[Frontiers in Bioscience 5, d694-711, August 1, 2000]

EPITHELIAL MAGNESIUM TRANSPORT AND REGULATION BY THE KIDNEY

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1. ABSTRACT

Magnesium is the fourth most abundant cation in the body and the second most common cation in the intracellular fluid. It is the kidney that provides the most sensitive control for magnesium balance. About a 80% of the total serum magnesium is ultrafilterable through the glomerular membrane. In all of the mammalian species studied to date, the proximal tubule of the adult animal reabsorbs only a small fraction, 10-15%, of the filtered magnesium. Unlike the adult proximal convoluted tubule that of young rats (aged 13-15 days) reabsorbs 50-60% of filtered magnesium along the proximal tubule together with sodium, calcium, and water. Micropuncture experiments, in every species studied to date, indicates that a large part (approximately 60%) of the filtered magnesium is reabsorbed in the loop of Henle. Magnesium reabsorption in the loop occurs within the cortical thick ascending limb (cTAL) by passive means driven by the transepithelial voltage through the paracellular pathway. Micropuncture experiments have clearly showed that the superficial distal tubule reabsorbs significant amounts of magnesium. Unlike the thick ascending limb of the loop of Henle, magnesium reabsorption in the distal tubule is transcellular and active in nature. Many hormones and nonhormonal factors influence renal magnesium reabsorption to variable extent in the cTAL and distal tubule. Moreover, nonhormonal factors may have important implications on hormonal controls of renal magnesium conservation. Dietary magnesium restriction leads to renal magnesium conservation with diminished urinary magnesium excretion. Adaptation of magnesium transport with dietary magnesium restriction occurs in both the cTAL and distal tubule. Elevation of plasma magnesium or calcium concentration inhibits magnesium and calcium reabsorption leading to hypermagnesiuria and hypercalciuria. The

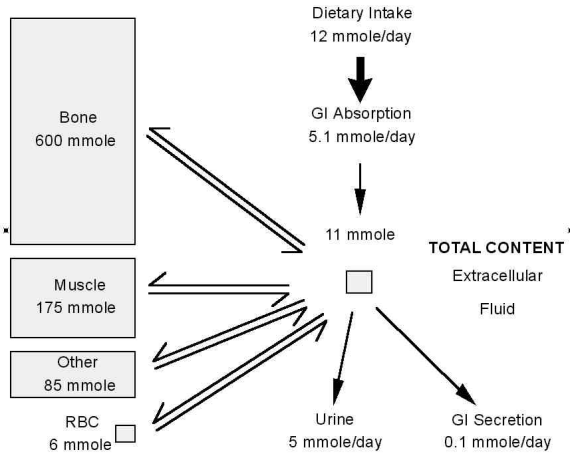

Figure 1. Distribution of magnesium in the body, including dietary intake, intestinal absorption, and urinary excretion. From Quamme (2).

identification of an extracellular Ca^{2+} /Mg^{2+} -sensing receptor located on the peritubular side of cTAL and distal tubule cells explains this phenomenon. Loop diuretics, such as furosemide and bumetanide, diminish salt absorption in the cTAL whereas the distally acting diuretics, amiloride and chlorothiazide stimulate magnesium reabsorption within the distal convoluted tubule. Finally, metabolic acidosis, potassium depletion or phosphate restriction can diminish magnesium reabsorption within the loop and distal tubule. Research in the 90's have greatly contributed to our understanding of renal magnesium handling.

2. INTRODUCTION

By its physicochemical characteristics, magnesium plays an essential role in the life of most, if not all, organisms. It participates in activation of many enzymes such as ATPases, regulation of channel activities such as K and Ca channels, and because it can establish stable ternary complexes with nucleotides, it is also involved in transcriptional, nucleic acid polymerization, and translational processes (1). All these functions make it an essential element of the "milieu intérieur".

The adult human body contains approximately one mole of magnesium, 50% in bone and the remaining in other soft tissues and extracellular fluids (Figure 1). Blood contains less than 10 mmoles of magnesium (2). In serum from healthy subjects the mean magnesium concentration is remarkably stable. Serum ionized and total magnesium concentrations average 0. 6 and 0. 85 mM, respectively (3,4). The total magnesium concentration seems to follow certain circadian changes with the lowest levels observed between 6-10 a.m. (3,4). The reasons for this observation are unknown but likely involves circadian hormonal cycles.

The average daily diet in European and North America contains approximately 228 (adult females) to 323 mg of elemental magnesium (5). The recommended daily estimated average requirement for magnesium is 265 and 350 mg for adult females and males, respectively (6). This suggests that the average North American diet is only adequate for maintenance of body magnesium levels (7). Moreover, magnesium requirements increase was rapid growth in infancy and adolescence and during pregnancy and lactation. Despite the suggestion of borderline dietary insufficiency, overt magnesium deficiency from dietary causes alone is uncommon. This is probably because of the ubiquity of magnesium in the European and North American diet. On the average, less than 30% of magnesium intakes is absorbed through the gastrointestinal tract through passive (paracellular) and active (transcellular) mechanisms (2). Magnesium balance is mainly assured by the kidneys.

The kidney provides an excellent example of the many ways that magnesium is handled by epithelial cells. In the proximal tubule, little magnesium is transported across the epithelium. In the of loop of Henle, specifically the thick ascending limb, transepithelial magnesium movement is abundant and principally passive in nature moving between the cells through the paracellular pathway. More distally, in the distal convoluted tubule and connecting segments magnesium transport is transcellular and active in nature quite different from that observed in the thick descending limb. Finally, there appears to be no transepithelial magnesium transport in the collecting ducts. There is no evidence for magnesium secretion within the mammalian kidney. This is unlike the kidney of fish and birds where secretion is a predominant mechanism of magnesium handling (8). Accordingly, nature has evolved many different ways of controlling epithelial magnesium transport. The purpose of this review is to detail the various ways that renal epithelial cells transport magnesium.

Magnesium is the fourth most abundant cation in the body and the second most common cation in the intracellular fluid. By its abundance and distribution of the Mg^{2+} ion plays an essential role in intracellular metabolism (1). Although no single hemostatic control has been demonstrated for Mg^{2+}, the ion's cellular availability is closely regulated by mechanisms acting at the level of the intestinal tract, bone and kidney (2). It is the kidney that provides the most sensitive control for magnesium balance (9).

In this monograph, we shall examine the renal magnesium handling at the tubular and cellular levels and the influences of hormonal and nonhormonal factors on magnesium transport. This information has been extensively reviewed elsewhere (9-12). We will highlight the new observations such as the participation of so-called "extracellular sensors" in the control of tubular magnesium transport (12). This is a new and very important issue because it emerges that extracellular ions in addition to hormones may act as first messengers.

3. BASIC MECHANISMS OF MAGNESIUM TRANSPORT IN THE KIDNEY

3.1. Glomerular filtration

Approximately 70% of plasma magnesium is in

the ionic form, Mg^{2+} (3, 4). The remaining magnesium is bound to circulating proteins (essentially albumin) or is complexed with citrate, oxalate and phosphate anions. About a 80% of the total serum magnesium is ultrafilterable through the glomerular membrane (14, 15). With a glomerular filtration rate, GFR, of 125 ml/min the filtered magnesium amounts to about 140 mmoles/day of which the kidney reabsorbs some 80-99%. Accordingly, 1-20% of the filtered magnesium is excreted in the final urine depending on the dietary magnesium intake (2).

