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PERSPECTIVES IN BASIC SCIENCE

Renal magnesium handling: New insights in understanding old problems

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Our understanding of renal magnesium handling has been greatly expanded by research performed over this decade. Control of total body magnesium homeostasis principally resides within the nephron segments of the kidney. Magnesium is handled in different ways along the nephron segments (Fig. 1). About 80% of the total plasma magnesium (0.65 to 1.2 mM) is filtered through the glomerular membrane. Of the ultrafilterable magnesium (0.5 to 0.9 mM), 5 to 15% is reabsorbed by the proximal tubule, including the convoluted and straight portions. This is distinct from sodium and calcium where ~ 70 and ~60%, respectively, are reabsorbed in the proximal nephron segments. Accordingly, the delivery of magnesium to the thick ascending limb of the loop of Henle is relatively much larger than that of sodium and calcium. Proportionally greater amounts of magnesium (50 to 60%) are reabsorbed in the loop compared with sodium (20 to 25%) or calcium (30 to 35%). The loop of Henle, specifically the cortical segment, plays a major role in the determination of magnesium reabsorption. Of the 10 to 15% of the filtered magnesium that is delivered to the distal tubule from the loop of Henle, 70 to 80% is reabsorbed, leaving about 3% of the filtered magnesium normally appearing in the urine. As there is little evidence for significant magnesium absorption in the segments beyond the distal tubule, this portion plays an important role in determining the final urinary excretion. The cellular mechanisms of magnesium absorption within proximal tubule, loop, and distal tubule are very different and are distinct from calcium, but many of the controls are similar. This review discusses recent advances in our understanding of renal magnesium handling and some of the clinical implications of these observations (Table 1). The discussion is limited to recent observations, as more exhaustive reviews are available elsewhere [1–3].

PROXIMAL TUBULE

The adult proximal tubule reabsorbs little magnesium and appears to play a limited role in control of renal magnesium conservation. Intraluminal magnesium concentration rises along the proximal tubule as water is reabsorbed so that large concentrations flow into the loop [4–7]. The mechanisms by which magnesium is reabsorbed within the proximal tubule are not known, but it is likely to be transcellular as no detectable magnesium influx could be measured in proximal convoluted or straight segments perfused with solutions free of magnesium [8, 9]. Extracelluar volume expansion, with an inhibition of proximal NaCl and water absorption, has little effect on fractional magnesium reabsorption [10]. Sufficient volume expansion, however, may affect loop reabsorption leading to increased magnesium excretion [9, 11].

Magnesium handling in the neonatal proximal tubule may be quite different than that observed in the adult kidney. This phenomenon has been overlooked since it was first reported by Leliéve-Pegorier and colleagues [12]. They showed that luminal magnesium concentration remained similar to the ultrafilterable plasma magnesium level along the neonatal rat proximal tubule so that fractional magnesium absorption was in the order of 60 to 70%, equal to that observed for sodium or calcium. The basis for the enhanced magnesium reabsorption in the immature nephron relative to the adult may be a lack of maturation of the paracellular pathway, which would allow significant amounts of magnesium to move across with sodium, calcium, and water [12]. Accordingly, in the immature rat magnesium reabsorption is predominately in the proximal tubule rather than the loop of Henle. As such, it may be predicted that in young animals control of magnesium reabsorption is influenced more by factors acting in the proximal tubule rather than the loop or distal tubule. In addition, factors influencing magnesium transport in the loop and distal tubule may be less profound in the young than the mature nephron because conservation may continue in the proximal tubule as well as the loop and distal tubule. This notion could be easily tested with loop diuretics. It would also be interesting to study young patients with magnesium-wasting diseases such as Bartter's or Gitelman's syndromes, which are due to mutational defects in the loop and distal tubule, respectively, to determine if hypermagnesiuria is as much of a problem as observed in adult patients [13–15]. Finally, further work is required to determine the basis and progression of maturation of the proximal paracellular pathway and its importance in renal magnesium handling.

LOOP OF HENLE

Magnesium is principally reabsorbed within the thick ascending limb (TAL) of the loop of Henle [1, 3]. Using microperfusion techniques of isolated mouse TAL segments, de Rouffignac and colleagues have shown that only the cortical thick ascending limb

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Table 1. Summary of major influences that alter renal magnesium reabsorption

	Thick ascending limb of Henle's loop	
Increase:	Hormones	— parathyroid hormone
		— calcitonin
		— glucagon
		— vasopressin
		— isoproternol
		— insulin
	Magnesium restriction	
	Metabolic alkalosis	
Decrease:	Hypermagnesemia (Ca^{2+}/Mg^{2+}-sensing receptor)	
	Hypercalcemia (Ca^{2+}/Mg^{2+}-sensing receptor)	
	Diuretics	— furosemide
		— bumetanide
	Distal tubule	
Increase:	Hormones	— parathyroid hormone
		— calcitonin
		— glucagon
		— vasopressin
	Diuretics	— amiloride
		— chlorothiazide
	Magnesium restriction	
	Metabolic alkalosis	
Decrease:	Metabolic acidosis	
	Phosphate depletion	
	Potassium depletion	

(cTAL) is involved with magnesium reclamation as no transport was observed in the medullary segment (mTAL) [16]. They have further demonstrated that magnesium transport is mainly passive in nature [17], supporting the earlier studies of Shareghi and Agus [18]. Net magnesium absorption was entirely dependent on the transepithelial voltage (Fig. 2). With luminal positive voltage, magnesium moved from lumen into the bath whereas luminal negative voltage resulted in magnesium secretion into the lumen. No transport was observed at zero voltage consistent with passive transport. It is envisioned that magnesium normally moves across the cTAL epithelium through the paracellular pathway driven by the positive luminal transepithelial voltage (Fig. 3). Factors controlling magnesium absorption in this segment act through changes on the voltage and/or the permeability of the paracellular pathway (Table 1) [19]. Di Stefano, Mandon, Wittner, de Rouffignac and their colleagues have provided evidence that both ways are used to physiologically control magnesium reabsorption within the cTAL [20–24].

Wittner et al have shown that the permeability of the paracellular pathway may be influenced by age and sex so that maturational changes may influence passive transport of magnesium in the cTAL [25]. As with all cells, intracellular magnesium is essential for normal cellular metabolism. We have shown that magnesium enters cTAL cells via selective entry pathways, likely through Mg^{2+} channels [26]. Whether this cellular transport of magnesium plays a role in transepithelial absorption is not known; certainly the majority, if not all, of the magnesium absorption within the loop is paracellular [3].

DISTAL TUBULE

The distal tubule normally reabsorbs about 10% of the magnesium filtered through the glomerulus [3]. Although this seems like a small amount, it represents 70 to 80% of the magnesium delivered to this segment from the loop of Henle. As there is little magnesium reabsorption beyond the distal tubule in the collecting ducts, the tubule segments comprising this portion of the nephron play an important role in determining the final urinary excretion of magnesium. The role of this nephron segment in the control of magnesium has been comparatively ignored because of the greater amounts reclaimed in the loop. However, recent studies indicate that the distal convoluted tubule may play a more important role than that recognized previously.

Most of our early knowledge concerning magnesium transport in the distal tubule has come from micropuncture and microperfusion studies of the superficial nephron. Micropuncture studies showed that significant amounts of magnesium are reabsorbed in the distal tubule [9, 27–31]. The mammalian distal tubule, located between the macula densa and the cortical collecting duct, is comprised of a short post-macula densa segment of thick ascending limb, the distal convoluted tubule (DCT), the connecting tubule and the initial collecting tubule. The micropuncture studies describing distal magnesium absorption may well have included portions of the superficial connecting tubule and the initial collecting tubule in addition to the DCT. Magnesium absorption within each of these individual segments have yet to be studied.

The distal convoluted tubule (DCT) has not been extensively studied because of its inaccessibility and difficulty in isolation for *in vitro* microperfusion studies. Immortalized DCT cell lines have recently been used to describe cellular magnesium transport [32–34]. Using the Madin-Darby canine kidney (MDCK) cell line, which exhibits a distal tubule-like phenotype, we have shown that magnesium entry is through specific and regulated magnesium pathways [34]. Electrolyte transport is usually quantitated by isotopic flux measurements, but as an appropriate isotope for Mg^{2+} is not available (^{28}Mg has a half-life of 21 hr), a cell model was developed to assess Mg^{2+} transport using the fluorescent dye, mag-fura-2, to determine intracellular free Mg^{2+} concentration, $[Mg^{2+}]_i$. Cytosolic free Mg^{2+} concentration of epithelial cells is in the order of 0.5 mM [34]. This is about 1 to 2% of the total magnesium, the remainder being complexed to various organic and inorganic ligands and chelated within the mitochondria [35–37]. Presumably, it is the free Mg^{2+} that enters into biochemical processes and is transported across plasma membranes. In order to determine Mg^{2+} transport, the epithelial cells were first depleted of Mg^{2+} by incubating in magnesium-free culture media. Subsequently, the cells were placed in solutions containing magnesium and $[Mg^{2+}]_i$ measured as a function of time over the duration of Mg^{2+} influx (Fig. 4). The rate of concentration change, $d([Mg^{2+}]_i)/dt$, is an estimate of transport rate. Influx of Mg^{2+} is concentration-dependent so that the rate of Mg^{2+} transport increases with external Mg^{2+} until saturation is attained at about 0.5 mM [32]. Mg^{2+} influx into Mg^{2+}-depleted cells was inhibited by Mn^{2+} and La^{3+} and by dihydropyridine channel blockers such as nifedipine. Ca^{2+} neither blocked Mg^{2+} entry nor was ^{45}Ca uptake or $[Ca^{2+}]_i$, intracellular free Ca^{2+}, changed in the presence of Mg^{2+}-depletion or the Mg^{2+}-refill process [32]. These results suggest that the influx pathway is specific for Mg^{2+} and not shared by Ca^{2+}. We used this approach to characterize the cellular mechanisms of Mg^{2+} uptake in an established mouse distal convoluted tubule (MDCT) cell line. This cell line was originally isolated from mouse distal tubules and immortalized by Pizzonia et al [38]. MDCT cells exhibit many of the functional properties characteristic of the *in vivo* distal convoluted tubule,

