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*Am J Physiol Renal Physiol* 277:907-913, 1999.

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**beta -Adrenergic agonists stimulate Mg<sup>2+</sup> 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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# Insulin stimulates $Mg^{2+}$ uptake in mouse distal convoluted tubule cells

LONG-JUN DAI, GORDON RITCHIE, BRIAN W. BAPTY, DIRK KERSTAN, 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, Gordon Ritchie, Brian W. Bapty, Dirk Kerstan, and Gary A. Quamme.** Insulin stimulates  $Mg^{2+}$  uptake in mouse distal convoluted tubule cells. *Am. J. Physiol.* 277 (Renal Physiol. 46): F907–F913, 1999.—Insulin has been shown to be a magnesium-conserving hormone acting, in part, through stimulation of magnesium absorption within the thick ascending limb. Although the distal convoluted tubule possesses the most insulin receptors, it is unclear what, if any, actions insulin has in the distal tubule. The effects of insulin were studied on immortalized mouse distal convoluted tubule (MDCT) cells by measuring cellular cAMP formation with radioimmunoassays and  $Mg^{2+}$  uptake with fluorescence techniques using mag-fura 2. To assess  $Mg^{2+}$  uptake, MDCT cells were first  $Mg^{2+}$  depleted to  $0.22 \pm 0.01$  mM by culturing in  $Mg^{2+}$ -free media for 16 h and then placed in 1.5 mM  $MgCl_2$ , and the changes in intracellular  $Mg^{2+}$  concentration ( $[Mg^{2+}]_i$ ) were measured with microfluorescence.  $[Mg^{2+}]_i$  returned to basal levels,  $0.53 \pm 0.02$  mM, with a mean refill rate,  $d([Mg^{2+}]_i)/dt$ , of  $164 \pm 5$  nM/s. Insulin stimulated  $Mg^{2+}$  entry in a concentration-dependent manner with maximal response of  $214 \pm 12$  nM/s, which represented a  $30 \pm 5\%$  increase in the mean uptake rate above control values. This was associated with a 2.5-fold increase in insulin-mediated cAMP generation ( $52 \pm 3$  pmol·mg protein $^{-1}$ ·5 min $^{-1}$ ). Genistein, a tyrosine kinase inhibitor, diminished insulin-stimulated  $Mg^{2+}$  uptake ( $169 \pm 11$  nM/s), but did not change insulin-mediated cAMP formation ( $47 \pm 5$  pmol·mg protein $^{-1}$ ·5 min $^{-1}$ ). PTH stimulates  $Mg^{2+}$  entry, in part, through increases in cAMP formation. Insulin and PTH increase  $Mg^{2+}$  uptake in an additive fashion. In conclusion, insulin mediates  $Mg^{2+}$  entry, in part, by a genistein-sensitive mechanism and by modifying hormone-responsive transport. These studies demonstrate that insulin stimulates  $Mg^{2+}$  uptake in MDCT cells and suggest that insulin acts in concert with other peptide and steroid hormones to control magnesium conservation in the distal convoluted tubule.

intracellular magnesium; fluorescence; insulin; genistein; tyrosine kinase; intracellular adenosine 3',5'-cyclic monophosphate; parathyroid hormone; aldosterone; extracellular calcium sensing; neomycin

INSULIN HAS PROFOUND EFFECTS on renal electrolyte transport, including magnesium conservation (3, 10, 11, 17, 22, 23, 27, 29). Insulin receptors are present in all tubule segments, so these actions may occur along the length of the nephron (4, 33). De Fonzo et al. (10, 11) first showed that insulin increases sodium reabsorption beyond the proximal tubule, and Kirchner (26) reported that insulin increased chloride transport in

the loop of Henle. These micropuncture studies were supported by Ito et al. (25) using in vitro microperfusion of medullary thick ascending limb segments. Finally, Mandon et al. (29) have shown that insulin stimulates active NaCl transport and passive calcium and magnesium transport in the mouse cortical thick ascending limb. The effects of insulin on magnesium transport in other tubular segments have not been investigated. Among the tubular segments studied, insulin receptor binding is highest along the medullary thick ascending limb and distal convoluted tubule; so by inference insulin may affect electrolyte transport within these segments (4, 16, 33). The medullary thick ascending limb does not absorb much magnesium (29, 43). The distal tubule normally reabsorbs ~10% of the filtered magnesium, which is 70–80% of that delivered to it from the loop of Henle (39). As there is little magnesium reabsorption beyond the distal tubule, this segment determines the final amount excreted in the urine. Hormonal control of magnesium transport in the distal tubule likely plays an important role in determining renal magnesium homeostasis.

In the present studies, we use an immortalized mouse distal convoluted tubule (MDCT) cell line to determine the cellular mechanisms of insulin stimulation on  $Mg^{2+}$  uptake. The MDCT cell line possesses many of the properties of the intact distal convoluted tubule (19, 39). The MDCT cells exhibit amiloride-inhibitable sodium transport and chlorothiazide-sensitive NaCl cotransport. Amiloride and chlorothiazide also stimulate  $Ca^{2+}$  and  $Mg^{2+}$  entry in these cells (8, 19). Furthermore, parathyroid hormone (PTH) and calcitonin stimulate calcium (18, 19) and magnesium (unpublished observations) uptake, and arginine vasopressin (AVP) and glucagon enhance  $Mg^{2+}$  entry into MDCT cells (6). We have also reported that these hormones stimulate  $Mg^{2+}$  uptake by signaling pathways involving intracellular cAMP formation (6).