3.2. Proximal tubule

The proximal convoluted tubule of immature and mature animals appears to reabsorb magnesium in very different ways that may be important in the interpretation of magnesium balance in young and adult individuals. In all of the mammalian species studied to date, the proximal tubule of the adult animal reabsorbs only a small fraction, 10-15%, of the filtered magnesium (16-28). The transepithelial potential difference (Vt) is about -2 mV (lumen negative) in the early convolutions and the late proximal tubule and straight tubules is about +3 mV (lumen positive). As magnesium concentration is above that of ultrafilterable plasma concentration, the orientation of the electrochemical gradient favors diffusion of magnesium from lumen to blood along most of the proximal tubule. To date, all effort to disclose in animals a transcellular transport of magnesium in proximal tubules have been unsuccessful. In the absence of evidence, it is generally assumed that magnesium is passively reabsorbed through the paracellular pathway. In tubules perfused with Ringer's solutions containing various amounts of $MgCl_2$, magnesium concentration in the tubular fluid downstream from the perfusion site increased proportionately to water reabsorption whether the magnesium concentration was far below or markedly above the plasma ultrafilterable magnesium levels (29). These data indicate a relatively low magnesium permeability of the tubule. However, the proximal tubule has a finite magnesium permeability as demonstrated by influx of magnesium into fluid recollected from magnesium-free droplets previously injected into oil filled proximal convoluted tubules (30). It is generally accepted that sodium is reabsorbed mainly through the transcellular route with a smaller fraction being reabsorbed through the paracellular pathway. It is well documented that the tight junctions in the paracellular pathways are very permeable to Na^+ and Ca^{2+} so it will be of interest to determine whether Mg^{2+} can move through paracellular pathways with Na^+.

Unlike the adult proximal convoluted tubule that of young rats (aged 13-15 days) reabsorbs 50-60% of filtered magnesium along the proximal tubule together with sodium, calcium, and water (31). Fractional reabsorption of magnesium in the proximal tubule declines as a function of age in contrast to that of Na and water. It is possible that the fall in Mg^{2+} permeability is caused by a maturational decrease of the paracellular permeability probably at the level of the tight junctions. In parallel with these changes, the proximal tubule of the young rat is unable to create or maintain a transepithelial concentration gradient for Cl^- (31). As the rat ages (beyond 3 weeks), the Cl^- gradient develops and increases with age. It is therefore possible that maturational alterations of the paracellular pathway affect the permeability of both Mg^{2+} and Cl^- but to differing degrees. At the other end of the age scale, fractional magnesium reabsorption in the senescent rat (30 months) is similar to that of the adult rat (10 months) (32).

In summary, net magnesium reabsorption in the proximal tubule of mammalians is very limited. Because there is no evidence for magnesium secretion, the small reabsorptive flux results from limited transport of this cation. This is not the case for lower animals. Beyenbach *et al* extensively studied the transport of magnesium in different species of glomerular and a glomerular fish (8). Isolated proximal tubules of aglomerular fish secrete fluid spontaneously (see review by Beyenbach in this series). Secreted fluid within the proximal tubule of the winter flounder contains magnesium at concentrations greater (26 mM) than those in the peritubular medium (1 mM). According to Beyenbach, magnesium could enter the proximal cell across the basolateral membrane through Mg^{2+} channels or Mg/Cl cotransport and be extruded from the cell into the tubule lumen through the apical membrane. The cellular details of these transport mechanisms remained to be identified.

3.3. Loop of Henle

Micropuncture experiments, in every species studied to date, indicates that a large part (approximately 60%) of the filtered magnesium is reabsorbed in the loop of Henle (9). The loop of Henle is composed of the straight proximal tubule, thin descending limb, thin descending limb, medullary thick ascending limb and cortical thick ascending limb segments. The major fraction of this reabsorption takes place in the cortical portion of the thick ascending limb (33).

3.3.1. Proximal straight tubule

The characteristics of magnesium transport in the proximal straight tubule appears to be similar to those described for the superficial proximal convoluted tubule (9). *In vitro* microperfusion studies of isolated straight proximal tubules showed that magnesium permeability was much lower than the permeability to either sodium or calcium (34). Accordingly, a greater fraction of filtered magnesium relative to sodium and calcium flows into the distal segments of the loop.

3.3.2. Thin descending and ascending limbs

There is no direct information about magnesium transport in the thin descending or thin ascending limbs of the Henle's loop. However, indirect information on the thin descending limb function can be inferred from micropuncture data obtained by comparing deliveries to the end-proximal and the bend of Henle's loop, i.e. end of the descending limb.

Micropuncture at the hairpin turn of

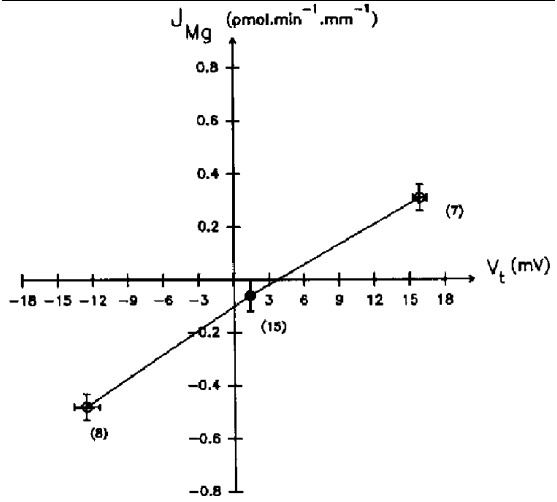

Figure 2. Magnesium net fluxes (J_{Mg}) measured in the absence of active NaCl transport (lumen: $10^{?4}$ mol.l$^{?1}$ furosemide) in mouse cTAL segments. Flux-voltage relationships were measured in two experimental series: i) with symmetrical solutions in lumen and bath (150 mM NaCl black dot), and in the presence of hypotonic luminal solutions (lumen: 50 mM NaCl; bath: 150 mM NaCl), generating positive dilution voltages (open circles). ii) with symmetrical solutions in lumen and bath (150 mM NaCl black dot) and in the presence of a bath solution in which NaCl was more dilute than in the luminal perfusate (lumen: 150 mM NaCl; bath: 50 mM NaCl + 200 mM mannitol), generating negative dilution voltages (open circles). Data from di Stefano *et al.* (46).

juxtamedullary nephrons in the rat indicates that a significant fraction of filtered magnesium, approximately 20%, can be reabsorbed along descending limb of these nephrons in the concentrating kidney. Magnesium reabsorption is associated with water reabsorption (35). This reabsorption is not observed when there is little water removal in this segment, i.e. *in situ*ations of hydration and increased water excretion (35). In that case, the amount of magnesium remaining at the hairpin turn is close to 90% of the filtered magnesium, i.e., close to the amount expected to remain at the end of the proximal convoluted tubule. A segment in the loop of juxtamedullary nephrons is therefore permeable to magnesium and the amount reabsorbed is dependent on water transport. If magnesium transport in convoluted and straight proximal tubules of the deep juxtamedullary nephrons is limited, as is the superficial nephron, it is inferred that the thin descending segment (perhaps the region just preceeding the hairpin turn) reabsorbs magnesium in association with water. This portion of the thin descending limb has been demonstrated in the chinchilla to be permeable to sodium, whereas the upper portion was impermeable (36). In magnesium-loaded rats (37) and in the concentrating sand rat, *Psammomys obesus*, (23, 27) significant magnesium addition to the fluid collected at hairpin turn of nephrons accessible to micropuncture was demonstrated confirming the presence of a segment permeable to magnesium along the descending limb of juxtamedullary nephrons.

