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Aldosterone potentiates hormone-stimulated Mg^{2+} uptake in distal convoluted tubule cells

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Dai, Long-Jun, Gordon Ritchie, Brian Bapty, and Gary A. Quamme. Aldosterone potentiates hormone-stimulated Mg^{2+} uptake in distal convoluted tubule cells. *Am. J. Physiol.* 274 (*Renal Physiol.* 43): F336–F341, 1998.—The distal convoluted tubule reabsorbs significant amounts of filtered magnesium that is under hormonal control. In this study, we describe the effects of aldosterone on Mg^{2+} uptake in an immortalized mouse distal convoluted tubule (MDCT) cell line. Intracellular free Mg^{2+} concentration ($[Mg^{2+}]_i$) was determined on single MDCT cells using microfluorescence with mag-fura 2. To determine Mg^{2+} entry rate into MDCT cells, they were first Mg^{2+} depleted ($[Mg^{2+}]_i$, 0.22 ± 0.01 mM) by culturing in Mg^{2+}-free media for 16 h and then placed in 1.5 mM $MgCl_2$. The rate of change in $[Mg^{2+}]_i$ as measured as a function of time, $d([Mg^{2+}]_i)/dt$, was 164 ± 5 nM/s in control cells. We have shown that glucagon or arginine vasopressin (AVP) stimulates Mg^{2+} entry by 63% and 15%, respectively. Incubation of MDCT cells with aldosterone for 16 h did not change the rate of Mg^{2+} uptake (172 ± 8 nM/s). However, aldosterone potentiated glucagon- and AVP-stimulated Mg^{2+} uptake rate up to 330 ± 39 and 224 ± 6 nM/s, respectively. Aldosterone also potentiated glucagon- and AVP-induced intracellular cAMP accumulation in a concentration-independent manner. As cAMP stimulates Mg^{2+} entry in MDCT cells, it is inferred that aldosterone may stimulate Mg^{2+} uptake through intracellular signaling pathways involving cAMP. The actions of aldosterone were dependent on de novo protein synthesis, as pretreatment of the cells with cycloheximide inhibited aldosterone potentiation of hormone stimulation of Mg^{2+} uptake and cAMP accumulation. These studies with MDCT cells suggest that aldosterone may modulate the effects of hormones acting within the distal convoluted tubule to control magnesium absorption.

intracellular magnesium; fluorescence; glucagon; arginine vasopressin; adenosine 3′,5′-cyclic monophosphate

THE DISTAL TUBULE of the nephron normally reabsorbs ~10% of the filtered magnesium (23). As there is little or no magnesium beyond the distal tubule, this segment is important in determining renal magnesium balance. Magnesium absorption within this nephron segment is influenced by a number of hormones including glucagon and arginine vasopressin (AVP) (23). Using an immortalized mouse distal convoluted cell (MDCT) line, we have shown that glucagon and AVP stimulates Mg^{2+} uptake, suggesting that among the segments composing the distal tubule, the convoluted portion is a site of hormone action (2). The distal convoluted segment also possesses mineralocorticoid receptors (7, 9, 10, 15). Although there is some dispute concerning their functional purpose within this segment (14, 21), there is evidence that mineralocorticoid hormones enhance the expression of both Na^+ conductance and NaCl cotransport in distal convoluted tubule cells (1, 27). Accordingly, mineralocorticoids may alter Mg^{2+} entry by acting through changes in ionic transport (4). Alternatively, mineralocorticoids may affect hormone-mediated control of Mg^{2+} transport within the distal tubule. Doucet et al. (6) have shown that adrenalectomy reduced glucagon-stimulated adenylate cyclase activity in thick ascending limbs; this reduced cAMP response was prevented with aldosterone administration (6). Other investigators have shown that AVP-sensitive adenylate cyclase is diminished in rat and rabbit collecting tubules harvested from adrenalectomized animals (8, 13, 20, 26). Accordingly, mineralocorticoids could alter hormonal control of transport in the distal tubule through its actions on receptor-mediated signaling pathways.