Furthermore, aldosterone potentiates glucagon- and AVP-stimulated  $Mg^{2+}$  uptake by increasing hormone-mediated cAMP production (9). Finally, activation of the extracellular polyvalent cation receptor with elevated  $Ca^{2+}$ ,  $Mg^{2+}$ , or neomycin inhibited hormone-stimulated cAMP formation and  $Mg^{2+}$  uptake (1, 2). Accordingly, MDCT cells are useful in vitro models with which to investigate the cellular mechanisms of individual hormones and hormonal interactions that are difficult to study in situ with intact distal tubules. In these studies, we determined  $Mg^{2+}$  entry into MDCT cells by first  $Mg^{2+}$  depleting the cells by culturing in magnesium-free media for 16 h. The  $Mg^{2+}$ -depleted MDCT cells were then placed in medium containing 1.5 mM magnesium, and the refill rate,  $d([Mg^{2+}]_i)/dt$  (where

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$[Mg^{2+}]_i$  is the intracellular  $Mg^{2+}$  concentration), was measured with microfluorescent studies using mag-fura 2. Evidence supports the notion that  $Mg^{2+}$  uptake reflects transepithelial transport (39).  $Mg^{2+}$  uptake rate is rapid, concentration dependent, and selective for magnesium (8). In the present study, we show that insulin stimulates  $Mg^{2+}$  uptake into MDCT cells, and we characterize some of the interactions with PTH and aldosterone and the effects of the  $Ca^{2+}/Mg^{2+}$ -sensing receptor.

## METHODS

**Materials.** Basal Dulbecco's minimal essential medium and Ham's F-12 medium (DMEM-F12) were purchased from GIBCO (Life Technologies, Gaithersburg, MD). Fetal calf serum (FCS) was from Flow Laboratories (McLean, VA). Mag-fura 2-AM was obtained from Molecular Probes (Eugene, OR). The protein kinase A inhibitor, RpAMPS (the Rp diastereoisomer of adenosine 3',5'-cyclic monophosphate) was purchased from Calbiochem (San Diego, CA). Insulin, genistein, and other materials were from Sigma (St. Louis, MO).

**Cell culture.** Immortalized MDCT cells were kindly provided to us by Dr. P. A. Friedman. They have been extensively characterized by Drs. P. A. Friedman and F. A. Gesek (19). The MDCT cell line was cultured in DMEM-F12, 1:1, media supplemented with 10% FCS, 1 mM glucose, 5 mM L-glutamine, 50 U/ml penicillin, and 50  $\mu$ g/ml streptomycin in a humidified environment of 5%  $CO_2$ -95% air at 37°C. For the cAMP determinations, the MDCT cells were cultured to confluence in 24-well plastic dishes. Sixteen hours prior to the cAMP measurements, the culture medium was changed to one containing 0.2% bovine serum albumin (BSA) in lieu of the FCS. 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 confluence over 4–6 days in supplemented media as described above. The normal media contained 0.6 mM magnesium and 1.0 mM calcium. In the experiments indicated, the cells were cultured in nominally magnesium-free media (<0.01 mM) for 16 h prior to study; 0.2% BSA replaced FCS during this period. Other constituents of the magnesium-free media were identical to those of the complete media.

**cAMP measurements.** cAMP was determined in confluent MDCT cell monolayers cultured in 24-well plates in DMEM-F12 media without serum as previously reported (6). After addition of the agonists, 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 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  $Mg^{2+}$  measurements.** Coverslips were mounted into a perfusion chamber, and the attached subconfluent cells were incubated with 5  $\mu$ M mag-fura 2-AM dissolved in Pluronic acid F-127 (0.125%; Molecular Probes) for determination of intracellular  $Mg^{2+}$  concentration ( $[Mg^{2+}]_i$ ) 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  $K_2HPO_4$ , 0.2  $KH_2PO_4$ , 1.0  $CaCl_2$ , 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 fluorescence of the MDCT cell monolayer. The chamber 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 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. Media changes were made without an interruption in recording.

The free  $[Mg^{2+}]_i$  was calculated from the ratio of the fluorescence at the two excitation wavelengths as previously described (8) using a dissociation constant ( $K_d$ ) of 1.4 mM for the mag-fura 2· $Mg^{2+}$  complex. The minimum ( $R_{min}$ ) and maximum ( $R_{max}$ ) ratios were determined for the cells at the end of each experiment using 20  $\mu$ 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.

**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

**Insulin 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  $[Mg^{2+}]_i$ ,  $0.22 \pm 0.01$  mM, than cells cultured in normal media,  $0.53 \pm 0.02$  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 leveled at a  $[Mg^{2+}]_i$  of  $0.49 \pm 0.08$  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  $164 \pm 5$  nM/s ( $n = 9$  cells), as determined over the first 500 s following addition of 1.5 mM  $MgCl_2$  (6).  $Mg^{2+}$  uptake is inhibited by a number of inorganic cations such as  $La^{3+}$  and  $Mn^{2+}$  and by organic channel blocks such as nifedipine but not  $Ca^{2+}$  (8). We used this approach to determine the effects of insulin on  $Mg^{2+}$  uptake into MDCT cells.

Insulin added to the refill solution increased the rate of  $Mg^{2+}$  entry into  $Mg^{2+}$ -depleted MDCT cells. Insulin,  $10^{-7}$  M, increased the mean  $Mg^{2+}$  entry rate from  $164 \pm 5$  to  $214 \pm 13$  nM/s ( $n = 5$ ) which represented a stimulation of  $30 \pm 5\%$  above control values measured after 500 s (Fig. 2). Insulin increases  $Mg^{2+}$  uptake so that  $d([Mg^{2+}]_i)/dt$  is not linear over the 500-s time frame, and therefore this value is an underestimation. In all cases where measured,  $[Mg^{2+}]_i$  returned to basal levels,  $0.47 \pm 0.05$  mM, in insulin-treated cells, similar to control observations. The effect of insulin on  $Mg^{2+}$  uptake was concentration dependent, with maximal rate of stimulation at  $10^{-7}$  M of  $214 \pm 13$  nM/s ( $n = 5$ ) and with half-maximal stimulation at a concentration