With respect to the thin ascending limb, little information is available concerning magnesium reabsorption because the ascending limb of the loop is composed of both thin and thick segments. Accordingly, micropuncture data at the beginning of the superficial distal tubule does not reflect the thin or the thick segment. Magnesium transport in the thin ascending limb remains to be identified.

3.3.3. Thick ascending limb

The thick ascending limb of Henle's loop consists of medullary and cortical segments. Although the early micropuncture studies clearly showed that the ascending loop absorbed large amounts of magnesium, in the order of 60% of that filtered, it did not describe the cellular mechanisms of transport. *In vitro*microperfusion of single segments dissected from the two regions, the medullary segment (mTAL) and cortical segment (cTAL) of the thick descending limb have greatly clarified our understanding of how magnesium is transported in the loop of Henle. In the mouse and rat, magnesium (and calcium) is reabsorbed in the cTAL not in the mTAL segment although both segments reabsorbed NaCl and display a lumen positive transepithelial voltage. However, Friedman reported significant calcium reabsorption in mouse mTAL segments (38).

3.3.3.1. Medullary thick ascending limb

In the rat and mouse mTAL cells studied under control (symmetrical) conditions and whatever the values of lumen-positive voltage, the net magnesium flux was always very limited, most often not significantly different from zero (39-44). In contrast, under conditions where net NaCl transport is suppressed, one may observe some alterations in the movements of divalent cations (45). When NaCl transport is totally inhibited with luminal furosemide, the voltage falls to zero. In spite of this diminished voltage, one observes a net secretion of magnesium and calcium into the lumen. Paradoxically, this secretion rises when a positive luminal voltage (up to +18 mV) is created, luminal furosemide still present. The reverse, that is at the least a decrease in this luminal influx, would have been expected. Such a secretion can also be elicited without furosemide, when the net reabsorptive flux of NaCl is abolished by perfusing the lumen with asymmetrical solutions (50 mM NaCl) elevating luminal voltage up to +30 mV. In spite of this elevated positive voltage, very significant additions of calcium and magnesium to the luminal fluid were observed. Moreover, if Na was substituted with K (25 mM K substituted for 25 mM Na) in the presence of furosemide, voltage remained close to zero but large amounts of calcium and magnesium were secreted into the lumen. If on the contrary, voltage were oriented in the reverse direction, i.e. towards lumen negative values, for example -15 mV, no more calcium or magnesium movements were observed in any direction. It is very likely that these secretions had a cellular origin, because of the impermeability of the paracellular pathway to magnesium in this nephron segment. It is likely that these events result from alterations of "housekeeping" mechanisms aimed at supplying the cell in magnesium for its metabolic needs. The research for active or secondary

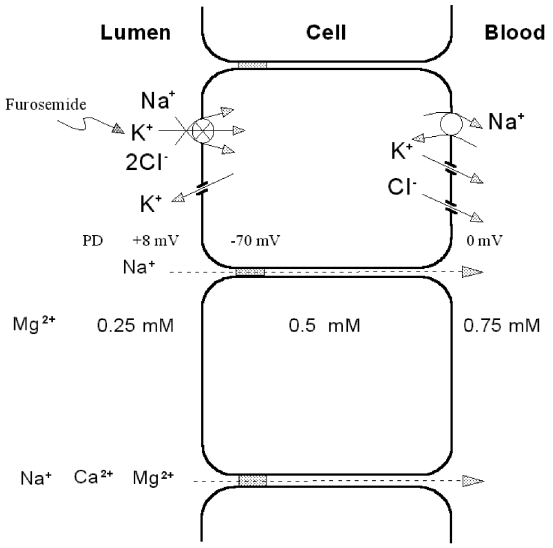

Figure 3. Schematic model of magnesium absorption in the cortical thick ascending limb of Henle's loop. Magnesium reabsorption is passive and occurs through the paracellular pathway. From Quamme (12).

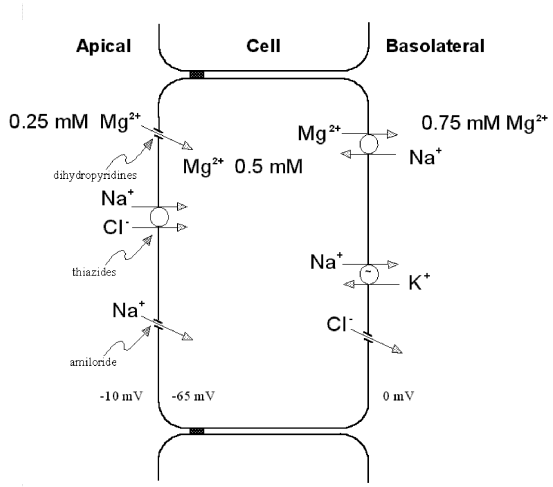

Figure 4. Schematic model of magnesium absorption in the distal convoluted tubule. Magnesium reabsorption is active and occurs through transcellular pathways. From Quamme (12).

active transport systems in these cells is a clear objective for future investigations.

3.3.3.1. Cortical thick ascending limb

In mouse, rat and rabbit cTAL segments, the directional flow of magnesium transport depends on the transepithelial voltage (42, 46, 47). When mouse cTAL segments are perfused under symmetrical conditions, i.e. when the bath and lumen solutions contain 150 mM NaCl, magnesium net reabsorptive flux is close to 0.2 pmole?mm^{-1} ?mm^{-1} ?mV^{-1} (Figure 2).

The magnesium fluxes were identical whether the voltage resulted from spontaneous active NaCl transport or was generated by an imposed transepithelial NaCl concentration gradient plus NaCl cotransport blockade with furosemide. Reversing the voltage, lumen negative with respect bath, only alters the direction not the absolute value of the net flux. In the mouse, the transepithelial voltage falls to values close to zero in the presence of furosemide. In parallel, magnesium transport also becomes negligible. The addition of the Cl channel blocker, diphenylamine-2-dicarboxylic acid, to the bath inhibits NaCl absorption and also reduces the voltage and decreases net flux of magnesium. Moreover, when tubules were perfused with 150 mM NaCl in the bath and 50 mM in the lumen (asymmetrical conditions) the transepithelial concentration gradient generates a high voltage (luminal +30 mV). These high voltages are accompanied with large reabsorptive fluxes of magnesium in spite of little NaCl net transport (46). If there was a cellular component participating in magnesium reabsorption, because of the value of the membrane voltage (about -70 mV) and intracellular Mg activity (0. 5 mM) the transport through the basolateral membrane should be an active process. This transport should be a Na and/or Cl independent process because magnesium transport is still observed during inhibition of NaCl entry into the cell or Cl efflux from the cell. Furthermore, during NaCl transport blockade with furosemide, net movements were directed towards the lumen that is in the opposite direction that could be expected if some active mechanism was operating in the basolateral membrane, and the absolute value of transport in either direction was around 0.2 pmole?mm-1 ?mm-1 ?mV-1 . All these observations are in favor of passive magnesium transport in the cTAL (Figure 3). Since this transport cannot be transcellular, the transepithelial route is the paracellular pathway.