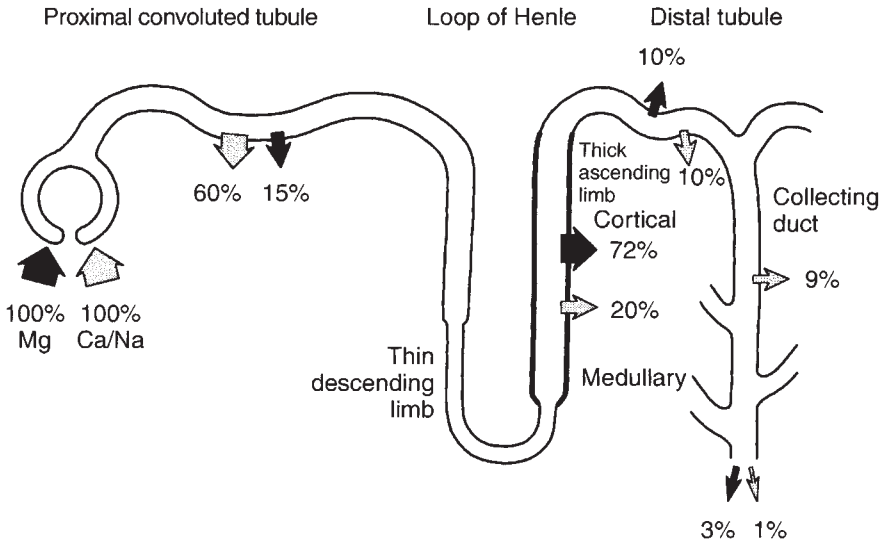

Fig. 1. Summary of segmental magnesium absorption along the nephron relative to sodium and calcium reabsorption.

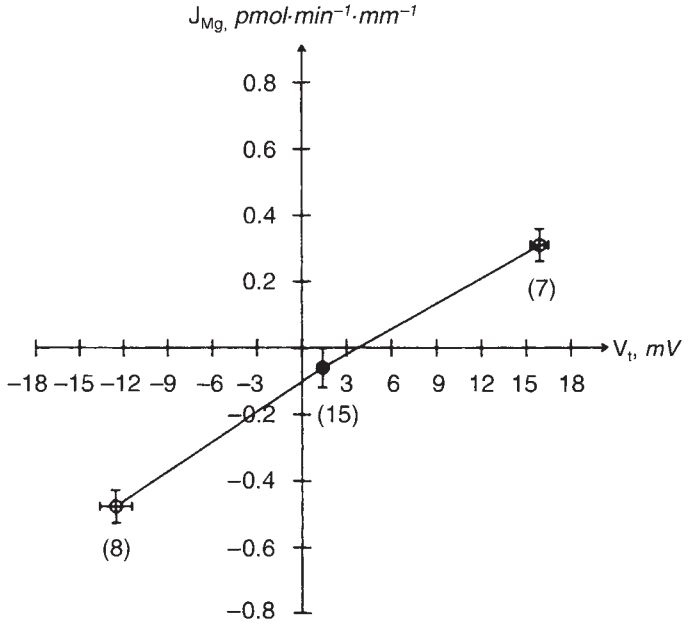

Fig. 2. Magnesium net fluxes (J_{Mg}) measured in absence of active NaCl transport (lumen, 10^{-4} M furosemide) in mouse cTAL segments. V_t indicates the transepithelial voltage, lumen with respect to bath. Flux-voltage relationships were measured in two experimental series: (1) with symmetrical solutions in lumen and bath (150 mM NaCl, solid circles); (2) in presence of hyposmotic luminal solutions (lumen, 50 mM NaCl; bath, 150 mM NaCl), generating positive dilution voltages (open circle in top right quadrant); and (3) with symmetrical solutions in lumen and bath (150 mM NaCl, solid circles) and in presence of a bath solution in which NaCl concentration was less than the luminal perfusate (lumen, 150 mM NaCl; bath, 50 mM NaCl + 200 mM mannitol), generating negative dilution voltages (open circle in bottom left quadrant). Data from di Stefano et al [17].

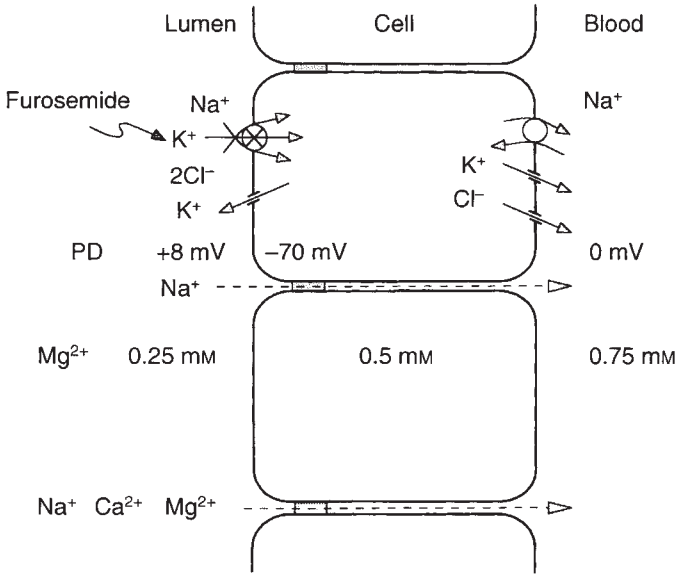

Fig. 3. Schematic model of magnesium absorption in thick ascending limb of Henle's loop. Conductive pathways are denoted by dashed arrows and carrier-mediated transport by solid arrows. Active transport processes are indicated by ~ symbol. Modified from Quamme [1].

such as amiloride-inhibitable Na^+ transport, chlorothiazide-sensitive NaCl cotransport and parathyroid hormone (PTH)- and calcitonin-stimulated Ca^{2+} transport [39].

Our initial studies looked at the changes in Mg^{2+} entry into MDCT cells following alterations in membrane voltage [32]. In these studies, with the same starting $[Mg^{2+}]_i$ and the same external Mg^{2+} concentration, the more negative the membrane voltage, that is, hyperpolarization, the higher the magnesium influx rate. Conversely, depolarization of membrane voltage diminishes Mg^{2+} uptake. The dependence of magnesium entry on the driving force induced by the electrochemical gradient indicates that cellular Mg^{2+} entry may be mediated by an ion channel. As the distal tubule is characterized by a negative luminal transepithelial voltage and high epithelial resistance, it is concluded that magnesium transport is active and transcellular in nature. The pathways involved with transcellular Mg^{2+} absorption are schematically illustrated in Figure 5. The evidence is that magnesium moves passively into the cell across the luminal

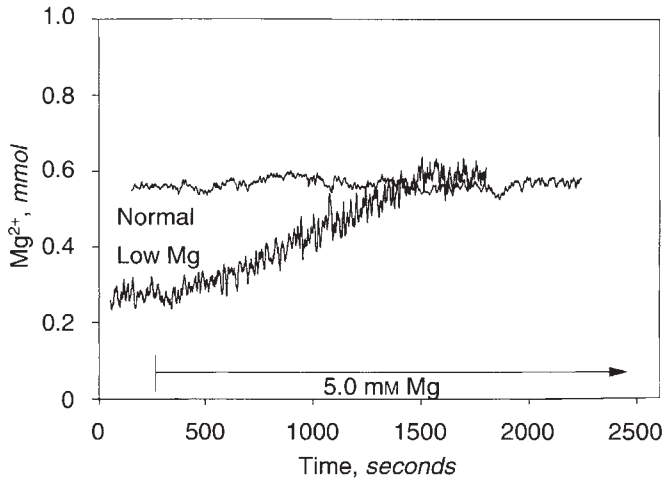

Fig. 4. Intracellular free Mg^{2+} concentration in normal and Mg^{2+}-depleted Madin-Darby canine kidney (MDCK) cells. Confluent MDCK cells were cultured in either normal (0.6 mM Mg^{2+}) or Mg^{2+}-free media (<0.01 mM) for 16 hours. Fluorescence studies were performed in buffer solutions in absence of Mg^{2+}, and, as indicated, $MgCl_2$ (5.0 mM final concentration) was added to observe changes in intracellular Mg^{2+} concentration. 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. Fluorescence was measured at 1 data point/second with 25-point signal averaging, and the tracing was smoothed according to methods previously described. Data from Quamme and Dai [34].