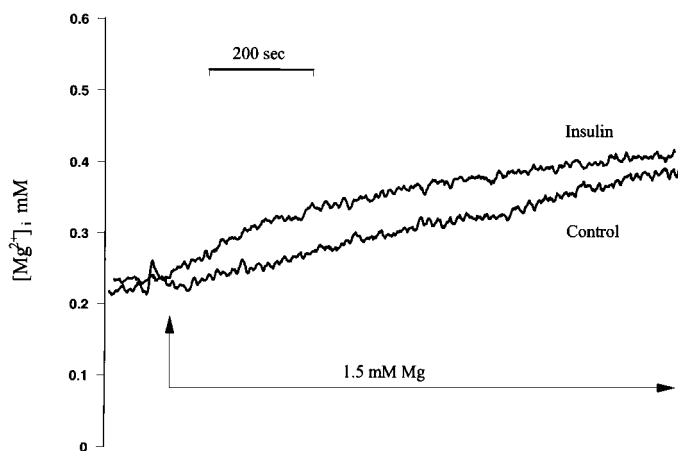


Fig. 1. Insulin stimulates  $Mg^{2+}$  uptake in  $Mg^{2+}$ -depleted mouse distal convoluted tubule (MDCT) cells. MDCT cells were cultured in  $Mg^{2+}$ -free media ( $<0.01$  mM). Fluorescence studies were performed in buffer solutions in absence of external magnesium, and where indicated,  $MgCl_2$  (1.5 mM final concentration) was added to observe changes in intracellular  $Mg^{2+}$  concentration ( $[Mg^{2+}]_i$ ). 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 insulin,  $10^{-7}$  M, was added to this buffer solution from a stock ethanol solution. Fluorescence was measured at 1 data point/s with 25-point signal averaging, and the tracing was smoothed according to methods previously described (8).

about  $10^{-8}$  M. We have previously reported that dihydropyridines inhibit  $Mg^{2+}$  uptake into  $Mg^{2+}$ -depleted MDCT cells (8). To determine whether insulin-induced  $Mg^{2+}$  entry is mediated through a dihydropyridine-sensitive pathway, we examined the effect of the channel blocker, nifedipine, on the changes in  $[Mg^{2+}]_i$  following placement in the refill buffer solution containing 1.5 mM  $MgCl_2$ . The presence of  $10^{-5}$  M nifedipine inhibited insulin-stimulated  $Mg^{2+}$  uptake to  $24 \pm 2$  nM/s, which was similar to that observed in control cells (8). These findings support the notion that insulin-stimulated

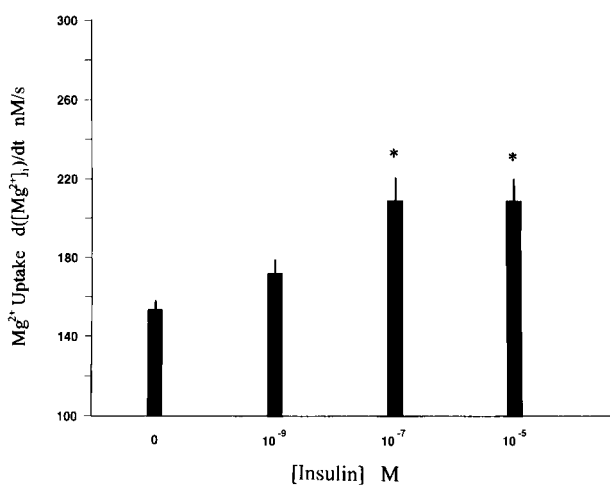


Fig. 2. Concentration dependence of insulin stimulation of  $Mg^{2+}$  entry in MDCT cells. Rate of  $Mg^{2+}$  influx as determined by the rate of change of  $[Mg^{2+}]_i$  over the first 500 s,  $d([Mg^{2+}]_i)/dt$ , following addition of insulin and  $MgCl_2$ . Fluorescence was performed according to the description given in legend to Fig. 1. Values are means  $\pm$  SE for 3–6 cells. \* $P < 0.01$ , significance of  $Mg^{2+}$  entry rates compared with respective control values.

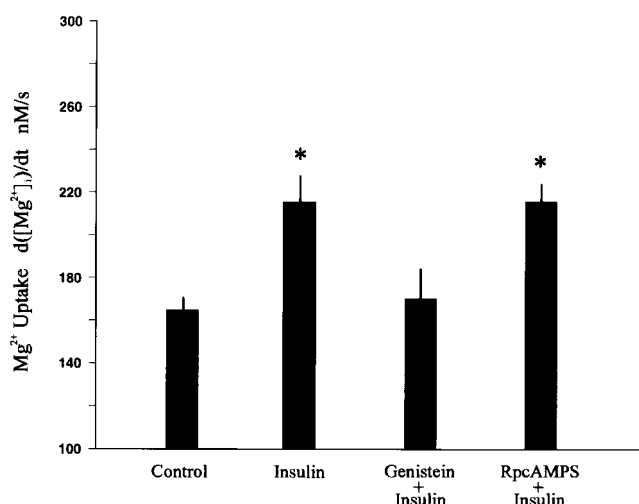


Fig. 3. Insulin stimulates  $Mg^{2+}$  uptake through tyrosine kinase- and adenylate cyclase-mediated pathways. The tyrosine kinase inhibitor, genistein (50  $\mu$ M, final), or the protein kinase A inhibitor, RpcAMPS (1.0  $\mu$ M), was added 60 min and 5 min, respectively, prior to the determination of  $Mg^{2+}$  uptake with the addition of insulin,  $10^{-7}$  M, and  $MgCl_2$ , 1.5 mM. Values are means  $\pm$  SE. \* $P < 0.01$ , significance of  $Mg^{2+}$  uptake measurements in presence of inhibitors vs. respective control values.

uptake is the same as the entry pathway observed in control cells.

Next, we determined the effect of genistein, a specific inhibitor of tyrosine protein kinases, on insulin-stimulated  $Mg^{2+}$  uptake (Fig. 3). Genistein inhibits by competing with ATP for the binding site in the catalytic domain of tyrosine kinases (46). Cells were pretreated with genistein, 50  $\mu$ M, for 1 h prior to the addition of insulin. Genistein inhibited insulin-stimulated uptake to  $170 \pm 14$  nM/s, suggesting that the effect of insulin depends on receptor  $\beta$ -subunit tyrosine autophosphorylation or tyrosine kinase substrate phosphorylation.

**Insulin stimulates cAMP formation in MDCT cells.** As cAMP increases  $Mg^{2+}$  entry into MDCT cells, we determined the effect of insulin on cAMP release in these cells (6). Insulin increased cAMP syntheses in a concentration-dependent manner with a maximal stimulation at about  $10^{-6}$  M (Fig. 4). These studies show that MDCT cells have insulin receptor signaling pathways that stimulate adenylate cyclase activity, resulting in an increase in intracellular cAMP levels (36, 44, 47, 49). Accordingly, insulin may affect  $Mg^{2+}$  uptake into MDCT cells by stimulating cellular cAMP formation (6). To test this notion, we determined the effect of protein kinase A inhibition on insulin-stimulated  $Mg^{2+}$  uptake. RpcAMPS, a competitive inhibitor of protein kinase A, was applied 5 min prior to performing  $Mg^{2+}$  uptake measurements (8). RpcAMPS did not inhibit insulin-stimulated uptake, suggesting that activation of protein kinase A is not involved with insulin actions (Fig. 3).