3.4. Distal tubule

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, the connecting tubule and the initial collecting tubule (48). The portions of the nephron located downstream to the distal tubule include the cortical and medullary segments of collecting duct system. There is no evidence to date for magnesium reabsorption or secretion in the collecting duct system (49-52). Recently, Bindels and colleagues isolated rabbit cortical collecting cells, grew them to confluency on permeable supports and measured calcium and magnesium fluxes across the epithelium. They observed no magnesium transport either in the apical (luminal)-to-basal (blood) or basal-to-apical direction despite 5-fold concentration gradients in either direction (personnal communication). These *in situ*studies support the earlier micropuncture and microcatherization experiments. Because there is no magnesium transport in the collecting ducts, the distal tubule is the last nephron segment to control urinary magnesium excretion.

Micropuncture experiments have clearly showed that the superficial distal tubule reabsorbs significant

Table 1. Controls of Magnesium Transport in Mouse/Rat cTAL and Distal Tubule and MDCT

Cells		Mouse/Rat cTAL	Intact Rat Distal Tubules	Isolated MDCT Cells
Peptide hormones	Parathyroid Hormone	Increase(84)	Increase(53)	Increase(96)
	Calcitonin	Increase(40)	Increase(56)	Increase(U.P.)
	Glucagon	Increase(41)	Increase(53)	Increase(94)
	Arginine Vasopressin	Increase(84)	Increase(55)	Increase(94)
adrenergic agonists	Isoproterenol	Increase(39)	?	Increase(U.P.)
Prostaglandins	PGE_2	?	?	Increase(95)
	Insulin	Increase(43)	?	Increase(96)
Mineralocorticoid s	Aldosterone	?	?	Increase(97)
Vitamin D	$1,25(OH)_2D_3$	?	?	Increase(U.P.)
Magnesium restriction		Increase(102)	Increase(57)	Increase(66)
Metabolic acidosis		?	Decrease(141)	Decrease(142)
Metabolic alkalosis		Increase(102)	Increase(57)	Increase(142)
Hypermagnesemia		Decrease(116)	Decrease(29)	Decrease(117)
Hypercalcemia		Decrease(116)	Decrease(24)	Decrease(117)
Phosphate-depletion		?	Decrease	Decrease(157)
Potassium-depletion		?	?	Decrease(67)
Diuretics	Furosemide	Decrease(89)	No Effect(59)	No Effect(66)
	Amiloride	No Effect	?	Increase(66)
	Chlorothiazide	No Effect	Increase(12)	Increase(67)

Data from isolated mouse or rat cTAL tubules and intact superficial rat distal tubules and immortalized mouse distal convoluted tubule (MDCT) cells. ? indicates not known, u.p. indicates unpublished observations.

amounts of magnesium (26, 29, 53-56). The distal tubule reabsorbs 10-15% of the total magnesium filtered at the glomerulus. Normally, 30-50% of the magnesium delivered from the loop is reabsorbed but with dietary magnesium restriction this may increase to 80-90% of the magnesium delivery (57). Distal magnesium reabsorption is load-dependent so that a constant fraction of magnesium is absorbed at any given delivery to this segment (29, 58, 59). As reviewed below, distal magnesium reabsorption is regulated by many hormones and influenced by many physiological and pathological factors but this load-dependence is maintained.

Unlike the thick ascending limb of the loop of Henle, magnesium reabsorption in the distal tubule is transcellular and active in nature (Figure 4). The distal tubule is a high resistance epithelium so that large concentration differences may be generated under certain conditions and maintained along its length. The luminal magnesium concentration may be significantly above or below the concurrent plasma concentration at any given point in the distal tubule depending on whether there is avid reabsorption of magnesium such as with dietary restriction (54, 57, 58) or there is a surfeit of magnesium as with magnesium-loading (29, 51, 52). As the luminal electrical voltage is negative with respect to the interstitium, reabsorption requires active transport such as a sodium-dependent exchange (91). There is no evidence for secretion, i.e. movement from blood-to-lumen even when superficial tubules were perfused with solutions containing no magnesium (29).

Because the distal tubule is difficult to study *in vivo*, established distal tubule cell lines were used to study the cellular mechanism of magnesium transport (60, 61). A method was developed to measure Mg^{2+} uptake by fluorescence measurements using Mandon-Darby canine kidney (MDCK) cells (61). The cells normally possess an intracellular Mg^{2+} concentration ($[Mg^{2+}]_i$) of about 0.5 mM. When the cells were placed in a media containing no magnesium, the $[Mg^{2+}]_i$ decreases to about 0. 25 mM in 8-16 hours (60). The cells were then placed in solutions containing magnesium and the increase in $[Mg^{2+}]_i$ with time measured with fluorescence. The increase of $[Mg^{2+}]_i$ as a function of time reflects the entry rate which is the rate-limiting step in transepithelial transport (61). We have more recently used this approach to study Mg^{2+} transport in an immortalized mouse distal convoluted tubule (MDCT) cell line (62). These cells possess amiloride-sensitive Na^+ conductance and chlorothiazide-inhibitable Na^+-Cl^- cotransport (63) which are characteristic of the intact distal convoluted tubule (64). Furthermore, MDCT cells possess many of the peptide and steroid hormone receptors which are found in the DCT (65). In our studies performed to date, the results with the MDCT cells have confirmed those obtained by micropuncture and microperfusion experiments (Table 1).

Using this fluorescence approach with MDCT cells, we have shown that Mg^{2+} entry is concentration-dependent (66). The mean Mg^{2+} uptake rate increases with external Mg^{2+} concentration below 2-3 mM; above this value transport saturates and becomes independent of the concentration (66). This is like that observed in the intact superficial rat distal tubule (29). Second, we reported that Mg^{2+} entry into MDCT cells is dependent on the transmembrane voltage (66, 67). Hyperpolarizationenhances uptake whereas depolarization decreases Mg^{2+} entry rates. Friedman and Gesek reported that Ca^{2+} uptake in MDCT cells was also similarly influenced by membrane voltage (63, 68). However, Mg^{2+} entry is independent of Ca^{2+} as high extracellular Ca^{2+} concentrations do not affect Mg^{2+} transport (66). Finally, Mg^{2+} uptake is inhibited by the channel-blocker , nifedipine, which suggests

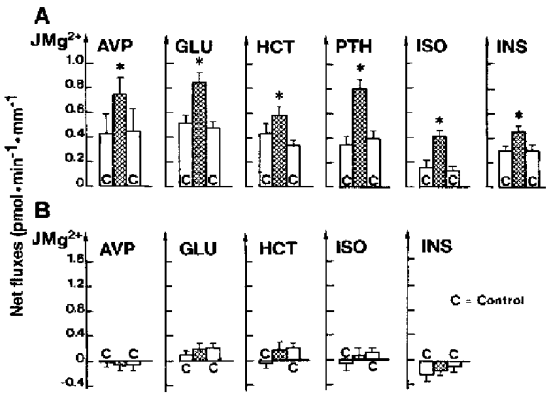

Figure 5. Individual effect of hormones on net magnesium transport within thick ascending limb segments. Isolated segments of either cortical (A) or medullary (B) thick ascending limb of mouse nephrons were microperfused at 2 nl/min in the absence (C) and presence of either antidiuretic hormone (AVP, $10^{?10}$ mM) glucagon (synthetic porcine glucagon $1.2 \times 10^{?8}$ mM), calcitonin (synthetic human calcitonin $3x10^{?8}$ mM, cibacalcin RC 47/75 Ba), parathyroid hormone (bovine PTH, 1-34 fragments, $10^{?8}$ mM), isoproterenol ($10^{?7}$ iso, Isuprel) or insulin ($10^{?7}$ mM). The hormones were added to the bath solutions. * Significance from the preceding period. Data from Bailly *et al.* (39), di Stefano *et al.* (40, 41), Mandon *et al.* (44), and Wittner *et al.* (43).

that the entry involves a conductance pathway (66). In summary, Mg^{2+} entry is concentration-dependent, modified by transmembrane voltage, and is inhibited by channel blockers which suggests that it is a selective Mg^{2+} channel.