In an accompanying report (2), we reported that glucagon and AVP increases cAMP accumulation in MDCT cells. As cAMP also stimulates Mg^{2+} entry, these hormones may act, in part, through cAMP-mediated responses. It was therefore of interest to examine the effects of aldosterone on Mg^{2+} transport in distal convoluted tubule cells and the interactions with hormone-stimulated Mg^{2+} uptake. In the present studies, it was shown that aldosterone potentiates glucagon- and AVP-stimulated intracellular cAMP accumulation and Mg^{2+} uptake in MDCT cells. The potentiation of hormone actions by mineralocorticoids may have significant effects on renal magnesium balance.

METHODS

Materials. Basal Dulbecco's minimal essential medium (DMEM) and Ham's F-12 media were purchased from GIBCO. Customized Mg^{2+}-free media were purchased from Stem Cell Technologies (Vancouver, BC, Canada). Fetal calf serum was from Flow Laboratories (McLean, VA). Mag-fura 2-AM was obtained from Molecular Probes (Eugene, OR). Glucagon, AVP, aldosterone, and other materials were from Sigma Chemical (St. Louis, MO).

Cell culture. Distal convoluted tubule cells were isolated from mice, immortalized, and characterized as previously described (12, 17). 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% CO_2-95% air at 37°C. For the fluorescence studies, confluent cells were washed three times with phosphate-buffered saline containing 5 mM ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid, 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, the

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cells were cultured in Mg^{2+}-free media (<0.01 mM) for 16–24 h prior to study. Other constituents of the Mg^{2+}-free were similar to the complete media. The Mg^{2+}-free media contained 0.2% bovine serum albumin rather than fetal calf serum.

Cytosolic Mg^{2+} measurements. Coverslips 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 (18). The cell-permeant acetoxymethyl ester (AM) form of the dye was dissolved in dimethyl sulfoxide 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 K_2HPO_4, 0.2 KH_2PO_4, 1.0 $CaCl_2$, 5 glucose, and 20 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid-tris(hydroxymethyl)aminomethane (HEPES-Tris), at pH 7.4. The MDCT cells were incubated for a further 20 min to allow for complete deesterfication and washed once before measurement of fluorescence.

Epifluorescence microscopy was used to monitor changes in the mag-fura 2 fluorescence of the MDCT cells. The chamber 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 (Deltascan; Photon Technologies, Princeton, NJ) with excitation at 335 and 385 nm (chopper speed set at 100 Hz/s) and emission at 505 nm. All experiments were performed at 23°C. Solution changes were made without an interruption in recording.

The $[Mg^{2+}]_i$ was calculated from the ratio of the fluorescence at the two excitation wavelengths as described using a dissociation constant (K_d) of 1.4 mM for the mag-fura 2 · Mg^{2+} complex (18). 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 $MgCl_2$ 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.

Determination of intracellular cAMP concentrations. cAMP was determined in confluent MDCT cell monolayers cultured in 24-well plates in DMEM-F12 media without fetal calf serum. After addition of various hormones, MDCT cells were incubated at 37°C for 5 min in the presence of 0.1 mM 3-isobutyl-1-methylxanthine. The cAMP was extracted with 5% trichloroacetic acid and further extracted with ether acidified with 0.1 N HCl. The aqueous phase was dried and dissolved in Tris-EDTA buffer, and cAMP was measured with a radioimmunoassay kit (Diagnostic Products, Los Angeles, CA).

Statistical analysis. Representative tracings of fluorescence intensities are given, and significance was determined by Tukey's analysis of variance. A probability of $P < 0.05$ was taken to be statistically significant. All results are means ± SE where indicated.

RESULTS

Mg^{2+} entry into MDCT cells. Since 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. Subconfluent MDCT monolayers were cultured in Mg^{2+}-free medium for 8–16 h. These cells possessed a significantly lower and reproducible $[Mg^{2+}]_i$, 0.22 ± 0.01 mM, as indicated in Fig. 1. When the Mg^{2+}-depleted MDCT