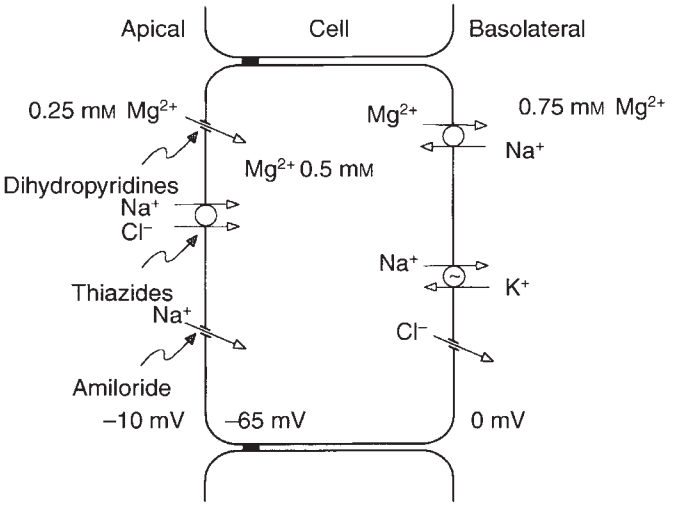

Fig. 5. Schematic model of magnesium absorption in the distal convoluted tubule. Conductive pathways and carrier-mediated transport are denoted by solid arrows. Active transport processes are indicated by ~ symbol. The sites of action of the transport inhibitors; dihydropyridines (such as nifedipine), thiazides, and amiloride are indicated.

membrane driven by a favorable transmembrane voltage. The luminal magnesium concentration in the distal tubule is in the order of 0.2 to 0.7 mM, depending on the condition studied, and intracellular free Mg^{2+} is about 0.5 mM. Thus, under some circumstances Mg^{2+} entry is against an appreciable concentration gradient [40]. We speculate that Mg^{2+} entry is through a unique channel and transport is dependent on the transmembrane voltage. The active step in transcellular movement is predicted to be at the basolateral membrane where Mg^{2+} leaves the cell against both electrical and concentration gradients. The means by which Mg^{2+} actively moves across the basolateral membrane is unknown. Evidence taken from studies using nonepithelial cells suggest that a Na^+-Mg^{2+} exchange may occur; Na^+ moving back into the cell coupled with Mg^{2+} exits from the cell into the interstitium [41, 42]. In this view, the factors that influence transcellular magnesium absorption include alterations of the entry step at the luminal membrane and changes in activity of the exit step at the basolateral membrane. As with other ions, it is likely that the entry step is rate-limiting and controls transepithelial magnesium reabsorption in DCT cells.

HORMONAL CONTROL OF RENAL MAGNESIUM REABSORPTION

A large number of hormones have been implicated in the control of renal magnesium conservation. Extensive description of hormone actions have been reviewed elsewhere [1–3]. Parathyroid hormone (PTH), calcitonin, glucagon, and vasopressin (AVP) stimulate magnesium absorption in both the TAL [9, 24, 31, 43–48] and DCT [9, 11, 27, 28, 46, 47, 49]. Insulin increases magnesium absorption in mouse cTAL; the effects of this hormone in the DCT have not been studied [22]. Finally, steroid hormones influence NaCl and magnesium absorption within the TAL [50–52] and DCT [51, 53]. All of these hormones stimulate magnesium absorption in the TAL and DCT by very different cellular mechanisms.

Thick ascending limb

Receptors for PTH, calcitonin, glucagon, AVP, and the β-adrenergic agonists such as isoproterenol are coupled to adenylate cyclase in the TAL [54–57]. In addition, these receptors likely are also coupled to other signaling pathways that may be interactive with cAMP-mediated actions [3, 58]. Shareghi and Agus microperfused rabbit cTAL segments and measured magnesium fluxes following addition of PTH to the bath [18]. They showed that magnesium absorption was stimulated in the absence of changes in transepithelial voltage. Using mouse cTAL segments, Wittner and colleagues showed that all of the above hormones enhanced both transepithelial voltage and magnesium absorption (Fig. 6) [24, 48]. However, the stimulation of magnesium transport was not proportional to the voltage change. The latter results led these investigators to conclude that hormonal actions in the mouse cTAL resulted from both a rise in transepithelial voltage and an increase in magnesium permeability of the paracellular pathway.

The cellular mechanisms underlying hormone-induced increase in transepithelial voltage have been clarified by Greger, Bleich and Schlatter [59] and Reeves and Andreoli [60]. Receptor-mediated activation of basolateral membrane Cl^- conductance and apical Na^+-$2Cl^-$-K^+ cotransport/K^+ conductance increases the luminal positive voltage. These hormones also increase the paracellular permeability but the mechanisms are not clear. de Rouffignac postulates that hormonal control of paracellular pathway proteins, possibly through phosphorylation, may change the permeability and allow for a greater movement of magnesium across the epithelium from the lumen to the interstitium [3]. Further studies are necessary to define the molecular mechanisms involved with this control.

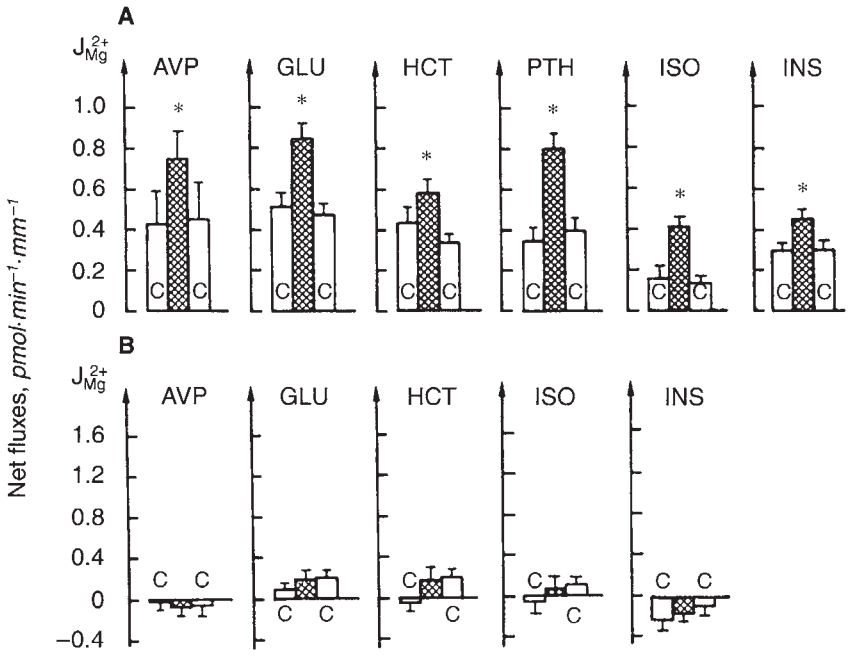

Fig. 6. Individual effects of peptide hormones on net magnesium transport in isolated thick ascending limb segments. Isolated segments of either cortical (cTAL) or medullary (mTAL) thick ascending limbs (panels *A* and *B*, respectively) of mouse nephrons were microperfused at 2 nl/min in absence (c, control) and presence of arginine vasopressin (AVP; 10^{-10} M), glucagon (GLU; synthetic porcine glucagon, 1.2×10^{-8} M), calcitonin (HCT; synthetic human calcitonin, 3×10^{-8} M), parathyroid hormone (PTH; bovine, 1 to 34 fragment, 10^{-8} M), isoproterenol (ISO; 10^{-7} M), or insulin (INS; 10^{-7} M). Hormones were added to bath solutions. *Significance from preceding period. Data are from Bailly et al [54], Mandon et al [22], and Wittner et al [24, 48].

In addition to hormones acting through G_s-coupled proteins, Mandon et al have reported that insulin increases transepithelial voltage and stimulates magnesium absorption in perfused mouse cTAL segments [22]. Accordingly, signaling pathways involving tyrosine kinases may also be involved in changes in voltage and paracellular pathway permeability. Further studies are needed to define the individual receptor-mediated pathways and their interactions that are involved with hormone-stimulated magnesium transport in the TAL.

In all of the above *in vitro* studies performed by Bailly, Di Stefano, de Rouffignac, and Wittner and colleagues, no magnesium absorption was observed in the mTAL (Fig. 6). Their conclusions indicate that the passive permeability of the mouse mTAL to divalent cations is very low and not influenced by AVP [23]. There may be species differences so that in some instances magnesium may be absorbed in the medullary segment of the TAL.