4. HORMONAL CONTROL OF RENAL MAGNESIUM TRANSPORT

Many hormones and nonhormonal factors influence renal magnesium reabsorption to variable extent in the various nephron segments mentioned above. Although we address these issues separately in the present review, these influences are closely integrated. Moreover, as will be evident, nonhormonal factors may have important implications on hormonal controls of renal magnesium conservation.

4.1. Proximal Tubule

Hormones, such as parathyroid hormone (PTH), calcitonin, glucagon, and arginine vasopressin (AVP) increase magnesium reabsorption within the kidney (26, 43, 69-74). The actions of these hormones are partly additive with antidiuretic hormone (44, 75). These hormones do not appear to have any effect on magnesium reabsorption in the proximal tubule even though they may alter salt and water reabsorption in this segment (19-22, 26, 28).

4.2. Loop of Henle

The major effects of these hormones are within the thick ascending limb of the loop of Henle and the distal convoluted tubule (9). The effects of PTH, calcitonin, glucagon and AVP were tested *in vivo*by micropuncture in the rat (19-22, 26, 35, 43, 76-78). These hormones stimulated sodium, chloride, magnesium and calcium transport in the loop of Henle. Considering the physiological properties of the upstream segments, it was very likely that these effects resulted from hormone-dependent increases of ionic transport in the thick ascending limb. Because of the heterogeneity of the thick descending limb, which is composed of cortical medullary segments, it was difficult to assign transport properties in each of the segments. Indirect information had been obtained from micropuncture studies of Brattleboro rats, deficient in endogenous AVP, treated for a long time with dDAVP. In these rats, Bankir and her group discovered that long-term treatment of dDAVP resulted in a preferential increase in tubular volume and length of the medullary limb not the cortical thick ascending limb (79). After cessation of treatment, there was no circulating dDAVP but the medullary hypertrophy was still present. In the loop of Henle, the transport of NaCl was elevated but transport of magnesium and calcium was identical to that measured in untreated rats (11). These data were confirmed by *in vitro*studies of isolated cortical and medullary thick ascending limb. Moreover, in the rat, mouse and rabbit, the physiological responses are in agreement with the distribution of adenylate cyclase activities in these segments (80-83).

4.2.1. Medullary thick ascending limb (mTAL)

In the medullary thick ascending limb of the mouse, AVP, glucagon, insulin and isoproterenol stimulates NaCl transport but magnesium transport remains unchanged, not different from zero (39-41, 43, 44, 84). Figure 5 summarizes thr effect of these hormones on magnesium transport in the medullary thick ascending limb. However, when the tubules are perfused with asymmetrical solutions (50 mM NaCl) creating and elevated positive luminal voltage, significant additions of magnesium and calcium to the luminal fluid are observed. The secretory fluxes were not altered by the presence of AVP (45).

4.2.2. Cortical thick ascending limb (cTAL)

In the mouse, PTH, calcitonin, glucagon, AVP, insulin, and isoproterenol stimulate NaCl, magnesium and calcium transport in the cortical thick ascending limb (39-41, 43, 44, 84). In the rabbit, PTH and calcitonin stimulate the divalent cation transport but not NaCl (47, 85). The cellular mechanisms responsible for these effects are relatively well understood (86). In the mouse, the hormone-dependent increase in NaCl transport results from an increase in voltage as a consequence of an increase in the unidirectional influx of Cl across the basolateral membrane and of K across the luminal side (87). Stimulation of NaCl transport in the thick descending limb can be observed only after one hour of equilibration following mounting of the tubule segment (88). During this period, the NaCl transport is maximal and cannot be further increased by hormones. Paradoxically, during the same period magnesium and calcium transport can be stimulated. Moreover, when the tubule reached a stationary level, the increase in magnesium and calcium

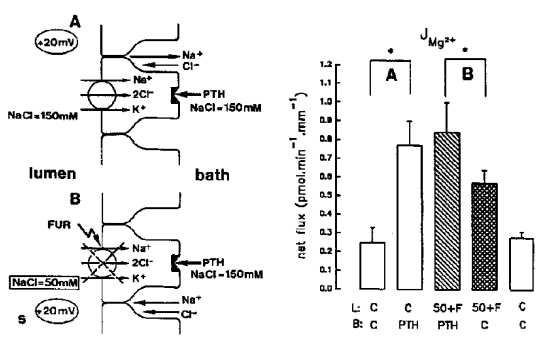

Figure 6. Effects of parathyroid hormone (PTH) on paracellular magnesium transport in mouse cortical thick ascending limbs. The segments were successively subjected to two experimental situations: **A**: the tubules were perfused with identical solutions on both sides of the epithelium (NaCl, 150 mM). PTH significantly increased the net reabsorption of magnesium by stimulating transepithelial voltage (+20 mV). **B**: the luminal solution was replaced with a hypotonic solution (NaCl, 50 mM) containing furosemide to block transcellular NaCl transport. Under these conditions, transepithelial voltage was maintained at a value close to +20 mV. The magnesium flux was unaltered by this new experimental condition. Removal of PTH from the bath resulted in a significant fall in the magnesium net flux, whereas voltage, imposed by the NaCl gradient, remained unchanged. Control magnesium transport was reestablished with removal of furosemide and PTH. Data from Wittner *et al.* (89).

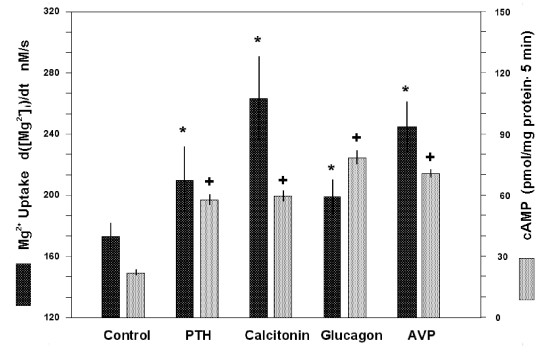

Figure 7. Peptide hormones stimulate intracellular cAMP formation and Mg^{2+} uptake in Mg^{2+} depleted mouse distal convoluted tubule (MDCT) cells. Confluent MDCT cells were cultured in Mg^{2+} -free media (<0.01 mM) for 16-20 hr. Measurement of cAMP was performed with radioimmunoassay. Fluorescence studies were performed in buffer solutions in absence of Mg^{2+}, and where indicated, $MgCl_2$ (1.5 mM final concentration) was added to observe changes in intracellular Mg^{2+} concentration. PTH, calcitonin, glucagon, and AVP were added to the buffer solution where indicated. Values are mean ± SE. * indicates changes in significance ($p<0.01$) of Mg^{2+} uptake and + indicates significance ($p<0.001$) of cAMP release from the respective control values. From Dai *et al.* (94).

