not have any effects on basal Mg^{2+} entry but abolished isoproterenol stimulation of cAMP generation and Mg^{2+} uptake (Fig. 7). Elevation of extracellular Ca^{2+} also inhibits aldosterone-potentiated, isoproterenol-stimulated cAMP formation and Mg^{2+} uptake (Fig. 7). The mechanisms by which Ca_{sr} inhibits isoproterenol actions remain unclear, but the receptor is coupled to $G\alpha_i$ proteins, which is consistent with the conclusion that isoproterenol responses in MDCT cells are dependent, in part, on cAMP-mediated signaling pathways.

DISCUSSION

Isoproterenol stimulates Mg^{2+} reabsorption in the distal tubule. In the present studies, we show that isoproterenol stimulates Mg^{2+} uptake in MDCT cells. The response is concentration dependent and involves selective β -adrenergic receptors as propranolol inhibited isoproterenol actions. As the MDCT cell line demonstrates the properties of the intact distal convoluted tubule, we infer that β -adrenergic innervations and neurally released catecholamines regulate distal magnesium conservation. Bailly et al. (2) have also reported that isoproterenol increases magnesium absorption in

Fig. 5. Phorbol ester potentiates isoproterenol-stimulated Mg^{2+} uptake. MDCT cells were incubated for 16 h in magnesium-free buffer solution. 12-*O*-tetradecanoylphorbol-13-acetate (TPA; 0.01 μ M), and RpcAMPS, 0.5 μ M, were added with and without 10^{-7} M isoproterenol, where indicated. Values are means \pm SE for 3–6 cells. *, +: $P < 0.01$ for Mg^{2+} entry rates and cAMP concentrations, respectively, compared with the respective control values. #: $P < 0.01$ for mean Mg^{2+} entry rate of TPA plus isoproterenol vs. isoproterenol alone and TPA+RpcAMPS vs. TPA alone, respectively.



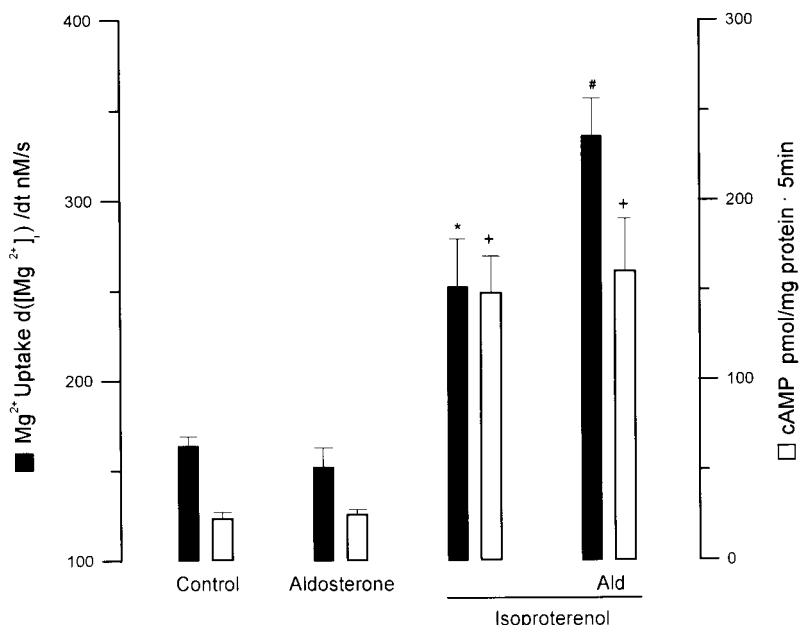


Fig. 6. Aldosterone potentiates isoproterenol-mediated cAMP generation and Mg²⁺ uptake. MDCT cells were incubated for 16 h in magnesium-free buffer solution containing 10⁻⁷ M aldosterone. Isoproterenol (10⁻⁷ M) was added where indicated, and cAMP was measured after 5 min in the presence of IBMX or Mg²⁺ uptake was determined after 500 s in 1.5 mM MgCl₂. Values are mean ± SE for 3–5 observations. *, +: P < 0.01 for mean Mg²⁺ entry rates and cAMP determinations, respectively, compared with the respective control values.

mouse cTAL. Accordingly, β-adrenergic agonists control magnesium reabsorption by its actions within both the thick ascending limb and the distal convoluted tubule.