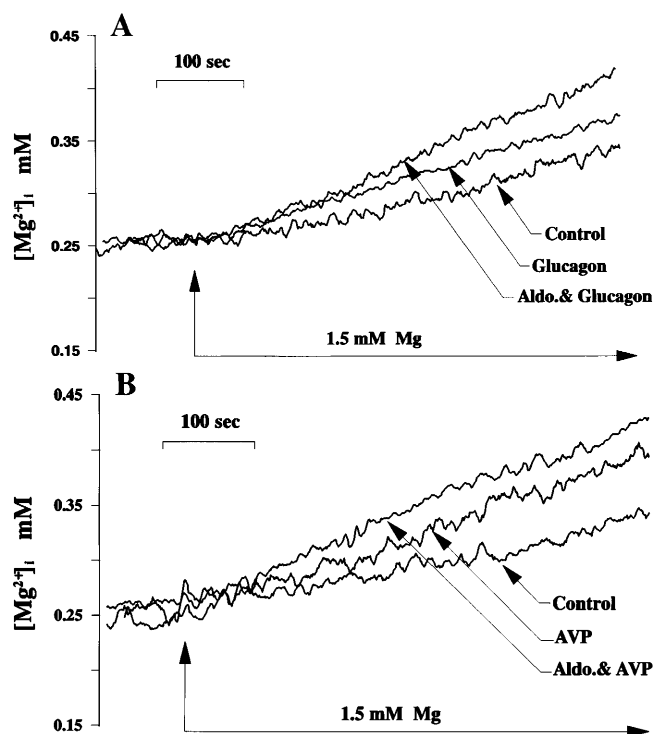

Fig. 1. Aldosterone potentiates glucagon- and arginine vasopressin (AVP)-stimulated Mg^{2+} uptake in Mg^{2+}-depleted mouse distal convoluted tubule (MDCT) cells. Confluent MDCT cells were cultured in Mg^{2+}-free media (<0.01 mM) for 16–20 h. Fluorescence studies were performed in buffer solutions in absence of Mg^{2+}, and, as indicated, $MgCl_2$ (1.5 mM final concentration) was added to observe changes in intracellular Mg^{2+} concentration ($[Mg^{2+}]_i$). 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 $MgCl_2$. Glucagon, 10^{-7} M (*A*), and AVP, 3×10^{-7} M (*B*), were added to this buffer solution where indicated. Where indicated, MDCT cells were treated with aldosterone (Aldo), 10^{-7} M, for 16 h prior to fluorescence. Fluorescence was measured at 1 data point/s with 25-point signal averaging, and tracing was smoothed according to methods previously described (18).

cells were placed in a bathing solution containing 1.5 mM $MgCl_2$, $[Mg^{2+}]_i$ increased with time and leveled at a $[Mg^{2+}]_i$ value of 0.52 ± 0.02 mM ($n = 6$), which was similar to normal cells (0.53 ± 0.03 mM, $n = 6$). The rate of refill, $d([Mg^{2+}]_i)/dt$, measured as the change in $[Mg^{2+}]_i$ with time, was 164 ± 5 nM/s ($n = 6$), as determined over the first 500 s following addition of magnesium.

In an accompanying report (2), we have shown that glucagon and AVP stimulate Mg^{2+} uptake into Mg^{2+}-depleted MDCT cells in a concentration-dependent fashion. Maximal concentration of glucagon, 10^{-6} M, and AVP, 3×10^{-7} M, enhanced mean Mg^{2+} uptake rates by 273 ± 6 and 189 ± 6 nM/s, respectively. Hormone-stimulated uptake was inhibited by nifedipine and was not affected by pretreatment with the protein synthesis inhibitor, cycloheximide. It was concluded that hormonal actions were through activation of putative Mg^{2+} channels that was independent of de novo protein synthesis.

Aldosterone potentiates hormone-stimulated Mg^{2+} uptake. Next, we determined the effects of mineralocorticoids on Mg^{2+} entry into distal convoluted tubule cells.

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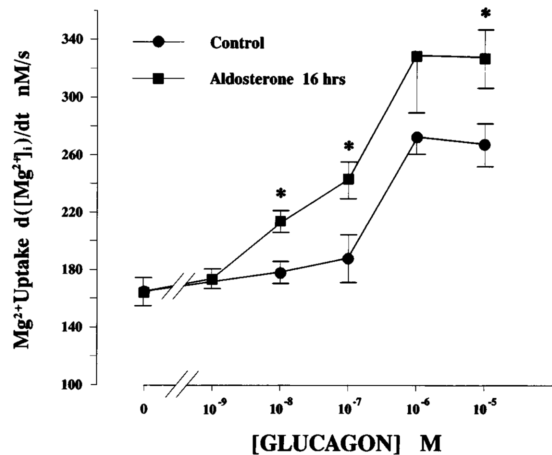