The evidence suggests that steroid hormones augment NaCl absorption and transepithelial voltage in isolated TAL segments [50, 52]. Although there have been no direct studies of the effects of mineralocorticoid on TAL magnesium transport, the increment in voltage would be expected to lead to an increase in magnesium absorption. However, chronic hyperaldosteronism leads to extracellular volume expansion and diminished salt absorption in the TAL [61]. This is associated with elevated urinary magnesium excretion [62]. Salt restriction and normalization of the extracellular volume mitigates aldosterone-induced hypermagnesuria [63]. In summary, the effects of steroid hormones within the TAL are complex; aldosterone enhances salt and probably magnesium retention that leads to volume expansion that in turn leads to increased salt and magnesium excretion.

Distal tubule

Adenylate cyclase-coupled hormone receptors are present along the distal tubule including the DCT [56, 57]. Studies have shown that there are apparent species differences in receptor-mediated adenylate activity, and as for the cTAL there are probably other receptor-mediated signaling pathways in addition to cAMP-dependent processes [64–67]. These pathways are not well defined at the present time. The early micropuncture studies showed that PTH, calcitonin, and glucagon increased magnesium absorption in the distal tubule, but they did not discern the cellular mechanisms involved [9, 11, 27, 28, 31, 45, 47, 49]. As magnesium absorption in the distal tubule is transcellular and active in nature, hormones act within the cell on active transport rather than passively through the paracellular pathway.

Recent studies have shown that PTH, calcitonin, glucagon, and AVP stimulate Mg^{2+} uptake into MDCT cells (personal observation; Fig. 7). As these hormones also stimulate cellular cAMP accumulation, we determined whether cAMP may influence Mg^{2+} entry into MDCT cells. The addition of cAMP increased Mg^{2+} uptake whereas inhibition of protein kinase A prevented hormone-stimulated uptake [68]. Accordingly, receptor-mediated cAMP release and activation of protein kinase A plays a role in hormone-stimulated Mg^{2+} uptake in MDCT cells. Other signaling pathways may also be involved in control of magnesium transport in the distal tubule [65–67]. The cellular mechanisms whereby cAMP and protein kinase A activation enhance Mg^{2+} entry is unclear.

Mineralocorticoid receptors are present in DCT cells that are thought to be involved in expression of NaCl cotransport, Na^+ conductance, and sodium pump activity [69]. The effects of aldosterone on magnesium transport with the intact distal tubule have not been studied. We have studied the effects of aldosterone on Mg^{2+} entry into MDCT cells [53]. Incubation of aldosterone, 10^{-7} M, for 16 hours prior to the determination of Mg^{2+} uptake failed to have any effect on basal magnesium transport. However, pretreatment of MDCT cells with aldosterone potentiated glucagon- and AVP-stimulated Mg^{2+} uptake (Fig. 8).

The explanation for these observations may be based on the

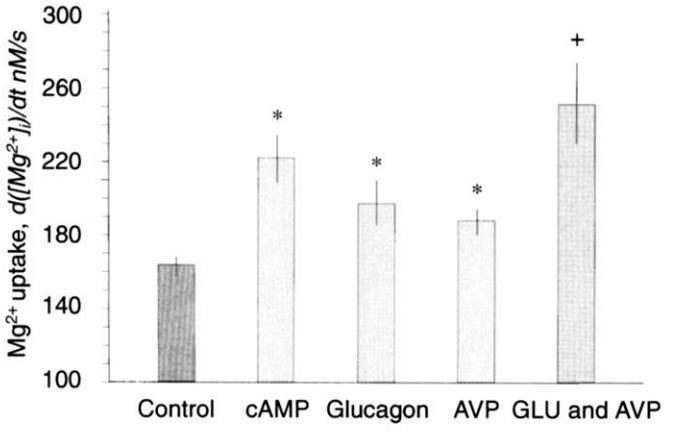

Fig. 7. Hormonal stimulation of Mg^{2+} uptake in Mg^{2+}-depleted mouse distal convoluted tubule (MDCT) cells. The studies were performed according to methods given in legend to Figure 4 but with the addition of 1.5 mM $MgCl_2$. The mean Mg^{2+} uptake rate, $d([Mg^{2+}]_i/dt$, was determined as the change in Mg^{2+} concentration with time over the first 200 seconds of study. 8-bromo cAMP, 10^{-4} M, glucagon, 10^{-7} M, and arginine vasopressin, 10^{-7} M, were added the buffer solution where indicated. Values are means ± SE, $N = 5$ to 10 cells. *Significant from control values and $^{+}$significant of glucagon plus AVP vs. either glucagon or AVP alone. Data are from Dai et al [68].

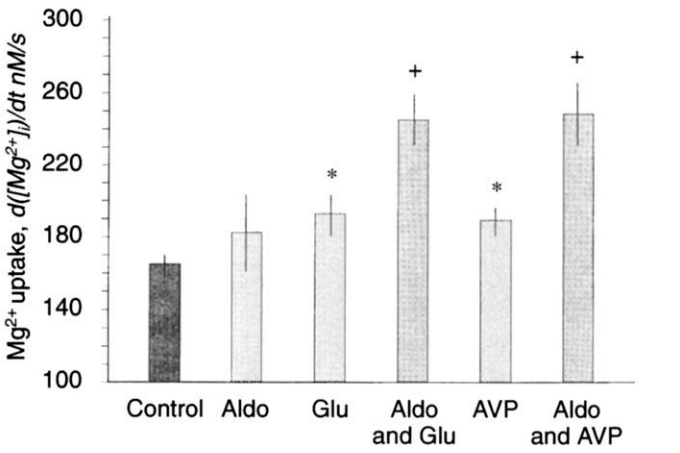

Fig. 8. Aldosterone potentiates glucagon- and AVP-stimulation of Mg^{2+} entry in MDCT cells. MDCT cells were incubated with and without aldosterone (Aldo.), 10^{-7} M, for 16 hr prior to the determination of Mg^{2+} uptake. Glucagon or AVP, 10^{-7} M, was added where indicated. The rate of Mg^{2+} influx as determined by $d([Mg^{2+}]_i)/dt$ was measured with the given hormone concentrations using fluorescence techniques performed according to those given in legend to Figure 4. Values are means ± SE for 3 to 6 cells. *Significant from control values and + significance of glucagon + Aldo. or AVP + Aldo. versus glucagon or AVP, respectively. Data are from Di et al [53].

observation that mineralocorticoids enhance hormone-stimulated cAMP generation in epithelial cells. For instance, adrenal insufficiency is associated with impairment of urinary diluting and concentrating capacity [70, 71; reviewed in 72]. Rajerison et al demonstrated that adrenalectomy reduced vasopressin-stimulated adenylate cyclase activity in membrane fractions prepared from rat kidney medulla [71]. Doucet et al have shown that glucagon- and AVP-responsive cAMP generation is diminished in thick ascending limb and collecting tubule segments harvested from adrenalectomized rats compared to animals treated with physiological doses of aldosterone [72, 73]. These investigators postulate that aldosterone induces a protein(s) that stimulates hormone-sensitive adenylate cyclase activity. Studies with kidney membrane fractions and isolated segments demonstrated that an impairment of coupling between hormone receptors and adenyl cyclase catalytic units was responsible for diminished cAMP generation in the absence of aldosterone [73]. The mechanism(s) through which steroids control G_S proteins (synthesis and/or degradation vs. activity of each unit) is not known [72]. Our studies with MDCT cells suggest that aldosterone potentiates glucagon- and AVP-stimulated cAMP generation in distal convoluted tubule cells [53]. This was associated with an increase in Mg^{2+} entry rate in response to these hormones. Aldosterone also induces an increase in Na^+ conductance and sodium pump activity among other metabolic changes [69]. Further research is warranted to determine the involvement of these changes in hormone-mediated control of Mg^{2+} transport. As mentioned above, mineralocorticoid excess with extracellular fluid volume expansion results in increased urinary magnesium excretion as well as salt excretion [63].

Multihormonal control of renal magnesium reabsorption

The above list of hormones is far from exhaustive and further research will in all likelihood identify others that affect renal magnesium handling [3]. There appears to be no single hormone that controls renal magnesium balance, rather many hormones act in concert to regulate magnesium. Elalouf et al have shown that glucagon and vasopressin are additive in promoting magnesium absorption within the cTAL whereas Mandon et al and Elalouf et al reported that insulin potentiates the actions of vasopressin [22, 45]. Moreover, many of the hormones interact to control magnesium transport. In the distal tubule, glucagon and AVP are additive or, as we have seen with aldosterone, potent in their effects on hormone stimulation of magnesium reabsorption (Figs. 7 and 8) [45, 74]. Individual hormonal control of magnesium conservation may be important in specific instances. For instance, PTH and calcitonin are principally involved in the regulation of calcium balance. The conservation of magnesium with calcium may facilitate calcium deposition. A further example is glucagon. A protein meal is a potent stimulus for glucagon secretion. Elevated circulating glucagon concentration contribute to the disposal of nitrogen metabolites, amino acids, and elevation of GFR [75]. Glucagon may be important in magnesium conservation following protein ingestion because of its associated increase in GFR and filtered magnesium. Additionally, stimulation of loop and distal magnesium conservation by AVP may be necessary to maintain normal magnesium balance when salt and water flow in the DCT is reduced during antidiuresis. Finally, mineralocorticoid potentiation of AVP actions may be necessary to maintain normal Mg^{2+} balance when salt and water flow in the distal tubule is reduced during aldosterone-mediated antidiuresis. Mineralocorticoid potentiation would increase AVP-stimulated Mg^{2+} reabsorption commensurate with enhanced salt and water retention. Perhaps the multihormonal control of renal magnesium absorption is a more efficient way of maintaining magnesium balance than what would be expected with a dedicated hormone.