transport elicited by hormones is not proportional to the increase in voltage. Without hormones, the basal net flux of magnesium is about 0.02 pmole?mm^{-1} ?mm^{-1} ?mV^{-1} whereas the hormone-dependent component of the transport is about 0.12 pmole?mm^{-1} ?mm^{-1} ?mV^{-1} . Magnesium transport is almost exclusively if not totally passive. When the transepithelial potential difference is imposed, lumen positive, for example by perfusing the lumen with 50 mM NaCl and the bath with 150 mM NaCl, the hormones increase magnesium and calcium reabsorptive fluxes in the presence as well as in the absence of active NaCl transport and in the absence of any variation in voltage (89). If the voltage is artificially maintained at an elevated value and then PTH withdrawn from the bath, magnesium and calcium fluxes decrease despite a constant voltage (Figure 6). In conclusion, these hormones affect net reabsorption in the mouse by two synergistic actions: an increase in the paracellular pathway permeability and an increase in voltage. In the rabbit, only the paracellular pathway permeability is modified since an increase in magnesium reabsorption in isolated tubules under the influence of PTH or calcitonin was observed in the absence of any changes in voltage.

4.3. Distal Tubule

The early micropuncture studies showed that hormones, such as PTH, calcitonin, glucagon and AVP, increase magnesium reabsorption in the superficial distal tubule (29, 53, 55, 58, 90). These hormones also stimulate intracellular cAMP formation in the distal tubule suggesting that they act, in part, through protein kinase A-mediated pathways (83, 91-93). More recently, it has been shown with isolated mouse distal convoluted tubule (MDCT) cells that in addition to these hormones, isoproterenol and prostaglandins (PGE_2) also stimulate Mg^{2+} uptake; all acting, in part, through cAMP-mediated signaling pathways (94, 95). Figure 7 summarizes this data. It was also shown in these studies that phospholipase C was essential to hormone action suggesting complex interactions among intracellular signaling pathways in hormone-mediated magnesium transport within the distal tubule (94, 95). Finally, insulin was shown to stimulate Mg^{2+} transport through activation of tyrosine kinase-mediated signalling pathways (96).

The steroid hormones have been shown to have important effects on Mg^{2+} uptake in distal tubule cells. Aldosterone potentiated hormone-mediated cAMP formation and Mg^{2+} transport (97). $1,25(OH)_2D_3$ on the other hand, stimulated Mg^{2+} uptake by cellular mechanisms which are separate from cAMP-mediated pathways (98). Accordingly, $1,25(OH)_2D_3$ has an additive action with hormones, like PTH, which act through cAMP (98). The evidence from micropuncture studies and experiments with isolated cells clearly show that a plethora of hormones control magnesium transport within the distal tubule.

Micropuncture and microperfusion studies and experiments with isolated cells have shown that many hormones regulate magnesium absorption in the thick

ascending limb and distal tubule. Interestingly, with the exception of prostaglandins, all of these hormones and factors also increase magnesium transport in the thick ascending limb of the loop of Henle (Table 1). 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. For example, the use of the loop diuretic, furosemide, would be expected to lead to severe magnesium wasting if the distal tubule were not situated to conserve part of the extra magnesium delivered to it from the loop of Henle.

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

5. NONHORMONAL INFLUENCES OF RENAL MAGNESIUM TRANSPORT

5.1. Intrinsic control: Magnesium restriction

It has long been known dietary magnesium restriction leads to renal magnesium conservation with diminished urinary magnesium excretion (7, 99, 100). This response is rapid, occurring within 4-8 hours; is sensitive, proceeds without a detectable change in plasma magnesium concentration; and is selective in the long term, altering magnesium reabsorption without changes in Na^+ or Ca^{2+} excretion. Conversely in states of magnesium surfeit, urinary magnesium markedly increases without significant increases in plasma magnesium (101). This ability to adapt to magnesium availability is intrinsic as it may be observed in isolated cells independent of hormones and other factors (60, 61).

Adaptation of magnesium transport with dietary magnesium restriction occurs at least in two segments, the ascending limb and distal tubule (54, 57, 58). Wittner and colleagues have recently shown that magnesium absorption is increased in cTAL segments harvested from dietary magnesium- deprived mice compared to control animals (102). Their studies showed that increased absorption was due to an increase in permeability of the paracellular pathway for magnesium which enhances magnesium movement at any given transepithelial voltage but independent of voltage and without changes in NaCl transport. Again, the increment in magnesium transport in the loop was entirely passive in nature. The cellular basis for the changes in the paracellular pathway are undefined (102). Interestingly, this response was not specific as calcium transport was also increased with magnesium depletion (102). Urinary calcium excretion was also observed to decrease in rats maintained on low magnesium diets but occurred 24-48 h following the fall in urinary magnesium (57). Chronic magnesium depletion in humans is associated with diminished urinary magnesium and normal to high excretion of calcium (7, 99, 103). This has been explained, in part, by acquired PTH resistance observed in magnesium deficiency (54).

We showed that isolated distal tubule cells, either MDCK or MDCT, cultured in magnesium-free media increased their Mg^{2+} transport rate (61, 66). This response is rapid (within 1-2 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. Furthermore, the adaptation was dependent on the concentration of media magnesium and the length of time in the culture media (60).

The response of epithelial cells to magnesium-free culture media is rapid; detectable within 1-2 hr. The increase in Mg^{2+} refill rate is not maximal until ? 4-6 hr. This may suggest 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 (60). 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 (60). From these studies, we infer that Mg^{2+} -depletion results in genetic expression of new proteins which 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.

The ability of the kidney to adjust its transport rate, i.e. control urinary magnesium excretion has been used by clinicians to assess overall magnesium balance (7, 104). Patients are given a parenteral magnesium load and urinary magnesium retention measured over 24 hours. Subjects with magnesium deficits will retain more magnesium than normal individuals. This practical use of renal adaptation is a very good example of the intrinsic ability of epithelial cells to respond to magnesium availability.

5.2..Ca2+/Mg2+-sensing:hypercalcemia, hypermagnesemia

Elevation of plasma magnesium or calcium concentration inhibits magnesium and calcium reabsorption leading to hypermagnesiuria and hypercalciuria. Inhibition of reabsorption occurs within the thick ascending limb of the loop of Henle and the distal convoluted tubule (29, 51, 105, 106). In the loop where magnesium is passively reabsorbed an elevation of extracellular magnesium or calcium may decrease 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 -

10 mM (107). However, this does not explain our earlier observations which indicated that magnesium and calcium acts only from the peritubular or blood side not the luminal side (29, 106). *In vivo*microperfusion of rat TAL segments clearly showed that increases in luminal magnesium concentrations were associated with increases in magnesium absorption rates (29). Intraluminal magnesium or calcium did not inhibit either magnesium or calcium absorption (108). However, elevation of plasma magnesium or calcium, i.e. on the basolateral side of the TAL, resulted in inhibition of both magnesium and calcium absorption. 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. In the distal tubule where magnesium is actively reabsorbed the early micropuncture and microperfusion experiments showed that hypermagnesemia and hypercalcemia inhibited magnesium absorption (29, 50, 105, 106). In one study where the luminal and plasma levels were controlled, fractional magnesium transport fell from 34±5 % to 14±7% with an elevation of plasma magnesium from 0.75 mM to 3.0 mM (29). Again, the explanation for these actions where not known.