**Insulin and PTH stimulate  $Mg^{2+}$  uptake by additive mechanisms.** We have shown that PTH increases  $Mg^{2+}$  entry into MDCT cells by intracellular pathways involving, in part, cAMP-mediated pathways (unpublished observations). As insulin associates with many differ-

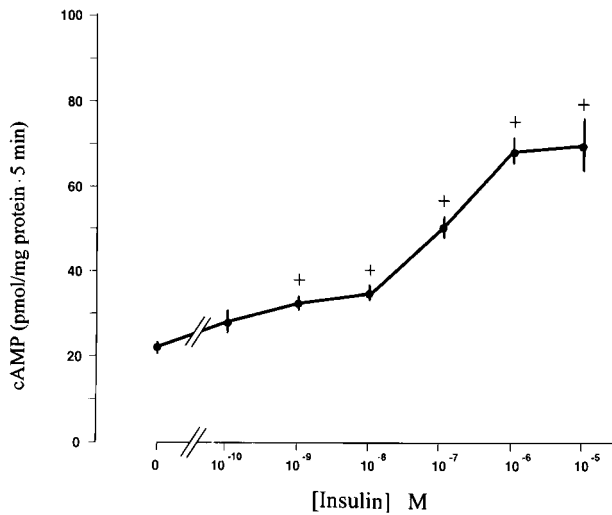


Fig. 4. Concentration dependence of insulin stimulation of cAMP syntheses in MDCT cells. Insulin was added, at concentrations indicated, 5 min prior to measurement of cAMP in presence of IBMX. Values are means  $\pm$  SE for 3–6 cells. + $P$  < 0.01, significance of cAMP concentrations compared with respective control values.

ent hormone signaling pathways, including cAMP-dependent mediation, we measured cAMP formation and  $Mg^{2+}$  uptake in cells treated with both hormones. PTH + insulin increased cAMP levels and  $Mg^{2+}$  entry rates to a greater extent than each of the hormones alone (Fig. 5). These data give credence to the notion that insulin potentiates PTH-stimulated  $Mg^{2+}$  uptake.

*Aldosterone does not potentiate insulin-stimulated  $Mg^{2+}$  uptake in MDCT cells.* We have previously shown that aldosterone, applied 16 h prior to experimentation, potentiates PTH-, calcitonin-, glucagon-, and AVP-mediated cAMP generation, which in turn increases  $Mg^{2+}$  uptake (4, 9). Although the cellular mechanisms are not known, it has been speculated that aldosterone-induced proteins increase the receptor-adenylate cyclase coupling in epithelial cells (12, 13). In the present study, we determined whether pretreatment of MDCT cells with aldosterone for 16 h potentiates the acute actions of insulin. Treatment of cells with aldosterone for 16 h prior

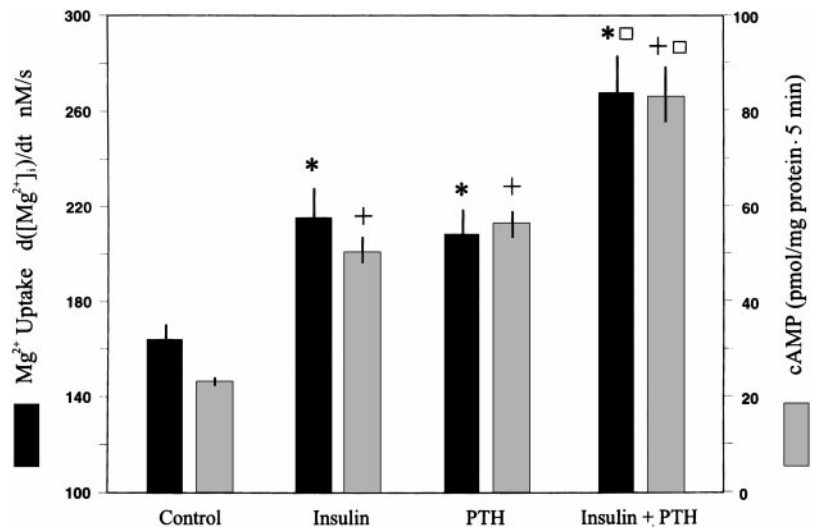
to study did not significantly increase insulin-mediated cAMP generation nor insulin-stimulated  $Mg^{2+}$  uptake, even though it increased PTH responses (Fig. 6).

*Activation of the extracellular  $Mg^{2+}/Ca^{2+}$ -sensing mechanism does not inhibit insulin-stimulated cAMP generation but diminishes  $Mg^{2+}$  uptake.* MDCT cells possess extracellular  $Mg^{2+}/Ca^{2+}$ -sensing mechanisms that, upon activation with polyvalent cations such as  $Mg^{2+}$ ,  $Ca^{2+}$ , or neomycin, inhibit PTH-, calcitonin-, glucagon-, and AVP-mediated cAMP generation and glucagon- and AVP-stimulated  $Mg^{2+}$  uptake (1, 2). To determine whether activation of  $Mg^{2+}/Ca^{2+}$  sensing alters insulin actions, we pretreated cells for 5 min with neomycin prior to the addition of insulin and  $MgCl_2$ . Neomycin did not affect insulin-mediated cAMP formation but modestly diminished insulin-stimulated  $Mg^{2+}$  uptake (Fig. 7). The mechanisms by which the  $Mg^{2+}/Ca^{2+}$ -sensing receptor inhibit insulin actions remain undefined. The  $Mg^{2+}/Ca^{2+}$ -sensing receptor is coupled to  $G_i$  and  $G_s$  proteins that may interact with insulin-mediated signaling pathways (1, 2).