PLASMA MAGNESIUM AND CALCIUM CONTROL RENAL MAGNESIUM REABSORPTION

Thick ascending limb

Elevation of plasma magnesium or calcium concentration inhibits magnesium and calcium reabsorption, leading to hypermagnesiuria and hypercalciuria. Inhibition of reabsorption occurs within the cTAL segment of the loop of Henle where both magnesium and calcium are reabsorbed primarily by passive mechanisms [9, 18, 23, 76]. The simplest explanation was that elevation of magnesium or calcium decreased the permeability for these cations in the paracellular pathway, so that for any given transepithelial voltage there is less magnesium and calcium absorption. In support of this notion, Di Stefano et al perfused isolated rabbit cTAL segments and reported an increase in transepithelial resistance by about 50% with an increase of luminal or bath magnesium and calcium of 2.5 to 10 mM [76]. However, this does not explain our earlier observations indicating that magnesium and calcium act only from the peritubular or blood side and not the luminal side [1, 9]. *In vivo* microperfusion of rat TAL segments clearly showed that increases in luminal magnesium concentrations were associated with increases in magnesium absorption rates [9]. Intraluminal magnesium or calcium did not inhibit either magnesium or calcium absorption [77]. However, elevation of plasma magnesium or calcium, that is, on the basolateral side of the TAL, resulted in inhibition of both magnesium and calcium absorption [9, 77]. The explanation for the asymmetrical actions of elevated magnesium and calcium across the TAL epithelium was not evident at the time that these studies were performed. The recent identification of a Ca^{2+}/Mg^{2+}-sensing receptor located on the peritubular side of TAL cells explains this phenomenon [78, 79].

A Ca^{2+}/Mg^{2+}-sensing receptor has been found in glomeruli, proximal tubules, cortical and medullary thick ascending limbs, distal convoluted tubules, cortical collecting ducts, and outer medullary collecting ducts [80]. This receptor was first cloned from the bovine parathyroid gland [78]. The cDNA encodes a G protein-coupled receptor that is involved with control of parathyroid hormone (PTH) secretion. The cDNA sequence suggested that the receptor is comprised of three major domains: (*1*) a large extracelluar amino-terminal domain of 613 amino acids that is thought to possess the cationic binding sites; (*2*) a 250 amino acid domain with seven predicted membrane-spanning segments characteristic of the superfamily of G protein-coupled receptors; and (*3*) a carboxyl terminal domain of 222 amino acids that likely resides within the cytoplasm and is involved with intracellular signaling processes [79]. The renal receptor is very similar to the one found in the parathyroid gland [81]. The evidence is that Ca^{2+} or Mg^{2+} binds to the extracelluar domain initiating a number of intracellular signals. Among other things, stimulation of G_i-proteins modulate adenylate cyclase activity and cAMP levels and G_q proteins activate phospholipase C releasing inositol 1,4,5-trisphosphate, cytosolic Ca^{2+} and cytochrome P-450 metabolites [79, 82, 83]. Ca^{2+}/Mg^{2+}-sensing receptor mediated intracellular signaling pathways have important effects on cellular function [79, 84]. Using immunocytochemistry, Riccardi and colleagues have shown the receptor is localized to the luminal membrane of proximal tubules and the inner medullary collecting ducts and to the basolateral membrane of cTAL and mTAL cells (data presented at the 1997 ASN). The site and location of the Ca^{2+}/Mg^{2+}-sensing receptor(s) have important effects on renal magnesium handling. Wang and Hebert reported that basolateral receptor activation inhibits apical K^+ channels and possibly Na-2Cl-K cotransport in the rat TAL [83]. This inhibition would be expected to diminish transepithelial voltage and, in turn, passive transport of sodium in the mTAL and sodium, magnesium, and calcium within the cTAL leading to increased distal delivery of these electrolytes. Elevation of divalent cations in the medullary collecting duct activates the luminal Ca^{2+}/Mg^{2+}-sensing receptor that diminishes water permeability and inhibits volume reabsorption [84]. The net effect is to increase volume flow along with an increase in calcium and magnesium excretion. The notion advanced by Sands et al is that a concomitment increase in volume flow with inhibition of calcium and magnesium reabsorption would minimize the incidence of stone formation [85]. It is clear from these studies that the presence of a Ca^{2+}/Mg^{2+}-sensing receptor in the TAL allows for an additional control of renal magnesium absorption.

Clearance studies have suggested that renal magnesium reabsorption is a Tm-limited process, that is, the kidney possesses a tubular maximum for transport that can be saturated with elevations of plasma magnesium [63, 86]. Determination of the Tm value as been useful in assessment of renal magnesium conservation [86, 87]. Micropuncture studies have shown that this phenomenon is due to segmental differences in magnesium absorption [11]. The cellular basis for a Tm is inhibition of magnesium transport within the loop of Henle [1, 9]. It is likely that activation of a Ca^{2+}/Mg^{2+}-sensing receptor within the thick ascending limb plays a role in this Tm phenomenon.

Distal tubule

The Ca^{2+}/Mg^{2+}-sensing receptor has been localized by immunocytochemical techniques along the entire length of the distal tubule [80]. The receptor was found on the basolateral membrane of the DCT but was less abundant than that of the TAL (presented by Riccardi et al at the 1996 ASN). The function of the Ca^{2+}/Mg^{2+}-sensing receptor within the distal tubule is not fully known. The Ca^{2+}/Mg^{2+}-sensing receptor is present in the MDCT cell line so that studies may be performed to determine its role in this segment [88]. The concentrations of extracellular Ca^{2+} or Mg^{2+} that activate this receptor are in the order of 0.2 to 3.0 mM, which is appropriate for physiological responses. Interestingly, either Ca^{2+} or Mg^{2+} may activate the receptor in the presence of high levels of extracellular Ca^{2+} or Mg^{2+} [88]. More recently, we have shown that activation of the Ca^{2+}/Mg^{2+}-sensing receptor does not inhibit basal Mg^{2+} entry in MDCT cells but it does inhibit hormone-stimulated Mg^{2+} uptake [89]. As receptor activation with neomycin, Ca^{2+} or Mg^{2+} also inhibits PTH-, calcitonin-, glucagon-, and AVP-stimulated cAMP release, it was concluded that the receptor diminishes hormone-stimulated Mg^{2+} uptake through inhibition of hormone-induced cAMP generation. It is inferred that the Ca^{2+}/Mg^{2+}-sensing receptor plays an important role in hormonal regulation of magnesium absorption in the DCT [89].

Diseases involving the Ca^{2+}/Mg^{2+}-sensing receptor

It has been shown that both inactivating and activating mutations of the Ca^{2+}/Mg^{2+}-sensing receptor are found in clinical medicine [90, 91]. Two rare hypercalcemia disorders, familial hypocalciuric hypercalcemia (FHH) [92–95] and neonatal severe

hyperparathyroidism (NSHPT) result from inactivating mutations when present in the heterozygous and homozygous states, respectively [96, 97]. The renal excretion of both calcium and magnesium is reduced in these patients leading to hypercalcemia and in some instances to hypermagnesemia [92–95]. As reviewed by Hebert, defective extracellular Ca^{2+}/Mg^{2+}-sensing likely results in inappropriate absorption of calcium and magnesium in the TAL and possibly other segments leading to diminished excretion and elevated plasma levels [82]. A knockout mouse model has been developed with all of the characteristics of FHH and NSHPT supporting this idea [98]. On the other side, an autosomal-dominant inherited activating mutation has also been reported that was associated with hypocalcemia and hypomagnesemia in about half the patients, presumably due to inappropriate renal wasting [91, 99]. It would be interesting to determine the effects of amiloride, a renal-conserving diuretic, in these patients to see if renal magnesium-wasting could be ameliorated.

INTRINSIC CELLULAR CONTROL OF RENAL MAGNESIUM REABSORPTION

All of the above receptor-mediated controls also influence calcium and sodium reabsorption in the TAL and calcium absorption in the DCT. What then selectively controls renal handling of magnesium? It has been recognized clinically for many years that diminished dietary magnesium intake or intestinal malabsorption leads to appropriate renal magnesium conservation in an effort to maintain magnesium balance [100–102]. This response is sensitive and independent of renal sodium and calcium excretion. The basis for this alteration in magnesium transport is not fully understood. However, it is clear that the fall in renal magnesium excretion is not simply due to a drop in plasma and filtered magnesium because a number of clinical studies have reported diminished urinary magnesium excretion with normal serum magnesium concentrations. In support of these clinical observations, we performed studies [40] in rats pair-fed control magnesium diets (0.05% Mg_2SO_4) and magnesium-restricted diets (< 0.01% Mg). Fractional urinary magnesium excretion of magnesium-restricted rats fell from 17 ± 3% to 8 ± 1% within eight hours without a change in plasma magnesium concentration. Accordingly, this cellular adaptation is rapid (detected within 2 hr), specific (without effect on sodium and calcium reabsorption), and sensitive (without detectable changes in plasma magnesium concentration). Micropuncture [40] and microperfusion [103] studies demonstrated that the cellular adaptation occurred within the thick ascending limb and the distal tubule. This ability to adjust transport appears to be intrinsic to renal cells. In summary, no extrinsic hormone controls renal magnesium transport separate from sodium and calcium handling. Rather, we believe that epithelial cells appropriately "adapt" their transport rate according to the availability of magnesium. This notion has received further support with *in vitro* microperfusion studies of cTAL segments and investigations using isolated distal tubule cells [26, 32, 34].