Barajas et al. (5) demonstrated that the thick ascending limb and distal convoluted tubule have the greatest adrenergic innervation. The receptor subtypes within the distal tubule, including thick ascending limb segments, distal convoluted tubule, and collecting tubule, have been reported to be β₁- and β₂-adrenoreceptors (7, 14, 27, 29, 37). Using RT-PCR, Gesek and White (17) reported that MDCT cells possess β₁- and β₂-receptor mRNA, the DNA of which was identical to the reported mouse receptor sequences (17). Receptor-binding studies confirmed the membrane expression of these subtypes, and receptor-mediated cAMP accumu-

lation demonstrated functional presence in the immortalized mouse cell line. These studies with MDCT cells are consistent with those in the intact kidney (6, 7, 14). Gesek and White (17) showed that both β₁- and β₂-receptor subtypes enhanced Na⁺ uptake into MDCT cells. Bailey et al. (2) and Levine et al. (26) reported that β-adrenergic agonists stimulated Na⁺, Cl⁻, and passive Ca²⁺ and Mg²⁺ absorption in the cTAL, and other investigators have demonstrated that isoproterenol increases K⁺, H⁺, and bicarbonate secretion in the collecting tubule (19, 20, 23, 24, 28). Levine et al. (26) reported that isoproterenol diminishes bicarbonate reabsorption in the distal convoluted tubule (26). Gesek and White (17) demonstrated that isoproterenol stimulated ²²Na⁺ entry but had no effect on ⁴⁵Ca²⁺ uptake

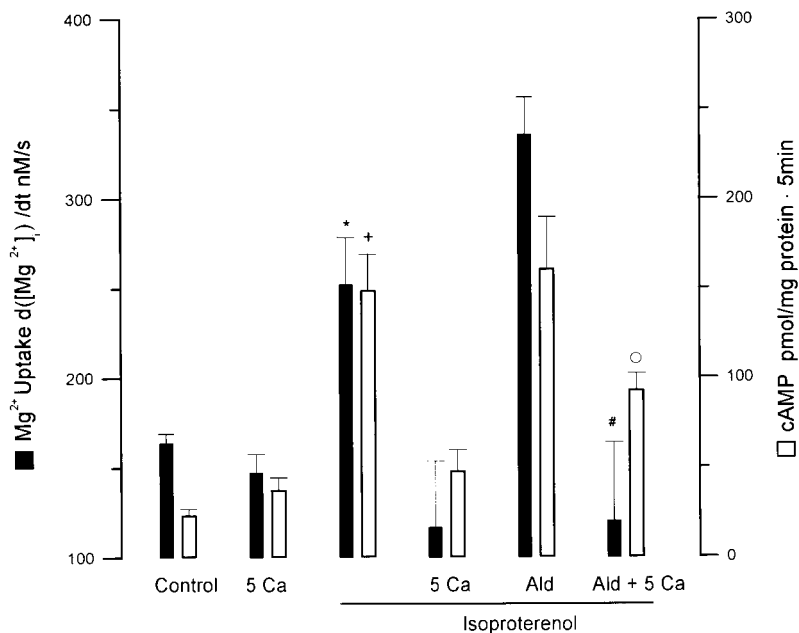


Fig. 7. Summary of the effects of extracellular Ca²⁺ on isoproterenol-stimulated cAMP formation and Mg²⁺ uptake. cAMP was measured by radioimmunoassay and d([Mg²⁺]_i)/dt was determined with 1.5 mM extracellular Mg²⁺ in the absence and presence of 5.0 mM CaCl₂ as indicated. CaCl₂ was added 5 min before the addition of 10⁻⁷ M isoproterenol. In those studies indicated, MDCT cells were treated with 10⁻⁷ M aldosterone 16 h before experimentation. Mg²⁺ uptake rate was determined over the initial 500 s after addition of isoproterenol. Values are mean ± SE for 3–5 cells. +, *: P < 0.001 for cAMP determinations and Mg²⁺ uptake, respectively, vs. respective control values. Ald, aldosterone; Ca, CaCl₂. #, #: P < 0.001 for cAMP determinations and Mg²⁺ uptake of Ald + 5 Ca vs. Ald alone, respectively.

in MDCT cells. These reports indicate that both β_1 -receptors, principally activated by neurally released norepinephrine, and β_2 -receptors, stimulated by circulating epinephrine, have direct effects on electrolyte transport in the distal tubule including the thick ascending limb, distal convoluted tubule, and collecting tubule.