## DISCUSSION

The distal tubule reabsorbs significant amounts of magnesium and plays an important role in determining the final urinary excretion rate (43). In contrast to more proximal segments of the nephron, distal magnesium transport processes are postulated to be active and transcellular in nature (39). 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 magnesium reabsorption within the distal tubule is controlled by peptide hormones including PTH, glucagon, and calcitonin (43). More recently, we have shown that PTH, calcitonin, glucagon, and AVP stimulate  $Mg^{2+}$  entry into MDCT cells (unpublished observations and Ref. 6). The actions of these hormones are, in part, through cAMP-mediated pathways, as RpcAMPS inhibits hormonal responses. Aldosterone potentiates hormone-stimulated cAMP generation,

Fig. 5. Insulin and parathyroid hormone (PTH) synergistically stimulate  $Mg^{2+}$  entry into MDCT cells. Maximal concentrations of insulin,  $10^{-7}$  M, and PTH,  $10^{-7}$  M, were added where indicated. cAMP was measured by radioimmunoassay, and  $d([Mg^{2+}]_i)/dt$  was measured by fluorescence. Values are means  $\pm$  SE for 3–5 preparations. \* $P$  < 0.01 and + $P$  < 0.01, significance of  $Mg^{2+}$  entry rates and cAMP concentrations, respectively, compared with respective control values.  $\square P$  < 0.01, significance of these data for insulin+PTH compared with either insulin or PTH alone.



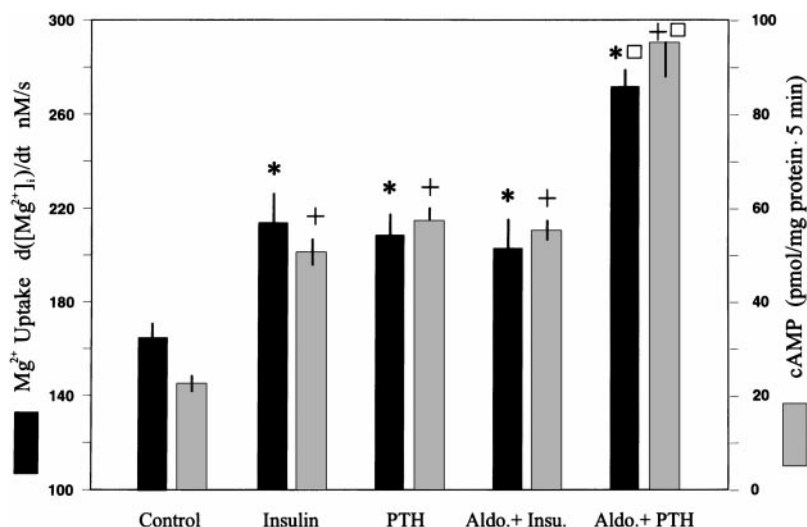


Fig. 6. Aldosterone does not potentiate insulin-mediated cAMP generation and  $Mg^{2+}$  uptake. MDCT cells were incubated for 16 h in magnesium-free buffer solution containing aldosterone,  $10^{-7}$  M. Insulin,  $10^{-7}$  M, or PTH,  $10^{-7}$  M, was added where indicated, and cAMP was measured following 5 min in presence of IBMX, or  $Mg^{2+}$  uptake was determined following 500 s in 1.5 mM  $MgCl_2$ . Values are means  $\pm$  SE for 3–5 observations. \* $P < 0.01$  and + $P < 0.01$ , significance of mean  $Mg^{2+}$  entry rates and cAMP determinations, respectively, compared with respective control values from either insulin or PTH alone. □ $P < 0.01$ , significance of  $Mg^{2+}$  uptake and cAMP formation rates of aldosterone+insulin vs. PTH alone.

thereby potentiating peptide hormone-responsive  $Mg^{2+}$  uptake (9). In the present study, we show that insulin stimulates  $Mg^{2+}$  uptake in MDCT cells through tyrosine protein kinase-mediated pathways. We infer from these results that insulin may modulate distal tubule magnesium transport and, together with peptide and steroid hormones, orchestrate renal magnesium conservation.

The evidence given here is that insulin stimulates  $Mg^{2+}$  entry into MDCT cells through genistein-sensitive tyrosine phosphorylation. Additionally, insulin stimulates cAMP formation in MDCT cells and presumably activates protein kinase A. We have shown that the peptide hormones PTH, calcitonin, glucagon, and AVP stimulate Mg entry by protein kinase A-, phospholipase C-, and protein kinase C-mediated pathways. The present studies suggest that insulin may potentiate or have additive effects on these peptide hormones. It is clear that insulin may modulate each of these pathways (5, 15, 21, 35, 38, 42). In relation to the kidney, Mandon et al. (29) have reported that insulin stimulates NaCl and magnesium transport in the per-

fused mouse cortical thick ascending limb. They further showed that insulin may potentiate AVP-responsive cAMP formation and magnesium transport in this segment. The potentiation of insulin and AVP may be explained by the two hormones having effects at different sites of the adenylate cyclase system (29). The interaction of insulin-mediated effects on peptide hormone responses is complicated, as aldosterone had no effect on insulin actions but potentiated PTH-stimulated cAMP (2) and  $Mg^{2+}$  uptake (unpublished observations). Finally, activation of  $Ca^{2+}/Mg^{2+}$ -sensing receptor inhibited insulin-stimulated  $Mg^{2+}$  uptake with no effect on intracellular cAMP formation. As in the cortical thick ascending limb, these actions may be explained by insulin inhibiting  $G_i$  and PTH activating  $G_s$  proteins of the adenylate cyclase system.

