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Mg^{2+} transport in the kidney

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Abstract

Magnesium is abundant in biological systems and an important divalent cation in the human body. Mg^{2+} helps mediate cellular energy metabolism, ribosomal and membrane integrity. Additionally Mg^{2+} modulates the activity of several membrane transport and signal transduction systems. Despite its importance however, little is known about the molecular mechanisms of Mg^{2+} transport and homeostasis in mammals. In mammals the amount of Mg^{2+} absorption is about the same as the amount of Mg^{2+} excretion in urine. Additionally, when total Mg^{2+} intake is deficient, the kidney is capable of reabsorbing all filtered Mg^{2+}. This balance between intake and excretion indicates that the kidney plays a principal role in maintenance of total body Mg^{2+} homeostasis. Within the kidney, Mg^{2+} filtered by the glomerulus is handled in different ways along the nephron. About 10–20% of Mg^{2+} is reabsorbed by the proximal tubule. the bulk of Mg^{2+} (about 50–70%) is reabsorbed by the cortical thick ascending limb of the loop of Henle. In this region, Mg^{2+} moves across the epithelium through the paracellular pathway, driven by the positive lumenal transepithelial voltage. A recently cloned human gene, paracellin-1 was shown to encode a protein localized to the tight junctions of the cortical thick ascending limb and is thought to mediate Mg^{2+} transport via the paracellular space of this epithelium. The distal convoluted tubule reabsorbs the remaining 5–10% of filtered Mg^{2+}. This segment seems to play an important role in determining final urinary excretion, since there is no evidence for significant Mg^{2+} absorption beyond the distal tubule. Although many renal Mg^{2+} transport activities have been characterized, no Mg^{2+} transporter cDNAs have been cloned from mammalian tissues. Recent research has certainly expanded our knowledge of Mg^{2+} transport in kidney; but details of the transport processes and the mechanisms by which they control Mg^{2+} excretion must await cloning of renal Mg^{2+} transporters and/or channels. Such information would provide new concepts in our understanding of renal Mg^{2+} handling.

Abbreviations: ADH – antidiuretic hormone/vasopressin; CaSR – Ca^{2+} sensing receptor; cTAL – cortical thick ascending limb of the loop of Henle; DCT – distal convoluted tubule; PCLN-1 – Paracellin-1; PT – proximal tubule.

Introduction

Magnesium (Mg^{2+}) is the fourth most abundant cation in human body and is second only to K^+ in intracellular concentration. It plays a critical role in cellular energy metabolism, ribosomal membrane integrity, protein translation and activity modulation of many membrane transporters and signal transduction systems.

The human body contains 20–28 g of Mg^{2+} (Brenner & Rector 1996). Less than 2% of the total body magnesium is in the extracellular space, 60% is in bone, and the rest is distributed almost equally between muscle and non-muscular soft tissue. Of soft tissues, striated muscle and liver have the highest magnesium content. About three-fourths of bone magnesium exists in apatite crystals. In magnesium de-

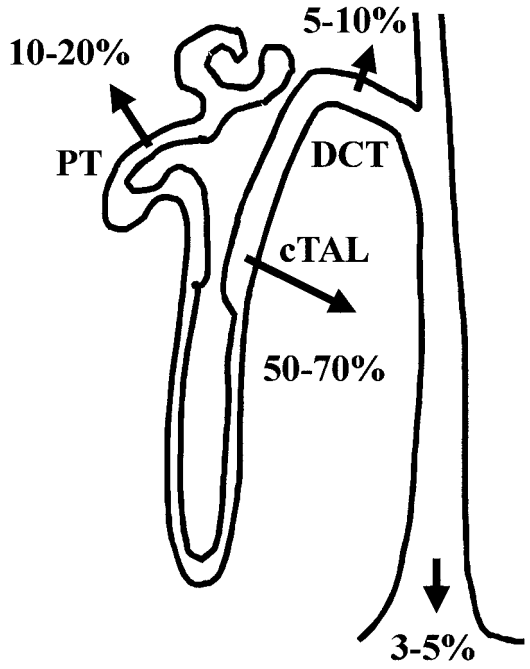


Fig. 1. Model of Mg^{2+} absorption along the nephron. The cartoon depicts the relative magnitudes of Mg^{2+} transport along the mammalian nephron.

ficiency, bone magnesium moves to the extracellular space through stimulation by parathyroid hormone.