The identification of an extracellular Ca^{2+}/Mg^{2+} sensing receptor located on the peritubular side of TAL cells can explain this phenomenon (13, 109). The receptor is comprised of three major domains: 1) a large extracellular amino-terminal domain of 613 amino acids which 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 signalling processes (109). Ca^{2+} or Mg^{2+} binds to the extracellular domain initiating a number of intracellular signals. 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 (109). 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 (110-112). 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 thick ascending and distal convoluted cells (111, 113). The site and location of the Ca^{2+}/Mg^{2+} -sensing receptor(s) have important effects on renal magnesium handling. Wang *et al.* reported that basolateral receptor activation inhibits apical K^+ channels and possibly Na-2Cl-K cotransport in the rat TAL (114). Moreover, De Jesus Ferreira *et al* reported that there is co-expression of the Ca^{2+}-sensing receptor with a Ca^{2+}-inhibitable adenylate cyclase in the rat cTAL (115). Thus, the Ca^{2+}-sensing receptor is 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. Desfleurs *et al* demonstrated that the addition of 3 mM calcium to the extracellular bath inhibited AVP-induced increase in intracellular cAMP in mouse cortical thick ascending limbs (116). Surprisingly, they found that the extracellular calcium did not alter AVP-dependent increase in in voltage of either mouse or rat ascending limb segments perfused with symmetrical conditions or the basal and AVP-dependent increase in transport in mouse limbs. When mouse cortical thick ascending limbs perfused under asymmetrical conditions (50 mM NaCl in the lumen, 150 mM NaCl in the bath) high tubular calcium resulted in a 50% decrease in magnesium absorption but did not blunt AVP-dependent increase in magnesium transport. Therefore, it is probable that the sensing receptor plays a role in controlling paracellular transport of calcium and magnesium.

Elevated magnesium and calcium inhibits hormone-stimulated Mg^{2+} uptake in MDCT cells suggesting that the Ca^{2+}/Mg^{2+} -sensing receptor also acts at the distal level (117). It is probable that the extracellular receptor acts by different mechanisms than those described in the loop. The evidence is that the receptor in MDCT cells acts through G_i protein to diminish hormone-stimulated cAMP levels and thus magnesium transport (118). Finally, Sands *et al* have reported that elevation of divalent cations in the medullary collecting duct activates the luminal Ca^{2+}/Mg^{2+} -sensing receptor which diminishes water permeability and inhibits volume reabsorption (119). The net effect is to increase volume flow along with an increase in calcium and magnesium excretion. The notion advanced by these authors is that a concomitant increase in volume flow with inhibition of calcium and magnesium reabsorption would minimize the incidence of stone formation (13). Accordingly, it has become clear that the Ca^{2+}/Mg^{2+}-sensing receptor plays an important role in the physiological control of renal magnesium handling.

The Ca^{2+}/Mg^{2+} -sensing receptor also explains the so-called "Tm" phenomenon of urinary magnesium excretion (101, 120-122). Clearance studies have suggested that renal magnesium reabsorption is a Tm-limited process, i.e. the kidney possesses a tubular maximum for transport that can be saturated with elevations of plasma magnesium. Determination of the Tm value as been useful in assessment of renal magnesium conservation (121, 122). Micropuncture studies have shown that this phenomenon is due to segmental differences in magnesium absorption (52). The cellular basis for a Tm is inhibition of magnesium transport within the loop of Henle (52). 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. chlorothiazide (CTZ) at final concentrations of 10^{-4} M were added from stock solutions where indicated. Data from Dai *et al.* (66, 66).

5.3. Extracellular Fluid Volume

Renal magnesium reabsorption is influenced by

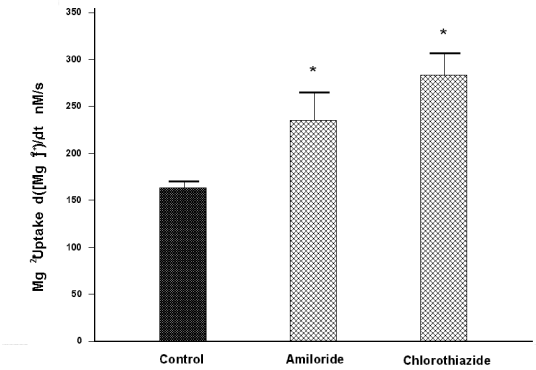

Figure 8. Effect of amiloride or chlorothiazide on Mg^{2+} influx into Mg^{2+}-depleted MDCT cells. Amiloride or

extracellular volume in that volume contraction increases magnesium conservation and volume expansion decreases magnesium absorption (101, 123). Changes of magnesium reabsorption within the proximal tubule are not markedly altered even though NaCl and water are significantly affected (124). This is probably due to the low reabsorption rate of magnesium relative to salt and water in the proximal tubule. The basis for changes of renal magnesium excretion with volume changes is principally associated with alterations of salt and magnesium transport within the loop of Henle, both the descending thin limb (see above) and thick ascending limb (52). Extracellular volume may have important pathophysiological effects. Hypermineralocorticoidism is accompanied by salt conservation due to enhanced distal tubular NaCl reabsorption (125). The resulting extracellular volume expansion diminishes proximal and loop magnesium reabsorption leading to renal magnesium wasting and in extreme cases to hypomagnesemia (126, 127).

5.4. Diuretics

5.4.1. Osmotic diuretics

Any solute that is not absorbed or reaches its maximal tubular transport rate becomes an osmotic diuretic. Accordingly, glucose and urea may increase salt and water excretion in addition to substances such as mannitol. Osmotic diuretics diminish salt and water reabsorption in the proximal tubule, loop, and distal tubule by increasing the flow rate along these segments (128, 129). The principal action on magnesium handling is in the thin descending and thick ascending limbs of the loop of Henle. Magnesium wasting in uncontrolled diabetes may be explained, in part, on increased filtered glucose and unreabsorbable ketoacids (130, 131).

5.4.2. Loop diuretics

Loop diuretics, such as furosemide and bumetanide, diminish salt absorption in the thick ascending limb by virtue of their action on electroneutral Na-2Cl-K cotransport across the luminal membrane (59, 86). 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 (86). It has been postulated that the lumen-positive voltage provides the driving force through the paracellular route for 40-50% 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 (59). Chronic usage of furosemide may lead to renal magnesium wasting and magnesium deficiency.

5.4.3. Distal diuretics

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 (108, 132). 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 (133). The cellular effects of amiloride on Mg^{2+} uptake were investigated in isolated MDCT cells (66). Amiloride stimulated nifedipine-sensitive Mg^{2+} influx by 41±3% (Figure 8). Amiloride blocks Na^+ entry into DCT cells and hyperpolarizes the cell by -28±8 mV (66). 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 (66, 134). These findings provide the basis for both clinical and experimental observations that show that amiloride is a magnesium-conserving diuretic (135).