**Insulin actions on cellular electrolyte transport.** Insulin has clearly been shown to have antinatriuretic effects by its actions on tubular absorption within the loop of Henle (10, 11, 17, 26, 34). Using microperfusion studies, Ito et al. (24, 25) reported that insulin stimulates NaCl transport in isolated rabbit medullary thick

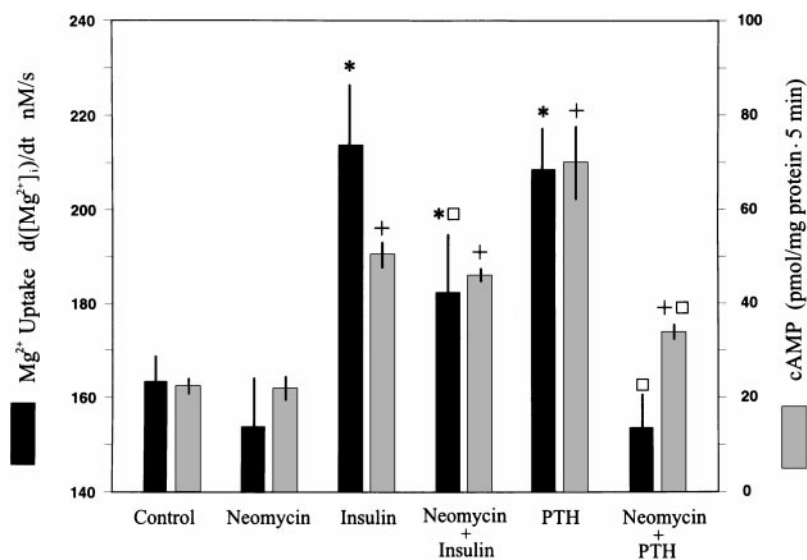


Fig. 7. Summary of effects of  $Mg^{2+}/Ca^{2+}$ -sensing mechanism activation on insulin-stimulated  $Mg^{2+}$  uptake and cAMP formation. cAMP was measured by radioimmunoassay, and  $Mg^{2+}$  uptake,  $d([Mg^{2+}]_i)/dt$ , was determined with 1.5 mM extracellular  $Mg^{2+}$  in absence and presence of neomycin, 50  $\mu$ M, as indicated. Neomycin was added 5 min prior to addition of insulin,  $10^{-7}$  M, or PTH,  $10^{-7}$  M, and  $MgCl_2$ , 1.5 mM.  $Mg^{2+}$  uptake rate was determined over the initial 500 s following addition of insulin or PTH. Values are means  $\pm$  SE for 3–5 cells. \* $P < 0.01$  and + $P < 0.01$ , significance of  $Mg^{2+}$  uptake and cAMP determinations, respectively, from respective control values. □ $P < 0.01$ , significance of  $Mg^{2+}$  uptake values of neomycin+insulin vs. insulin alone.

ascending limb segments and showed that the actions were dependent on tyrosine kinase, phosphatidylinositol 3-kinase, and protein kinase C but independent of intracellular cAMP. As mentioned above, Mandon et al. (29) reported that insulin stimulates active NaCl and passive calcium and magnesium transport in perfused mouse cortical thick ascending limbs in vitro. They suggested that insulin acts on different levels on the adenylate cyclase-mediated pathways. The notion is that insulin may increase NaCl transport in the loop, thereby stimulating passive magnesium absorption. Insulin also stimulates sodium transport in  $A_6$  cells, which are a distal cell line from *Xenopus laevis* that has properties similar to the mammalian distal nephron (23, 31, 40). Insulin-stimulated sodium transport was partly inhibited by genistein, indicating tyrosine kinase was important (40). Insulin did not increase cAMP formation in  $A_6$  cells, nor did adenylate cyclase inhibition diminish transport (31, 41, 50). As genistein did not completely inhibit insulin actions, Rodriguez-Commes et al. (41) suggested that parallel non-tyrosine-kinase-dependent pathways were also involved. They showed that insulin actions in  $A_6$  cells required intracellular  $Ca^{2+}$  signaling, suggesting to these investigators that protein kinase C is needed for some of the responses. Clearly, the insulin receptor signaling pathways are different in the various epithelial cell types studied. In the present studies, we show that insulin stimulates  $Mg^{2+}$  uptake in MDCT cells through tyrosine kinase pathways not involving cAMP formation.

*The role of insulin in renal magnesium handling.* Micropuncture and microperfusion studies and experiments with isolated cells have shown that many hormones including insulin regulate magnesium absorption in the distal tubule. Interestingly, all of these hormones and factors also increase magnesium transport in the thick ascending limb of the loop of Henle (43). Accordingly, these hormone-mediated responses are serially organized to similarly regulate magnesium absorption in both the loop and distal tubule. This organization may be of benefit in that influences acting in either the loop or distal tubule may be modified by changes in the other segment.

The interactions of the various peptide and steroid hormones are complex (39, 43). It can be inferred that overall distal magnesium absorption is controlled by all of these influences initiated individually but coming together through shared intracellular signaling pathways. Few studies have been directed at describing these interactions. Clearly, control of renal magnesium handling is sufficiently important to warrant multiple hormonal control.

Finally, hypomagnesemia has been reported in ~25% of diabetic patients, and renal magnesium wasting has been associated with both type I and type II diabetes mellitus (20, 30, 37, 45, 48). As insulin stimulates magnesium conservation in the loop (29) and distal tubule (present report), insulin deficiency could explain the increase in urinary magnesium excretion. A number of indirect influences commonly present in diabetes mellitus may also explain an increase in magnesium

excretion. First, uncontrolled hyperglycemia and hyperglycemia may increase excretion through osmotic diuresis (14, 28, 32). Second, metabolic acidosis commonly observed in diabetes may increase magnesium excretion by its actions within the distal tubule (7). Finally, hypophosphatemia and hypokalemia are often associated with diabetes, and both may decrease distal magnesium reabsorption (37, 39). Insulin deficiency with any one of these indirect entities may underlie the diminished magnesium reabsorption and hypomagnesemia commonly observed in diabetes mellitus.

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Address for reprint requests and other correspondence: G. A. Quamme, Department of Medicine, Vancouver Hospital and Health Sciences Centre, Koerner Pavilion, 2211 Wesbrook Mall, Vancouver, BC, Canada V6T 1Z3 (E-mail: quamme@interchange.ubc.ca).