Fig. 2. Aldosterone potentiates glucagon-stimulated Mg^{2+} entry in MDCT cells. MDCT cells were incubated with and without aldosterone, 10^{-7} M, for 16 h prior to determination of Mg^{2+} entry. Rate of Mg^{2+} uptake, $d([Mg^{2+}]_i)/dt$, was determined over the initial 500 s following addition of the hormone and $MgCl_2$. Glucagon was added at concentrations indicated. Values without aldosterone are from Ref. 2. Values are means ± SE for 3–6 cells. * $P < 0.05$, significantly different for Mg^{2+} uptake with glucagon vs. aldosterone + glucagon.

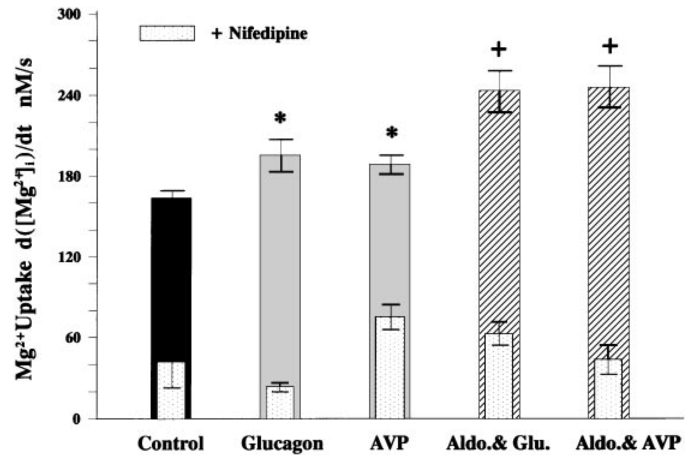

Fig. 4. Aldosterone potentiation of hormone-stimulated Mg^{2+} entry is through dihydropyridine-sensitive pathways. MDCT cells were treated with aldosterone (Aldo), 10^{-7} M, for 16 h prior to determining hormone-responsive Mg^{2+} uptake. Nifedipine, 10 µM, was added with the 1.5 mM $MgCl_2$ refill solution. Values are means ± SE for 3–6 cells. *Significantly different from control values. +Significantly different for aldosterone plus hormone vs. respective glucagon (Glu) or AVP values.

The addition of aldosterone, 10^{-7} M, 20 min prior to the measurement of Mg^{2+} entry did not alter the rate of Mg^{2+} uptake (189 ± 21 nM/s, n = 3). Incubation of MDCT cells with aldosterone, 10^{-7} M, for 16 h prior to determination of Mg^{2+} entry also had no effect on basal Mg^{2+} uptake (172 ± 8 nM/s, n = 3). However, pretreatment of MDCT cells with aldosterone potentiated both glucagon- and AVP-stimulated Mg^{2+} uptake rates (Fig. 1). Hormone-stimulated Mg^{2+} refill was enhanced with aldosterone, but aldosterone treatment had no effect on the final $[Mg^{2+}]_i$ attained following refill; this first concentration was not different from control values (0.52 mM). Furthermore, aldosterone potentiated glucagon and AVP at all hormone concentrations above 10^{-9} M (Figs. 2 and 3). The glucagon concentration required to produce half-maximal stimulation of Mg^{2+} uptake in aldosterone-treated cells was ~5×10^{-7} M, which was not different from control cells. Unlike glucagon, AVP stimulates Mg^{2+} uptake after a latent period of 5–10 min (Fig. 1*B*). Pretreatment of cells with aldosterone appeared to shorten the duration of this latent period (Fig. 1*B*). The AVP concentrations for half-maximal stimulated Mg^{2+} uptake was not changed (Fig. 3).