The cellular mechanisms by which β -adrenergic agents influence distal electrolyte transport are not fully understood. In most tissues, the major route for β -agonist effects is the stimulation of adenylate cyclase and increases in intracellular cAMP accumulation (6, 18). The evidence is that β -adrenergic receptors directly influence epithelial transport, in part, through $G\alpha$ -coupled proteins (8, 18, 19, 22, 27, 34). Again, Gesek and White (17) have carefully characterized some of the receptor-mediated signaling pathways in the immortalized MDCT cell line. They showed that β_1 - and β_2 -receptor subtypes increase intracellular cAMP accumulation but not intracellular transient Ca^{2+} signaling in MDCT cells. This response is unlike those observed in rat collecting ducts, where calcium signals are associated with β -agonist stimulation of cAMP (27). However, in support of the observations of Gesek and White (17), we have shown that isoproterenol does not increase intracellular Ca^{2+} in MDCT cells so that transient Ca^{2+} signaling is not likely involved with isoproterenol-mediated Mg^{2+} uptake (17, data not shown). The notion that adrenergic agonists act through cAMP-dependent mechanisms is persuasive; first, isoproterenol stimulates cAMP accumulation and cAMP enhances Mg^{2+} entry (9). Second, inhibition of cAMP-dependent protein kinase A abolishes isoproterenol-stimulated Mg^{2+} uptake (Fig. 4). Third, aldosterone potentiates isoproterenol-mediated cAMP concentrations that are associated with greater Mg^{2+} uptake (Fig. 6). Finally, elevation of extracellular Ca^{2+} diminishes isoproterenol-induced cAMP and Mg^{2+} transport (Fig. 7). However, receptor-mediated cAMP-protein kinase A activity is not the full explanation as phospholipase C and protein kinase C inhibition also mitigate hormone-stimulated Mg^{2+} uptake (Fig. 4). Furthermore, phorbol esters potentiate isoproterenol-mediated Mg^{2+} uptake without changing cAMP concentrations (Fig. 5). The intracellular mechanisms involved with isoproterenol responses are yet to be fully explored.

Aldosterone potentiates isoproterenol-stimulated Mg^{2+} entry in MDCT cells. Mineralocorticoid hormones stimulate NaCl cotransport, Na^+ conductance, and Na^+ pump activity in the distal convoluted tubule (27). Aldosterone also modulates hormone-responsive Mg^{2+} transport into MDCT cells (13). Incubation of aldosterone, for 16 h before determination of Mg^{2+} uptake, failed to have any effect on basal magnesium transport; however, pretreatment of MDCT cells with aldosterone potentiated isoproterenol-stimulated Mg^{2+} entry (Fig. 6). This was not associated with potentiation of hormone-mediated cAMP release in the aldosterone-treated MDCT cells, suggesting that the aldosterone actions were either downstream from the generation of this second messenger or involve the induction of other

signaling pathways (Fig. 6). The prominent mechanism of steroids, which operate through nuclear receptors, is to control transcriptional regulation, expression, and posttranslational targeting of heterotrimeric G proteins (30). Regulation of G protein subunits by steroid hormones has been studied in a variety of systems. Changes in the levels of expression of G protein subunits in adrenalectomized animals reflect changes in subunit mRNA, suggesting that adrenocorticoids activate genes encoding $G\alpha_s$, $G\alpha_i$, $G\beta$, $G\gamma$, and phospholipase C (30). Other aldosterone-induced proteins may also be involved (38). These studies indicate that distal magnesium transport is regulated at two levels: first by membrane-receptor signaling and, second, by nuclear transcriptional expression of receptor subunits.

Extracellular Ca^{2+} affects isoproterenol-stimulated Mg^{2+} uptake in MDCT cells. Ca_{sr} within the distal tubule is important in the control of renal electrolyte handling (3). We have reported that elevation of extracellular Ca^{2+} , Mg^{2+} , or the addition of the polyvalent cation neomycin, completely inhibits peptide hormone-stimulated cAMP formation and glucagon- and AVP-stimulated increases in Mg^{2+} uptake in MDCT cells (3, 4). Activation of Ca_{sr} inhibits isoproterenol-mediated cAMP and isoproterenol stimulation of Mg^{2+} uptake in MDCT cells (Fig. 7). The extracellular Ca^{2+} - and Mg^{2+} -sensing mechanisms provide a negative feedback loop to diminish the renal conserving actions of hormones such as β -agonists.

Role of β -adrenergic agonists on renal magnesium reabsorption. The loop of Henle reabsorbs ~70% of the filtered magnesium. Isoproterenol increases magnesium absorption within the thick ascending limb (2). However, the distal convoluted tubule reabsorbs significant amounts of magnesium and plays an important role in determining the final urinary excretion rate (32). In contrast to more proximal segments of the nephron, distal magnesium transport processes are postulated to be active and transcellular in nature (35). Hormonal control of magnesium transport in this segment provides the fine-tuning of renal conservation, contributing to whole body magnesium balance. In the present study, we show that isoproterenol stimulates Mg^{2+} uptake in MDCT cells, in part, through increases in cellular cAMP levels. We infer from these results that β -adrenergic agonists may modulate distal tubule magnesium transport and, together with its actions within the loop, regulate renal magnesium conservation.

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