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## REFERENCES

1. **Bapty, B. W., L.-J. Dai, G. Ritchie, L. Canaff, G. N. Hendy, and G. A. Quamme.** Activation of  $Mg^{2+}/Ca^{2+}$ -sensing receptors inhibits hormone-stimulated  $Mg^{2+}$  uptake in distal convoluted tubule cells. *Am. J. Physiol.* 275 (*Renal Physiol.* 44): F356–F360, 1998.
2. **Bapty, B. W., L.-J. Dai, G. Ritchie, F. Jirik, L. Canaff, G. N. Hendy, and G. A. Quamme.** Extracellular  $Mg^{2+}$ - and  $Ca^{2+}$ -sensing in mouse distal convoluted tubule cells. *Kidney Int.* 53: 583–592, 1998.
3. **Baum, M.** Insulin stimulates volume absorption in the rabbit proximal convoluted tubule. *J. Clin. Invest.* 79: 1104–1109, 1987.
4. **Butlen, D., S. Vadrot, S. Roseau, and F. Morel.** Insulin receptor along the rat nephron: [ $^{125}I$ ]insulin binding in microdissected glomeruli and tubules. *Pflügers Arch.* 412: 604–612, 1988.
5. **Clawges, H. M., K. M. Depree, and S. G. Graber.** Human 5-HT<sub>1</sub> receptor subtypes exhibit distinct G protein coupling behaviours in membranes from Sf9 cells. *Biochemistry* 36: 12930–12938, 1997.
6. **Dai, L.-J., B. Bapty, G. Ritchie, and G. A. Quamme.** Glucagon and arginine vasopressin stimulate  $Mg^{2+}$  uptake in mouse distal convoluted tubule cells. *Am. J. Physiol.* 274 (*Renal Physiol.* 43): F328–F335, 1998.
7. **Dai, L.-J., P. A. Friedman, and G. A. Quamme.** Acid-base changes alter  $Mg^{2+}$  uptake in mouse distal convoluted tubule cells. *Am. J. Physiol.* 272 (*Renal Physiol.* 41): F759–F766, 1997.
8. **Dai, L.-J., L. Raymond, P. A. Friedman, and G. A. Quamme.** Mechanisms of amiloride stimulation of  $Mg^{2+}$  uptake in immortalized mouse distal convoluted tubule cells. *Am. J. Physiol.* 272 (*Renal Physiol.* 41): F249–F256, 1997.
9. **Dai, L.-J., G. Ritchie, B. Bapty, and G. A. Quamme.** Aldosterone potentiates hormone-stimulated  $Mg^{2+}$  uptake in distal convoluted tubule cells. *Am. J. Physiol.* 274 (*Renal Physiol.* 43): F336–F341, 1998.
10. **De Fronzo, R. A., C. R. Cooke, R. Anderes, G. R. Faloona, and P. S. Davis.** The effect of insulin on renal handling of sodium, potassium, calcium, and phosphate in man. *J. Clin. Invest.* 55: 845–855, 1975.
11. **De Fronzo, R. A., M. Goldberg, and Z. S. Agus.** The effects of glucose and insulin on renal electrolyte transport. *J. Clin. Invest.* 58: 83–90, 1976.
12. **Doucet, A., C. Barlet-Bas, S. Siaume-Perez, C. Khadouri, and S. Marsy.** Gluco- and mineralocorticoids control adenylate cyclase in specific nephron segments. *Am. J. Physiol.* 258 (*Renal Fluid Electrolyte Physiol.* 27): F812–F820, 1990.
13. **El Mernissi, G., C. Barlet-Bas, C. Khadouri, L. Cheval, S. Marsy, and A. Doucet.** Short-term effect of aldosterone on vasopressin-sensitive adenylate cyclase in rat collecting tubule. *Am. J. Physiol.* 264 (*Renal Fluid Electrolyte Physiol.* 33): F821–F826, 1993.