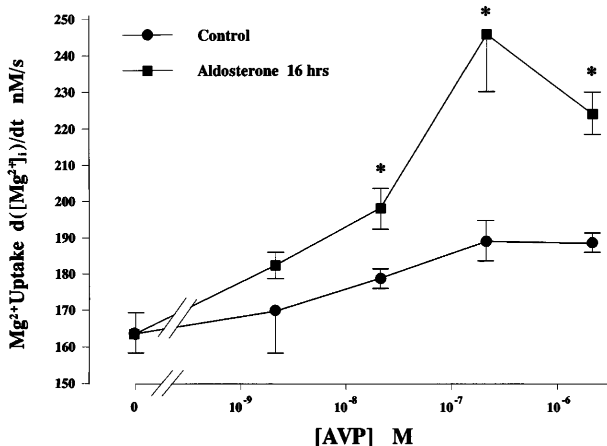

Fig. 3. Aldosterone potentiates AVP-stimulated Mg^{2+} uptake in MDCT cells. Fluorescence determinations were performed as described in Fig. 1. AVP was added at concentrations indicated. Cells were incubated with and without aldosterone, 10^{-7} M, for 16–20 h prior to uptake measurements. Mg^{2+} uptake rates were determined 6 min following addition of AVP. Tracings are representative of 4–6 cells. * $P < 0.05$, significantly different for Mg^{2+} uptake with AVP vs. aldosterone + AVP.

Mg^{2+} uptake in MDCT cells is sensitive to the dihydropyridine channel blockers such as nifedipine (4). Nifedipine also blocks glucagon- and AVP-stimulated Mg^{2+} entry (2). Accordingly, if aldosterone potentiation activates hormone-stimulated uptake through these pathways, then nifedipine should block this response. Figure 4 shows that aldosterone-potentiated, hormone-stimulated Mg^{2+} uptake is sensitive to nifedipine, suggesting that Mg^{2+} entry is being affected.

Aldosterone potentiates hormone-stimulated cAMP accumulation in MDCT cells. Evidence has been given that mineralocorticoids may increase the coupling effi-

Table 1. *Aldosterone potentiates hormone stimulation of cAMP accumulation in MDCT cells*

	cAMP Release, pmol·mg protein^{-1}·5 min^{-1}			
	Glucagon	AVP	PTH	Calcitonin
Control				
19 ± 1 (5)	105 ± 5* (4)	71 ± 2* (5)	56 ± 2* (5)	60 ± 0.1* (4)
Aldosterone				
26 ± 4 (4)	251 ± 32† (3)	139 ± 14† (4)	153 ± 26† (3)	286 ± 29† (3)

Values are means ± SE; number of experiments is in parentheses. Hormones, glucagon (10^{-7} M), AVP (10^{-8} M), parathyroid hormone (PTH, 10^{-7} M), and calcitonin (10^{-7} M), were added 5 min prior to cAMP determinations. Where indicated, aldosterone (10^{-7} M) was added to the culture media 16 h prior to cAMP measurements (measured as pmol·mg protein^{-1}·5 min^{-1}). * $P < 0.01$, significant vs. control values. † $P < 0.01$, significant for hormone vs. aldosterone + hormone for each of the respective hormones.

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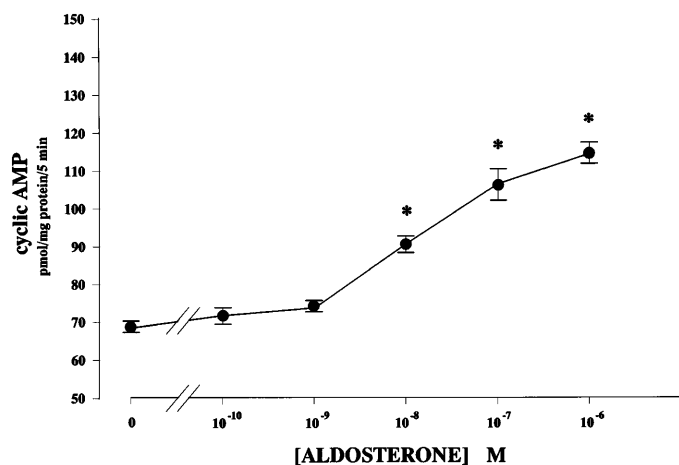

Fig. 5. Aldosterone potentiates glucagon-stimulated intracellular cAMP accumulation in a concentration-dependent manner. MDCT cells were incubated for 16 h in Mg^{2+}-free buffer solution containing the indicated aldosterone concentrations. Glucagon, 10^{-7} M, was added, and cAMP was measured following 5 min in presence of 3-isobutyl-1-methylxanthine. Values are means ± SE for 3–5 observations. * $P < 0.05$, significantly different from control values.

ciency of hormone receptors through G proteins to adenylate cyclase (6). Accordingly, we determined hormone-stimulated cAMP release in MDCT cells pretreated with aldosterone. Aldosterone did not have any effect on basal cAMP concentrations in control cells (Table 1). However, aldosterone potentiated cAMP accumulation in response to glucagon and AVP. Aldosterone also enhanced cAMP release by parathyroid hormone and calcitonin; accordingly, potentiation of hormone-mediated cAMP production by mineralocorticoids may be a general phenomenon (Table 1). These results indicate that mineralocorticoids increase receptor-mediated cAMP release, which may provide the basis for potentiation of Mg^{2+} uptake in MDCT cells.