Thick ascending limb

de Rouffignac and colleagues have recently shown that magnesium absorption is increased in cTAL segments harvested from dietary magnesium- deprived mice compared to control animals [103, personal communication]. Their studies showed that increased absorption was due to an increase in permeability of the paracellular pathway for magnesium that enhances magnesium movement at any given transepithelial voltage. Again, the increment in magnesium transport in the loop was entirely passive in nature.

Distal tubule

We showed that isolated distal tubule cells, either MDCK or MDCT, cultured in magnesium-free media increased their Mg^{2+} transport rate [32, 34]. This response is rapid (within 1 to 2 hr), and specific for magnesium as there was no effect on sodium or calcium transport. The "adaptation" of magnesium transport rates is intrinsic as there were no hormones in the culture media (Fig. 2). Furthermore, the adaptation was dependent on the concentration of media magnesium and the length of time in the culture media [26].

The response of epithelial cells to magnesium-free culture media is rapid; it is detectable within one to two hours. The increase in Mg^{2+} refill rate is not maximal until ~four to six hours. This suggests that in addition to increasing the entry rate there may also be recruitment of new or formed transporters into the apical membrane. To test this possibility we pretreated MDCT cells with either actinomycin D, an inhibitor of transcription, or cycloheximide, an inhibitor of protein synthesis. These inhibitors led to a decrease of about 50% in the adaptive response as measured by Mg^{2+} refill rate in Mg^{2+}-depleted cells (unpublished observations). From these studies, we infer that Mg^{2+}-depletion results in genetic expression of new proteins that are involved in the adaptative response leading to enhanced Mg^{2+} uptake. Further studies are necessary to characterize the gene product(s) involved and the turnover of the components known to be important in up-regulation of Mg^{2+} transport.

In summary, these studies with isolated distal cells support the notion of intrinsic controls within the cells that adapt their magnesium transport rate appropriately to the environmental magnesium. We postulate that this intrinsic adaptation provides the discriminatory control of magnesium transport independent of sodium and calcium. Intrinsic adaptation provides the selective control that hormonal regulation does not have. These studies with isolated cells are in keeping with the experimental observations within intact kidney [40].

Diseases involving intrinsic cellular control of renal magnesium reabsorption

A practical use of this property of renal cells to adapt to magnesium availability is the clinical assessment of magnesium homeostasis. As most of the magnesium within the body is intracellular, determination of plasma magnesium may not reflect magnesium status [104]. Some clinical laboratories use magnesium retention following parental administration of magnesium salts [105, 106], and others measure mononuclear blood cell or platelet magnesium levels to assess magnesium status [102, 107–109]. These approaches are not particularly useful in the routine laboratory. As described above, the kidney is very sensitive to magnesium, so that urinary magnesium excretion relative to the plasma concentration reflects magnesium status. Accordingly, the simplest test is to evaluate renal magnesium physiology. This can be done by collecting a 24-hour urine specimen and calculating a

24-hour magnesium clearance [104]. This of course holds true only in the absence of renal disease.

Familial hypomagnesemia due to renal magnesium-wasting is an uncommon disease [110, 111]. The MEDLINE lists over fifty case reports in the last decade and it is probable that many presentations go unreported. Familial magnesium-wasting disease is by all accounts an autosomal recessive disease [112–116]. However, one study reported dominant penetrance [117]. The complexity of the clinical reports suggest that familial hypomagnesemia may be a spectrum of genetic diseases [118–123]. It is often associated with hypercalcemia and at times with hypokalemia [112, 115, 122–124]. Amiloride may be beneficial in some cases but not in others [110, 118]. Renal magnesium-wasting may be due to defective transport in the thick ascending limb or the distal tubule resulting in the complexity of presentation. Mutations of transport proteins or regulatory elements controlling transport are likely to be basis for these familial diseases. We have recently identified a gene using differential display of amplified cDNAs from MDCK cells grown in low magnesium [125]. The message for this gene, termed the magnesium-responsive element, is enhanced in magnesium-deficient DCT cells. Furthermore, transfection of MDCK cells with antisense oligonucleotides of this response-element prevented the up-regulation of Mg^{2+} entry expected with low magnesium. Further studies are underway to define its role in normal control of magnesium transport. Changes in expression of this gene could well provide the basis for altered renal magnesium handling.

DIURETICS

Thick ascending limb

Loop diuretics, such as furosemide and bumetanide, diminish salt absorption in the TAL by virtue of their action on electroneutral Na-2Cl-K cotransport across the luminal membrane [59, 60]. Inhibition of luminal Cl^- entry leads to diminished cellular Cl^- activity and diminished basolateral conductive Cl^- efflux, which results in a decrease in lumen-positive voltage [17]. It has been postulated that the lumen-positive voltage provides the driving force through the paracellular route for 40 to 50% of the total net sodium absorption. Magnesium, a divalent cation, may be influenced to a greater degree than the monovalent cations, such as sodium and potassium. This notion is supported by our *in vivo* microperfusion findings illustrating that net magnesium transport is inhibited to a greater degree than sodium at any given luminal furosemide concentration [30]. The observation of fractionally greater inhibition of TAL magnesium absorption compared with sodium is relevant to the management of patients receiving loop diuretics because hypomagnesemia is a possible complication of diuretic therapy.

Distal tubule

The distally acting diuretics, amiloride and chlorothiazide stimulate magnesium reabsorption within the distal convoluted tubule. A large number of clinical studies have led to the notion that amiloride possesses magnesium-conserving properties in addition to its natriuretic and potassium-sparing effects [118, 126]. Despite these observations very few experimental studies have been published concerning amiloride effects on renal magnesium handling. Devane and Ryan have shown that infusion of amiloride reduced the fractional excretion of magnesium in anaesthetized rats, which they attributed to a direct renal action of the drug [127]. The nephron segments and cellular mechanisms were not delineated in this study.

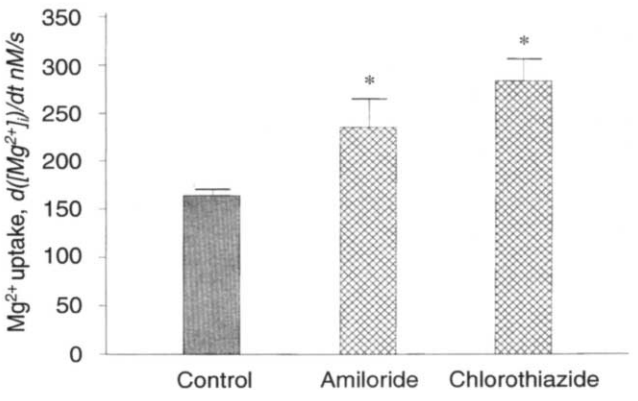

Fig. 9. Effect of amiloride or chlorothiazide on Mg^{2+} influx into normal and Mg^{2+}-depleted MDCT cells. Fluorescence was measured according to techniques illustrated in Figure 4. Amiloride or chlorothiazide (CTZ) at final concentrations of 10^{-4} M were added from stock solutions where indicated. Tracings are representative of 6 studies. Data are from Dai et al [32, 33].

We determined the cellular effects of amiloride on Mg^{2+} uptake in isolated MDCT cells [32]. Amiloride stimulated nifedipine-sensitive Mg^{2+} influx by 41 ± 3% (Fig. 9). Amiloride blocks Na^+ entry into DCT cells and hyperpolarizes the cell by −28 ± 8 mV [128]. As amiloride does not stimulate Mg^{2+} uptake in the absence of a change in voltage, we concluded that it acts through hyperpolarization of the membrane voltage, thereby increasing the driving force for Mg^{2+} entry [32]. These findings provide the basis for both clinical and experimental observations that show that amiloride is a magnesium-conserving diuretic [127, 129, 130].

Thiazides are extensively used in the management of diseases due to fluid retention, diabetes insipidus, and nephrolithiasis. Despite their widespread use, little is known about the actions of thiazides on cellular magnesium transport. Some studies have reported that patients receiving chlorothiazide may develop magnesium deficiency due, most likely, to renal magnesium-wasting [126, 131].