14. Ericsson, Y., B. Angmar-Månsson, and M. Flores. Urinary mineral ion loss after sugar ingestion. *Bone Miner.* 9: 233–237, 1990.
15. Farese, R. V., J. S. Davis, D. E. Barnes, M. L. Standaert, J. S. Babischkin, R. Hock, N. K. Rosic, and R. J. Pollock. The de novo phospholipid effect of insulin is associated with increases in diacylglycerol but not inositol phosphates or cytosolic  $Ca^{2+}$ . *Biochem. J.* 231: 269–278, 1985.
16. Feraille, E., S. Marsy, L. Cheval, C. Barlet-Bas, C. Khadouri, H. Favre, and A. Doucet. Sites of antinatriuretic action of insulin along rat nephron. *Am. J. Physiol.* 263 (*Renal Fluid Electrolyte Physiol.* 32): F175–F179, 1992.
17. Friedberg, C. E., M. Van Buren, J. A. Ijlsma, and H. A. Koomans. Insulin increases sodium reabsorption in diluting segment in humans: evidence for indirect mediation through hypokalemia. *Kidney Int.* 40: 251–256, 1991.
18. Friedman, P. A., B. A. Coutermarsh, S. M. Kennedy, and F. A. Gesek. Parathyroid hormone stimulation of calcium transport is mediated by dual signaling mechanisms involving protein kinase A and renal protein kinase C. *Endocrinology* 137: 13–20, 1996.
19. Friedman, P. A., and F. A. Gesek. Calcium transport in renal epithelial cells. *Am. J. Physiol.* 264 (*Renal Fluid Electrolyte Physiol.* 33): F181–F198, 1993.
20. Fujii, S., T. Takemura, M. Wada, T. Akai, and K. Okuda. Magnesium levels in plasma, erythrocyte and urine in patients with diabetes mellitus. *Horm. Metab. Res.* 14: 161–162, 1982.
21. Green, M. A., P. Chidiac, and J. W. Wells. Cardiac muscarinic receptors. Relationships between the G protein and multiple states of affinity. *Biochemistry* 36: 7380–7394, 1997.
22. Hammerman, M. R. Interactions of insulin with the renal proximal tubular cell. *Am. J. Physiol.* 249 (*Renal Fluid Electrolyte Physiol.* 18): F1–F11, 1985.
23. Herrer, F. C. Effect of insulin on short-circuit current and sodium transport across toad urinary bladder. *Am. J. Physiol.* 209: 819–824, 1965.
24. Ito, O., Y. Kondo, M. Oba, N. Takahashi, K. Omata, and K. Abe. Tyrosine kinase, phosphatidyl inositol 3-kinase, and protein kinase C regulate insulin-stimulated NaCl absorption in the thick ascending limb. *Kidney Int.* 51: 1037–1041, 1997.
25. Ito, O., Y. Kondo, N. Takahashi, K. Kudo, Y. Igarashi, Y. Imai, K. Omata, and K. Abe. Insulin stimulates NaCl transport in isolated perfused MTAL of Henle's loop of rabbit kidney. *Am. J. Physiol.* 267 (*Renal Fluid Electrolyte Physiol.* 36): F265–F270, 1994.
26. Kirchner, K. A. Insulin increases loop segment chloride reabsorption in the euglycemic rat. *Am. J. Physiol.* 255 (*Renal Fluid Electrolyte Physiol.* 24): F1206–F1213, 1988.
27. Lau, K., J. Guntupalli, and B. Eby. Effects of somatostatin on phosphate transport: evidence of the role of basal insulin. *Kidney Int.* 24: 10–15, 1983.
28. Lindeman, R. D., S. Adler, M. J. Yiengst, and E. S. Beard. Influence of various nutrients on urinary divalent cation excretion. *J. Lab. Clin. Med.* 70: 236–245, 1967.
29. Mandon, B., E. Siga, D. Chabardes, D. Firsov, N. Roinel, and C. de Rouffignac. Insulin stimulates  $Na^+$ ,  $Cl^-$ ,  $Ca^{2+}$ , and  $Mg^{2+}$  transports in TAL of mouse nephron: cross-potentialiation with ADH. *Am. J. Physiol.* 265 (*Renal Fluid Electrolyte Physiol.* 34): F361–F369, 1993.
30. Mather, H. M., J. A. Nisbet, G. H. Burton, G. J. Poston, J. M. Bland, P. A. Bailey, and T. R. Pilkington. Hypomagnesemia in diabetes. *Clin. Chim. Acta* 95: 235–242, 1979.
31. Matsumoto, P. S., A. Ohara, P. Duchatelle, and D. C. Eaton. Tyrosine kinase regulates epithelial sodium transport in  $A_6$  cells. *Am. J. Physiol.* 264 (*Cell Physiol.* 33): C246–C250, 1993.
32. McNair, P., M. S. Christensen, C. Madsbad, and I. Transbøl. Renal hypomagnesemia in human diabetes mellitus: its relation to glucose homeostasis. *Eur. J. Clin. Invest.* 12:81–85, 1982.
33. Nakamura, R., D. S. Emmanouel, and A. I. Katz. Insulin binding sites in various segments of the rabbit nephron. *J. Clin. Invest.* 72: 388–392, 1983.
34. Nizet, A., P. Lefebure, and J. Crabbé. Control of insulin of sodium, potassium, and water excretion by the isolated dog kidney. *Pflügers Arch.* 323: 11–20, 1971.
35. O'Brien, R. M., M. D. Houslay, G. Milligan, and K. Siddle. The insulin receptor tyrosyl kinase phosphorylates holomeric forms of the guanine nucleotide regulatory proteins  $G_i$  and  $G_o$ . *FEBS Lett.* 212: 281–288, 1987.
36. Pertseva, M. N., S. A. Plesneva, L. A. Kuznetsova, A. O. Shpakov, and K. V. Derkach. On the tyrosine kinase mechanism of the novel effect of insulin and insulin like growth factor I. Stimulation of the adenylyl cyclase system in muscle tissues. *Biochem. Pharm.* 52:1867–1874, 1966.
37. Ponder, S. W., B. H. Brouhard, and L. B. Travis. Hyperphosphaturia and hypermagnesuria in children with IDDM. *Diabetes Care* 13: 437–441, 1990.
38. Pröfrock, A., S. Schnefel, and I. Schulz. Receptors for insulin interact with  $G_i$ -proteins and for epidermal growth factor with  $G_i$  and  $G_s$ -proteins in rat pancreatic acinar cells. *Biochem. Biophys. Res. Commun.* 175: 380–386, 1991.
39. Quamme, G. A. Renal magnesium handling: new insights in understanding old problems. *Kidney Int.* 52: 1180–1195, 1997.
40. Record, R. D., M. Johnson, S. Lee, and B. L. Blazer-Yost. Aldosterone and insulin stimulate amiloride-sensitive sodium transport in  $A_6$  cells by additive mechanisms. *Am. J. Physiol.* 271 (*Cell Physiol.* 40): C1079–C1084, 1996.
41. Rodriguez-Commes, J., C. Isales, L. Kalghati, J. Gasalla-Herraiz, and J. P. Hayslett. Mechanism of insulin-stimulated electrogenic sodium transport. *Kidney Int.* 46: 666–674, 1994.
42. Rothenberg, P. L., and C. R. Kahn. Insulin inhibits pertussis toxin-catalysed ADP-ribosylation of G-proteins. Evidence for a novel interaction between insulin and G-proteins. *J. Biol. Chem.* 263: 15546–15552, 1988.
43. Rouffignac, C. de, and G. A. Quamme. Renal magnesium handling and its hormonal control. *Physiol. Rev.* 74: 305–322, 1994.
44. Saltiel, A. R. Diverse signaling pathways in the cellular actions of insulin. *Am. J. Physiol.* 270 (*Endocrinol. Metab.* 33): E375–E385, 1996.
45. Sjøgren, A., C. H. Florén, and Å. Nilsson. Magnesium, potassium and zinc deficiency in subjects with type II diabetes mellitus. *Acta Med. Scand.* 224: 461–466, 1988.
46. Srinivas, P. R., and G. Grunberger. Inhibitors of the insulin receptor tyrosine kinase. *Pharmacol. Ther.* 64: 23–35, 1994.
47. Strålfors, P. Insulin second messengers. *Bioessays* 19: 327–335, 1997.
48. Tosiello, L. Hypomagnesemia and diabetes mellitus. A review of clinical implications. *Arch. Intern. Med.* 156: 1143–1148, 1996.
49. Trovati, M., P. Massucco, L. Mattiello, F. Cavalot, E. M. Mularoni, A. W. Hahn, and G. Anfossi. Studies on the influence of insulin on cyclic adenosine monophosphate in human vascular smooth muscle cells: dependence on cyclic guanosine monophosphate and modulation of catecholamine effects. *Diabetologia* 39: 1156–1164, 1996.
50. Weisman, W. P., S. Sinha, and S. Klahr. Effects of insulin, ADH, and cyclic AMP on sodium transport in the toad bladder. *Am. J. Physiol.* 232 (*Renal Fluid Electrolyte Physiol.* 1): F307–F314, 1977.