The serum magnesium concentration is held at a constant level of 1.8–2.5 mg/dl (0.75–1.05 mM). Approximately 55% of serum total magnesium is free cation; 13% complexed to phosphate, citrate and unidentified anions; and 32% bound to albumin and α-globulins. Only the ionic forms are filtered by glomeruli in the kidney. The average daily dietary intake of magnesium is about 200 mg in adults (4 mg/kg day). Of this 30–50% is absorbed by the jejunum and ileum, but this can rise to 70% when dietary Mg^{2+} is deficient (Brannan *et al.* 1976). The remaining Mg^{2+} is lost in feces. Only about 3–5% of filtered Mg^{2+} normally appears in the urine. That is, Mg^{2+} absorption is balanced by a relatively equal excretion in urine. When total body Mg^{2+} is deficient, the kidney is capable of reabsorbing all filtered Mg^{2+}. These observations indicate that the kidney is the principal organ responsible for maintenance of total body magnesium homeostasis.

In the kidney, filtered Mg^{2+} is handled by varied mechanisms in each nephron segment (Quamme 1997). Figure 1 illustrates the relative magnitudes of Mg^{2+} absorption along the nephron. About 10–20% of Mg^{2+} is reabsorbed by the proximal tubule (PT). Most of the filtered Mg^{2+} (about 50–70%) is reabsorbed by the cortical thick ascending limb of the loop of Henle (cTAL). The distal convoluted tubule (DCT) reabsorbs the remaining 5–10%. These nephron segments have their own distinct mechanisms of Mg^{2+} absorption. This review will discuss known and inferred mechanisms of Mg^{2+} transport in the kidney.

Proximal tubule (PT)

The 10–20% of Mg^{2+} filtered at the glomeruli is absorbed by the PT in mammalian adult. In the mammalian neonatal PT, Mg^{2+} reabsorption in the PT is greater than that in the adult. Lelievre-Pegorier and coworkers reported that the 60–70% of filtered Mg^{2+} is reabsorbed in the PT of the rat neonate (Lelievre-Pegorier *et al.* 1983). This is similar to the PT isotonic reabsorption of Na^+, water and Ca^{2+}. It has been conjectured that the paracellular pathway is less selective in early stages of development, so that large amounts of Mg^{2+} are reabsorbed with Na^+ and water. With growth, the principal site for Mg^{2+} reabsorption gradually changes to the cTAL.

PT reabsorption of Mg^{2+} in adult is probably an active process, paralleling that of Na^+ and water (Wen *et al.* 1970a) but has a very low rate. Fractional Mg^{2+} reabsorption in the PT is unaffected by volume expansion (Massry *et al.* 1967a), diuretic administration (Wong *et al.* 1979), or Ca^{2+} concentration (Quamme 1982). Thus Mg^{2+} reabsorption in the PT appears independent of reabsorption pathways and seems dependent on only lumenal Mg^{2+} concentration.

Mg^{2+} wasting diseases associated with the PT have not been reported. One likely reason is that the mechanisms of Mg^{2+} reabsorption in the PT are unknown. Figure 2 shows a schematic model of Mg^{2+} absorption in the PT. The Mg^{2+} pathway seems to be through Mg^{2+} channels in the lumenal membrane. These presumed Mg^{2+} channels may be dependent only on lumenal Mg^{2+} concentration because Mg^{2+} reabsorption in the PT increases in hypermagnesemia (Wen *et al.* 1970a).