Recent studies with isolated MDCT cells have shown that chlorothiazide increases Mg^{2+} uptake in a dose-dependent fashion (Fig. 9). Maximal concentrations (10^{-4} M) of chlorothiazide increased Mg^{2+} transport by 58% [33]. This was associated with hyperpolarization of the plasma membrane voltage from −65 ± 5 to −80 ± 5 mV. Inhibition of Na-Cl cotransport and diminished intracellular sodium and chloride concentration results in hyperpolarization of the apical membrane of the distal convoluted tubule cells [132, 133]. An increase in the membrane voltage enhances Mg^{2+} uptake into MDCT cells. Accordingly, chlorothiazide may stimulate Mg^{2+} transport through changes in the membrane voltage similar to the basis of amiloride actions. The studies with MDCT cells demonstrate that chlorothiazide enhance Mg^{2+} entry in distal convoluted tubule cells. It is inferred that enhanced influx would translate into an increase in magnesium reabsorption and diminished urinary magnesium excretion.

The clinical use of thiazide diuretics in patients over a long

period of time sometimes lead to hypomagnesemia, probably from renal magnesium-wasting [130, 134–137]. Chlorothiazide inhibits Na-Cl cotransport, leading to an increase in urinary NaCl excretion and contraction of the extracelluar fluid volume. The renin-angiotensin system is activated, resulting in elevated aldosterone levels and increased potassium and hydrogen ion secretion, which in turn may result in hypokalemia and exacerbation of metabolic alkalosis. Volume depletion, elevated aldosterone, and metabolic alkalosis increase renal magnesium conservation so that it is unlikely that these influences would lead to magnesium-wasting [104]. However, hypokalemia has been associated with altered renal magnesium handling [115, 138–140]. Using MDCT cells, it was shown that cellular potassium depletion may inhibit Mg^{2+} influx (see below). We postulate that chronic use of thiazides may result in potassium-depletion leading, in turn, to renal magnesium-wasting.

METABOLIC ACIDOSIS AND ALKALOSIS

It has long been known that systemic acidosis is associated with renal magnesium-wasting. Acute metabolic acidosis produced by infusion of NH_4Cl or HCl leads to significant increases in urinary magnesium excretion [63, 141]. Chronic acidosis also leads to urinary magnesium-wasting that, as with acute acidosis, may be partially corrected by the administration of bicarbonate [142, 143]. In contrast to metabolic acidosis, acute and chronic metabolic alkalosis consistently leads to a fall in urinary magnesium excretion [144].

Although it has long been known that metabolic acidosis and alkalosis alter renal magnesium handling, relatively little information is available regarding the tubular segment involved. Wong, Quamme and Dirks showed that metabolic alkalosis resulted in increased magnesium reabsorption in the loop of Henle and distal tubule of the dog [145]. Magnesium reabsorption was closely associated with bicarbonate delivery to the distal tubule in this study. We have shown that acute bicarbonate infusions into chronic acidotic rats leads to a marked increase in magnesium reabsorption in the loop and distal tubule [143]. Thus, on balance, the evidence is that metabolic acidosis and alkalosis act within both the loop of Henle and the distal tubule.

Thick ascending limb

Di Stefano et al perfused isolated mouse cTAL segments harvested from mice maintained on alkaline drinking water (20 mM sodium bicarbonate) for three days [103]. They showed that alkalosis doubled magnesium absorption from control levels of 0.47 ± 0.06 to 0.89 ± 0.06 pmol $\cdot$ min^{-1} $\cdot$ mm^{-1} without a change in transepithelial voltage. They interpreted this data to indicate that alkalosis changes the permeability of the paracellular pathway so that magnesium moves passively through the pathway to a greater degree, resulting in greater magnesium absorption. The effects of metabolic acidosis were not determined in this study [103].

Distal tubule

We have used the MDCT cell line to determine the effects of pH changes on cellular Mg^{2+} uptake [146]. The results of these experiments show that acute alkalosis markedly enhance Mg^{2+} uptake whereas acidosis diminish transport (Fig. 10). Bicarbonate had no effect on Mg^{2+} entry into MDCT cells. This information indicates that protons directly affect Mg^{2+} entry through the

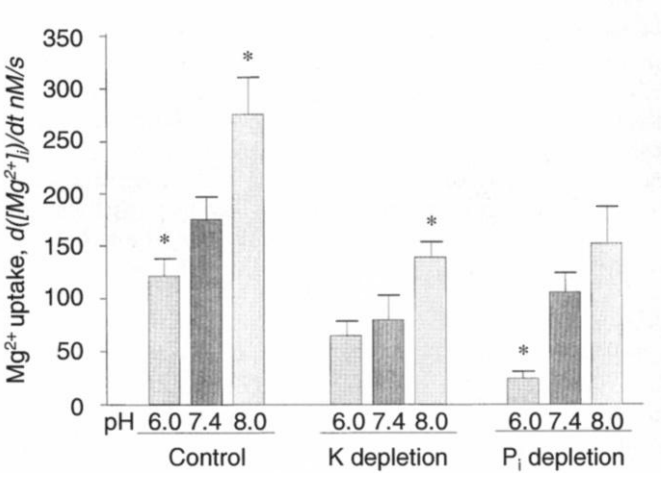

Fig. 10. Summary of acid-base, potassium depletion, and phosphate (Pi) deficiency on the rate of Mg^{2+} entry into MDCT cells. Cell preparation and fluorescence determinations were performed according to techniques given in legend to Figure 4. The refill solutions were buffered to 6.0, 7.4, or 8.0, respectively in these studies. The control cells were cultured in normal magnesium-deficient media containing 4.8 mM potassium and 1.0 mM phosphate. Potassium-depleted cells were cultured in 2.5 mM potassium and phosphate-deficient cells in 0.3 mM phosphate; the other constituents were normal. Values are means $\pm$ SE for 9 to 10 cells. *Significant, $P < 0.05$, of Mg^{2+} uptake rate compared to control values. Data are from Dai et al [33, 146, 169].

Mg^{2+} transport pathway. A change in extracelluar pH has significant effects on many kinds of ion channels. Hess and colleagues proposed that protons titrate an external histidine residue of L-type Ca^{2+} channels, outside of the permeation pathway, and reduce channel conductance by an allosteric mechanism [147]. Alternatively, Tsien postulated that protonation of glutamates within the Ca^{2+} pore itself blocks the permeation pathway [148]. Our evidence indicates that protonation of the Mg^{2+} pathway may alter Mg^{2+} influx leading to diminished transport as it does for Ca^{2+} flux through Ca^{2+} channels.

Metabolic acidosis

As distal luminal fluid pH is normally in the range of 5 to 8, it is apparent that distal magnesium absorption is physiologically affected by proton concentration. Metabolic acidosis of any etiology would be expected to lead to diminished magnesium reabsorption in the distal tubule. Mather et al and McNair et al have reported that 25 and 38%, respectively, of outpatients with diabetes mellitus have hypomagnesemia [149, 150]. This is due in part to renal magnesium-wasting stemming from decreased magnesium reabsorption. As we have seen, insulin stimulates magnesium reabsorption in the TAL so that insulin deficiency directly affects magnesium transport in addition to producing ketoacidosis that inhibits magnesium conservation [151].

POTASSIUM DEPLETION

Hypokalemia and potassium depletion is associated with diminished magnesium absorption within the loop and distal tubule that may lead to increased magnesium excretion [138–140].

Thick ascending limb

The increase in urinary excretion of divalent cations may be explained by the well known effects of potassium-depletion on

NaCl absorption in the thick ascending limb. Chloride conservation is impaired in potassium-depleted rats which may be related to altered basolateral Na-K transport resulting in impaired NaCl transport [152–154]. To date, there is no direct evidence for changes in magnesium absorption in the thick ascending limb with potassium-depletion; however, as magnesium and calcium are absorbed by passive mechanisms, it is probable that impaired NaCl transport may lead to diminished divalent cation absorption in this segment [155].

Distal tubule

Our studies, using isolated MDCT cells suggest that potassium-depletion may have additional important effects on magnesium transport within the distal convoluted tubule [33]. Cellular K^+ depletion results in the inhibition of Mg^{2+} uptake into MDCT cells as determined by microfluorescence. The mechanism for diminished Mg^{2+} entry is not known; it is not due to an alteration in the membrane voltage [33]. Further studies are required to fully explain the defective distal Mg^{2+} transport associated with cellular potassium depletion.

BARTTER'S AND GITELMAN'S SYNDROMES

The relationship of potassium and magnesium balance is far from clear. Many patients with hypokalemia have no problems with renal magnesium conservation. This is no more evident than in those patients with Bartter's or Gitelman's diseases [156, 157]. About 30% of patients with Bartter's syndrome develop hypomagnesemia whereas those with Gitelman's universally present with diminished plasma magnesium levels, and yet both diseases are associated with hypokalemia [158, 159].