Aldosterone potentiates glucagon-stimulated cAMP release in a concentration-dependent manner (Fig. 5). Incubation of cells for 16 h with various aldosterone concentrations potentiated maximal glucagon responses with a half-maximal aldosterone concentration at $\sim 10^{-8}$ M.

Aldosterone potentiates hormone-stimulated Mg^{2+} uptake and cAMP generation through de novo protein synthesis. As it has been well established that mineralocorticoids act, in part, through translational processes, we tested whether potentiation of hormone action may be through induced proteins. In this study, we Mg^{2+} depleted the MDCT cells for 16 h prior to the addition of aldosterone with and without cycloheximide. The cells were incubated for a further 3 h, and fluorescence measurements were performed in the presence of 1.5 mM $MgCl_2$ to determine Mg^{2+} uptake. Mg^{2+} uptake was 244 ± 14 nM/s in the presence of aldosterone plus glucagon. Addition of cycloheximide with aldosterone, 3 h prior to fluorescence determination, resulted in the inhibition of aldosterone potentiation of glucagon-stimulated Mg^{2+} entry (Table 2). Cycloheximide did not have any effect on glucagon-stimulated Mg^{2+} entry in the absence of mineralocorticoids (Table 2). These observations indicate that aldosterone may potentiate hormone actions through mechanisms dependent on de novo protein synthesis.

Next, we determined the effect of cycloheximide on aldosterone potentiation of hormone-stimulated cAMP accumulation. Again, the cells were Mg^{2+} depleted for 16 h, then treated with aldosterone, 10^{-7} M, with and without cycloheximide. Three hours later, hormone-responsive cAMP release was determined by radioimmunoassay measurements. Glucagon, 10^{-7} M, stimulated cAMP release from 19 ± 1 to 105 ± 5 pmol·mg protein^{-1}·5 min^{-1} (Table 1). Cycloheximide pretreatment did not alter cAMP generation. However, cycloheximide prevented the potentiation of aldosterone- or glucagon-induced cAMP release (Table 2).

DISCUSSION

Mineralocorticoid receptors are present in distal convoluted tubule cells, which are thought to be involved in enhanced expression of NaCl cotransport, Na^+ conductance, and sodium pump activity (1, 7, 9, 10, 15, 21, 25). Reports of the effects of adrenal steroids on renal magnesium conservation are equivocal (reviewed in Refs. 16 and 19). On balance, acute administration of aldosterone has little or no effect on renal magnesium conservation, whereas chronic hyperaldosteronism is associated with renal magnesium wasting (16). The latter has been explained by mineralocorticoid-induced volume expansion leading to diminished proximal and loop magnesium reabsorption. Alternatively or in conjunction with volume expansion, cellular potassium depletion may provide the basis of renal magnesium

Table 2. *Role of protein synthesis on glucagon-stimulated Mg^{2+} uptake and cAMP release in MDCT cells*

	d([Mg^{2+}]$_i$)/dt, nM/s		cAMP Release, pmol·mg protein^{-1}·5 min^{-1}	
	Control	Cycloheximide	Control	Cycloheximide
Control	164 ± 5 (6)	163 ± 6 (3)	19 ± 1 (5)	26 ± 7 (3)
Glucagon	196 ± 11* (3)	197 ± 11* (3)	105 ± 5* (4)	98 ± 6* (3)
Aldosterone + glucagon	244 ± 14* (6)	195 ± 18*† (5)	251 ± 32* (3)	113 ± 14*† (4)

Values are means ± SE; number of observations is in parentheses. Mg^{2+} entry, d([Mg^{2+}]$_i$)/dt, and cellular cAMP accumulation were determined with and without the presence of 10^{-7} M aldosterone for 3 h and with the addition of 10^{-7} M glucagon with and without pretreatment with 1 µg/ml cycloheximide as indicated. Protein synthesis inhibitor was applied with the aldosterone 3 h prior to fluorescence studies or measurement of cAMP accumulation. * $P < 0.05$, significant for hormone vs. control values. † $P < 0.05$, significant for cycloheximide vs. control values for each of the hormone treatments.