Thiazides are extensively used in the management of diseases due to fluid retention, diabetes insipidus, and nephrolithiasis. Recent studies with isolated MDCT cells have shown that chlorothiazide increases Mg^{2+} uptake in a dose-dependent fashion. Maximal concentrations of chlorothiazide increased Mg^{2+} transport by 58% (67). 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 (68, 134). 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 clinical use of thiazide diuretics in patients over a long period of time sometimes lead to hypomagnesemia probably from renal magnesium-wasting (100, 136, 137). Chlorothiazide inhibits Na-Cl cotransport leading to an increase in urinary NaCl excretion and contraction of the extracellular fluid volume. The renin-angiotensin system is activated resulting in elevated aldosterone levels and increased potassium and hydrogen ion secretion which 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. However, hypokalemia has been associated with altered renal magnesium handling that may explain some of the cases of potassium

deficiency with chronic chlorothiazide usage (see below).

5.5. Metabolic Acidosis/Alkalosis

It has long been known that systemic acidosis is associated with renal magnesium- wasting (101). Acute metabolic acidosis produced by infusion of NH_4Cl or HCl leads to significant increases in urinary magnesium excretion (138). Chronic acidosis also leads to urinary magnesium-wasting which, as with acute acidosis, may be partially corrected by the administration of bicarbonate (139-141). In contrast to metabolic acidosis, acute and chronic metabolic alkalosis consistently leads to a fall in urinary magnesium excretion (138).

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 *et al.* showed that metabolic alkalosis resulted in increased magnesium reabsorption in the loop of Henle and distal tubule of the dog (138). Magnesium reabsorption was closely associated with bicarbonate delivery to the distal tubule in this study. Shapiro *et al* has shown that acute bicarbonate infusions into chronic acidotic rats leads to a marked increase in magnesium reabsorption in the loop and distal tubule (141). Thus, on balance, the evidence is that metabolic acidosis and alkalosis act within both the loop of Henle and the distal tubule.

In order to determine the cellular mechanisms of pH on magnesium transport in the 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 (102). 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 both transepithelial voltage and NaCl transport. They interpreted this data to indicate thatalkalosis 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 (102).

With regards to the distal tubule, Dai *et al* used the MDCT cell line (Figure 9) to determine the effects of pH changes on cellular Mg^{2+} uptake (142). In these cells, acute alkalosis markedly enhance Mg^{2+} uptake whereas acidosis diminish transport. Bicarbonate had no effect on Mg^{2+} entry. This information indicates that protons directly affect Mg^{2+} entry through the Mg^{2+} channel. A change in extracellular pH has significant effects on many kinds of ion channels by protonation of the amino acids comprising the pore region (143, 144).

Metabolic acidosis of any etiology would be expected to lead to diminished magnesium reabsorption in the loop and distal tubule. For example, Mather *et al.* and McNair *et al.* have reported that 25 and 38%, respectively, of outpatients with diabetes mellitus have hypomagnesemia (145, 146). Magnesium wasting with diabetes is due to insulin deficiency but more importantly as a result of keto acidosis often associated with the disease (145, 146).

5.6. Potassium Depletion

Hypokalemia and potassium depletion is associated with diminished magnesium absorption within the loop and distal tubule that may lead to renal magnesium wasting (147-149).

In the 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 (150-152). 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 and governed by the transepithelial voltage, it is probable that impaired NaCl transport may lead to diminished divalent cation absorption in this segment.

In isolated MDCT cells, potassium depletion may also inhibit Mg^{2+} uptake as determined by microfluorescence (Figure 9). The mechanism for diminished Mg^{2+} entry is not due to an alteration in the membrane voltage (67). Further studies are required to fully explain the defective distal Mg^{2+} transport associated with cellular potassium depletion.

5.7. Phosphate Depletion

One of the hallmarks of hypophosphatemia and cellular phosphate-depletion is the striking increase in urinary excretion of calcium and magnesium (153). Magnesium excretion may be sufficiently large to lead to hypomagnesemia (154, 155). The increase in divalent ion excretion in both human and 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 (153).

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 (153). Using micropuncture, we have demonstrated that defective magnesium absorption occurred in the loop of Henle and the distal tubule of phosphate-depleted dogs (156). The cellular mechanisms involved with diminished magnesium absorption within the loop of Henle have not been determined.

Cellular phosphate-depletion leads to diminished Mg^{2+} uptake in MDCT cells (157). Thus the DCT may be involved, in part, the increase in magnesium excretion associated with hypophosphatemia. Removal of phosphate from the media rapidly leads to diminished Mg^{2+} transport

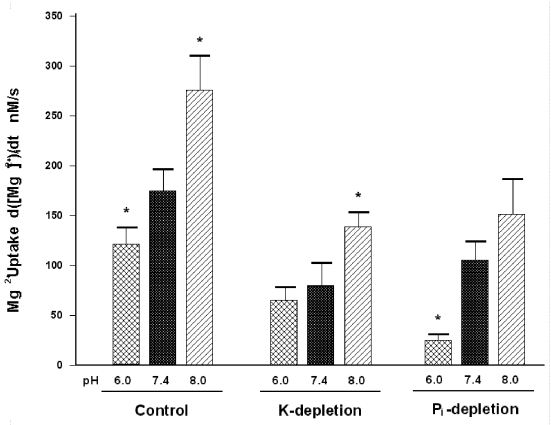

Figure 9. Summary of acid-base, potassium depletion, and phosphate (Pi) deficiency on the rate of Mg^{2+} entry into MDCT cells. Cell preparation and fluorescence determinations were performed according to techniques given in Dai *et al.* (61). The refill solutions were buffered to 6.0, 7.4, or 8.0, 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 ± SE for 9-10 cells. *indicates significance, $p<0.05$, of Mg^{2+} uptake rate compared to control values. Data from Dai *et al.* (67, 142, 157).

which is dependent on the degree of phosphate-depletion. Mg^{2+} uptake is inhibited by 50% when cultured in about 0.3 mM phosphate (Figure 9). These actions are fully reversible with the return of phosphate to the media. The induction of defective transport which is associated with phosphate depletion must reside within the cell either to prevent the normal upregulation of Mg^{2+} transport with Mg^{2+} deficiency or to inhibit Mg^{2+} uptake through actions on transport processes. In order to determine if phosphate-depletion acts through post-translational mechanisms, MDCT cells were first Mg^{2+} depleted for 16 hr to maximally upregulate Mg^{2+} transport. The cells were then phosphate-depleted for various time periods and Mg^{2+} uptake was assessed by microfluorescence. 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 (158, 159). Crook reported a two-fold increase in the prevalence of hypophosphatemia (plasma phosphate < 0.8 mM) and a six-fold increase in hypokalemia (plasma potassium < 3.5 mM) in patients with hypomagnesemia (plasma magnesium < 0.70 mM) (160). 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 (12). 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.

6. ACKNOWLEDGEMENTS

This work was supported by research grants from the Medical Research Council of Canada (MT-5793) and from The Kidney Foundation of Canada to GA Quamme.

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Key Words: Review, Magnesium, Kidney, Proximal Tubule, Loop Of Henle, Distal Tubule, Parathroid Hormone, Calcitonin, Glucagon, Arginine Vasopressin, Isoproterenol, Prostaglandins, Insulin, Vitamin D, Magnesium Restriction, Metabolic Aciosis, Hypermagnesemia, Hypercalcemia, Phosphate Depletion, Potassium Depletion, Diuretics, Review

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