Thick ascending limb

Bartter's syndrome is characterized by hypokalemia, metabolic alkalosis, hyperprostaglandin production, hyperreninemia, secondary hyperaldosteronism, and normal blood pressure [156, 159]. The evidence from clinical studies implicates defective salt transport in the thick ascending limb of the loop [158, 159]. Simon and colleagues have recently shown with linkage and mutational analysis that Na-2Cl-K cotransport or apical K^+ conductance is defective [160]. These alterations would be expected to decrease transepitheial voltage and magnesium reabsorption. It is surprising that Bartter's syndrome, a defect in loop absorption where the majority of filtered magnesium is reclaimed, is not more frequently associated with renal magnesium-wasting. About a fifth of Bartter's patients have abnormal magnesium concentrations whereas patients with Gitelman's syndrome, due to a distal defect, uniformly demonstrate hypomagnesemia [159, 161]. Despite the high incidence of hypercalciuria in Bartter's patients, there is little effect on renal magnesium handling. Aberrant salt cotransport in the thick ascending limb would lead to defective magnesium and calcium absorption and increased delivery to the DCT. It remains to be determined why magnesium absorption in the DCT proceeds normally in most of these patients, while calcium is excreted in the urine. Hypokalemic metabolic alkalosis tends to be less severe in Bartter's patients so that magnesium reabsorption in the DCT may not be compromised.

Distal tubule

Gitelman's syndrome refers to a familial disease in which patients present with hypokalemic alkalosis in conjunction with hypocalciuria and hypomagnesemia [157]. Some of the features of Gitelman's syndrome may be observed in patients chronically receiving thiazide diuretics raising the possibility that loss in Na-Cl cotransport in the distal tubule could result in this disease. Again, Simon et al used linkage and mutational analysis to confirm that Gitelman's syndrome is indeed due to mutations in the thiazide-sensitive Na-Cl cotransporter [13]. The renal magnesium-wasting and hypomagnesemia in Gitelman's patients remains to be explained [14, 162, 163]. However, the results with MDCT cells suggest that the basis of increased magnesium excretion may be due to associated hypokalemia. If this is true then it may be speculated that correction of the potassium deficits in these patients may also correct renal magnesium-wasting independent of the genetic aberrations of Na-Cl cotransport. Colussi et al reported that antialdosterone therapy is effective in ameliorating hypokalemia and hypomagnesemia in Gitelman's syndrome [164]. Further studies are warranted to determine the cellular basis of hypokalemic renal magnesium-wasting.

PHOSPHATE-DEPLETION

One of the hallmarks of hypophosphatemia and cellular phosphate-depletion is the striking increase in urinary excretion of calcium and magnesium [165]. Magnesium excretion may be sufficiently large to lead to overt hypomagnesemia [166, 167]. The increase in divalent ion excretion in both human and experimental animals occurs within hours following initiation of dietary phosphate restriction. Three mechanisms have been proposed to account for the increased renal excretion: (*1*) mobilization of calcium and magnesium from bone, (*2*) suppression of parathyroid hormone secretion, and (*3*) aberrant tubular transport [165].

Thick ascending limb

It is evident from clearance experiments that the urinary excretion of divalent cations of phosphate-depleted subjects is inappropriate for the plasma concentration, supporting the notion of defective tubular transport [165]. Using micropuncture, we have demonstrated that defective magnesium absorption occurred in the loop of Henle and the distal tubule of phosphate-depleted dogs [168]. The cellular mechanisms involved with diminished magnesium absorption within the loop of Henle have not been determined.

Distal tubule

We have shown that cellular phosphate-depletion leads to diminished Mg^{2+} uptake in MDCT cells [169]. This observation supports the notion that the DCT may be involved, in part, in decreased magnesium absorption and increased magnesium excretion associated with hypophosphatemia. The effects of phosphate depletion on Mg^{2+} uptake in MDCT cells are reminiscent of those observed in the intact kidney. Removal of phosphate from the media rapidly leads to diminished Mg^{2+} transport, which is dependent on the degree of phosphate-depletion. Mg^{2+} uptake is inhibited by 50% when cultured in about 0.3 mM phosphate. These actions are fully reversible with the return of phosphate to the media. The induction of defective transport that is associated with phosphate depletion must reside within the cell either to prevent the normal up-regulation of Mg^{2+} transport with Mg^{2+} deficiency or to inhibit Mg^{2+} uptake through actions on transport processes. To determine if phosphate-depletion acts through post-translational mechanisms, MDCT cells were first Mg^{2+}

depleted for 16 hours to maximally up-regulate Mg^{2+} transport. The cells were then phosphate-depleted for various time periods and Mg^{2+} uptake was assessed by microfluorescence (Fig. 10). Phosphate-depletion resulted in diminished Mg^{2+} uptake in preadapted cells, which suggests it affects transport through actions on preformed pathways rather than through transcriptional or translational mechanisms. Further studies are necessary to define these post-translational events. It is evident from these studies with isolated MDCT cells that magnesium-wasting commonly observed with hypophosphatemia and phosphate depletion could be due, in part, to diminished Mg^{2+} uptake in the distal convoluted tubule.

Experimental and clinical data suggest an association between hypomagnesemia and hypokalemia and also hypophosphatemia [126, 130, 170]. Crook reported a twofold increase in the prevalence of hypophosphatemia (plasma phosphate < 0.8 mM) and a sixfold increase in hypokalemia (plasma potassium < 3.5 mM) in patients with hypomagnesemia (plasma magnesium < 0.70 mM) [170]. A trilogy consisting of hypomagnesemia, hypophosphatemia and hypokalemia was also found in 8% of patients with hypomagnesemia and 17% of patients with severe hypomagnesemia (plasma magnesium < 0.50 mM). The evidence suggests that hypokalemia and hypophosphatemia may have profound effects on tubular magnesium transport. Many of the syndromes associated with potassium depletion and phosphate depletion are complicated by concurrent alterations in acid-base balance [126, 171]. Our evidence indicates that acid-base changes (H^+ ions) have different effects on magnesium transport relative to potassium or phosphate depletion so that the three disturbances may act in an additive manner to compromise renal magnesium conservation.

SUMMARY

Recent research has provided new concepts in our understanding of renal magnesium handling. Although the majority of the filtered magnesium is reabsorbed within the loop of Henle, it is now recognized that the distal tubule also plays an important role in magnesium conservation. Magnesium absorption within the cTAL segment of the loop is passive and dependent on the transepithelial voltage. Magnesium transport in the DCT is active and transcellular in nature. Many of the hormonal (PTH, calcitonin, glucagon, AVP) and nonhormonal (magnesium-restriction, acid-base changes, potassium-depletion) influences that affect magnesium transport within the cTAL similarly alter magnesium absorption within the DCT. However, the cellular mechanisms are different. Actions within the loop affect either the transepithelial voltage or the paracellular permeability. Influences acting in the DCT involve changes in active transcellular transport either Mg^{2+} entry across the apical membrane or Mg^{2+} exit from the basolateral side. These transport processes are fruitful areas for future research. An additional regulatory control has recently been recognized that involves an extracellular Ca^{2+}/Mg^{2+}-sensing receptor. This receptor is present in the basolateral membrane of the TAL and DCT and modulates magnesium and calcium conservation with elevation in plasma divalent cation concentration. Further studies are warranted to determine the physiological role of the Ca^{2+}/Mg^{2+}-sensing receptor, but activating and inactivating mutations have been described that result in renal magnesium-wasting and hypermagnesemia, respectively. All of these receptor-mediated controls change calcium absorption in addition to magnesium transport. Selective magnesium control is through intrinsic control of Mg^{2+} entry into distal tubule cells. The cellular mechanisms that intrinsically regulate magnesium transport have yet to be described. Familial diseases associated with renal magnesium-wasting provide a unique opportunity to study these intrinsic controls. Loop diuretics such as furosemide increase magnesium excretion by virtue of its effects on the transepithelial voltage thereby inhibiting passive magnesium absorption. Distally acting diuretics, like amiloride and chlorothiazide, enhance Mg^{2+} entry into DCT cells. Amiloride may be used as a magnesium-conserving diuretic whereas chlorothiazide may lead to potassium-depletion that compromises renal magnesium absorption. Patients with Bartter's and Gitelman's syndromes, diseases of salt transport in the loop and distal tubule, respectively, are associated with disturbances in renal magnesium handling. These may provide useful lessons in understanding segmental control of magnesium reabsorption. Metabolic acidosis diminishes magnesium absorption in MDCT cells by protonation of the Mg^{2+} entry pathway. Metabolic alkalosis increases magnesium permeability across the cTAL paracellular pathway and stimulates Mg^{2+} entry into DCT cells. Again, these changes are likely due to protonation of charges along the paracellular pathway of the cTAL and the putative Mg^{2+} channel of the DCT. Cellular potassium-depletion diminishes the voltage-dependent magnesium absorption in the TAL and Mg^{2+} entry into MDCT cells. However, the relationship between potassium and magnesium balance is far from clear. For instance, magnesium-wasting is more commonly found in patients with Gitelman's disease than Bartter's but both have hypokalemia. Further studies are needed to sort out these discrepancies. Phosphate deficiency also decreases Mg^{2+} uptake in distal cells but it apparently does so by mechanisms other than those observed in potassium depletion. Accordingly, potassium depletion, phosphate deficiency, and metabolic acidosis may be additive. The means by which cellular potassium and phosphate alter magnesium handling are unclear. Research in the nineties has increased our understanding of renal magnesium transport and regulation, but there are many interesting experimental and clinical areas for future research.

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