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wasting. We have shown that Mg^{2+} entry into potassium-depleted MDCT cells is significantly diminished compared with control cells (3). Alteration of sodium absorption in the distal tubule may also affect Mg^{2+} transport in this segment. The present studies suggest that aldosterone may have little effect on distal Mg^{2+} transport by itself but potentiates the actions of magnesium-conserving hormones, such as glucagon and AVP. Glucagon- and AVP-stimulated Mg^{2+} entry was greater in MDCT cells pretreated with aldosterone relative to control values (Fig. 1). These actions may be through an augmentation of hormone-mediated signaling pathways.

A number of studies have shown that mineralocorticoids enhance hormone-stimulated cAMP generation. Adrenal insufficiency is associated with impairment of urinary diluting and concentrating capacity (13, 26; reviewed in Ref. 6). Rajerison et al. (20) demonstrated that adrenalectomy reduced AVP-stimulated adenylate cyclase activity in membrane fractions prepared from rat kidney medulla. Doucet et al. (6) have shown that glucagon-, calcitonin-, and AVP-responsive adenylate cyclase activity is diminished in thick ascending limb and collecting tubule segments harvested from adrenalectomized rats compared with animals treated with aldosterone (6). They used segments within 2.5 h (8). These workers postulate that aldosterone induces a protein(s) that stimulates hormone-sensitive adenylate cyclase activity. Their studies with kidney membrane fractions and isolated segments demonstrated that an improvement of coupling between hormone receptors and adenyl cyclase catalytic units was responsible for enhanced cAMP generation in the presence of aldosterone (8). The mechanism(s) through which steroids control G_s proteins (synthesis and/or degradation vs. activity of each unit) is not known (6). Mineralocorticoids may also increase the number of hormone receptors expressed on the cell surface, thereby amplifying hormonal actions. The present studies suggest that aldosterone potentiates glucagon- and AVP-stimulated cAMP generation in distal convoluted tubule cells. This was associated with an increase in Mg^{2+} entry rate in response to these hormones. We have previously shown that cAMP increases Mg^{2+} uptake in MDCT cells; accordingly, aldosterone may enhance hormone-stimulated Mg^{2+} entry by increases in hormone-mediated cAMP release or by amplification of cAMP-mediated actions (2). As there is no association of Mg^{2+} uptake rate with hormone-induced cAMP release, the latter actions may be valid (2). Further research is warranted to determine the signaling pathways whereby hormones mediate the control of Mg^{2+} transport.

Aldosterone potentiates AVP-stimulated Mg^{2+} entry in MDCT cells. Although the physiological role of this response to AVP is not clear, stimulation of Mg^{2+} reabsorption by AVP may be necessary to maintain normal Mg^{2+} balance when salt and water flow in the distal tubule is reduced during antidiuresis. Mineralocorticoid potentiation would increase AVP-stimulated Mg^{2+} reabsorption commensurate with enhanced salt and water retention. The importance of potentiation of glucagon effects is also not clear, but a similar rationale may hold as for AVP, because de Rouffignac (22) has postulated that a number of hormones including glucagon and AVP act in combination to maintain salt and water equilibrium. Accordingly, aldosterone potentiation of these hormones is associated with increased Mg^{2+} conservation along with salt and water retention.

In summary, these studies show that aldosterone potentiates glucagon- and AVP-stimulated Mg^{2+} uptake into MDCT cells. From these observations, we infer that mineralocorticoids may have important effects on hormone regulation of magnesium absorption within the distal convoluted tubule. Aldosterone acts through de novo protein synthesis on nifedipine-sensitive pathways. However, the intracellular pathways involved in stimulation of Mg^{2+} uptake remain to be described.

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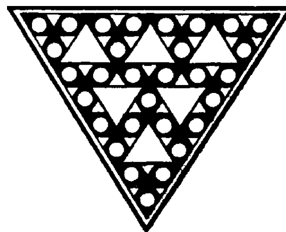

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