Cortical thick ascending limb of the loop of Henle (cTAL)

Most filtered Mg^{2+} (about 50–70%) is reabsorbed by the cTAL. The medullary TAL (mTAL) does not

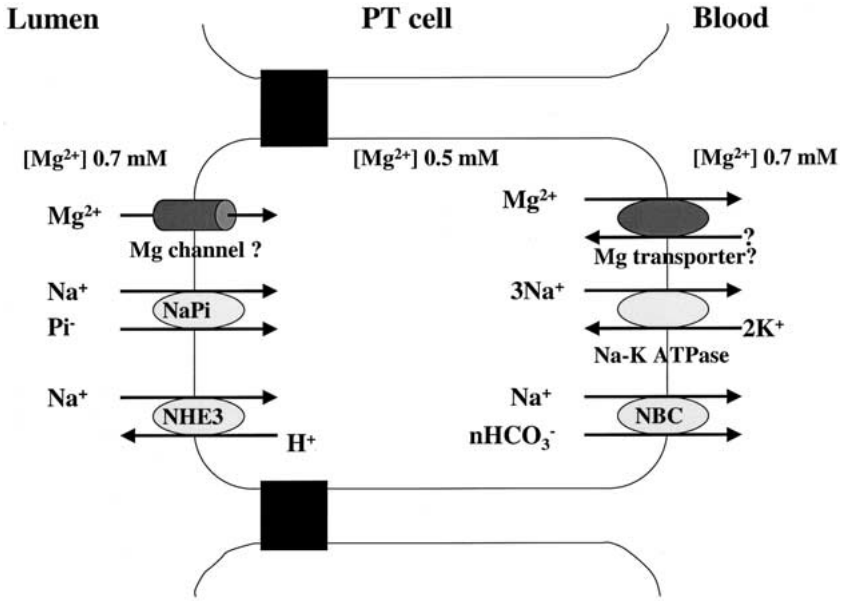


Fig. 2. Schematic model of Mg^{2+} absorption in proximal tubule (PT). Little gradient exists for net transcellular Mg^{2+} in the PT. This cartoon illustrates the proposed Mg^{2+} transporters and other basic ion transporters of the mammalian proximal tubule.

appear to have any capacity for Mg^{2+} reclamation (Shareghi & Agus 1982a). Mg^{2+} transport in the cTAL parallels that of NaCl reabsorption and is dependent on the lumen-positive voltage in this segment. Fractional Mg^{2+} reabsorption in the cTAL is as affected by volume expansion (Poujeol *et al.* 1976) or loop-diuretic administration, e.g., furosemide (Quamme 1981), both resulting in a decrease in the lumen-positive potential across the cTAL. These findings suggest that Mg^{2+} transport in the cTAL is primarily passive and driven by the electrical gradient. That is, Mg^{2+} moves across the cTAL through the paracellular pathway driven by the positive lumenal voltage (Figure 3). The maintenance and magnitude of this positive voltage depend on active NaCl reabsorption. As illustrated in Figure 3, obstruction of this NaCl reabsorption causes renal Mg^{2+}, Ca^{2+} and Na^{+} wasting. Mutations in the Na-K-Cl cotransporter (NKCC2) (Simon *et al.* 1996a), the inwardly rectifying potassium channel (ROMK) (Simon *et al.* 1996b), and a chloride channel (CLCNKB) (Simon *et al.* 1997) all obstruct NaCl reabsorption. These mutations are known as Bartter's syndrome type 1, 2, and 3, respectively. As noted above, loop-diuretics cause Mg^{2+} wasting in the cTAL by disturbing NKCC2.

Recently, positional cloning has identified a human gene for a paracellular protein, paracellin-1 (PCLN-1) in the TAL (Simon *et al.* 1999). This gene encodes a protein of 305 amino acids with four transmembrane domains and intracellular N- and C-termini. The PCLN-1 protein shows sequence and structural similarity to members of the claudin family and has been given the name claudin-16. Mutations in PCLN-1 result in abnormal permeability of the paracellular pathway leading to renal Mg^{2+} and Ca^{2+} wasting. However, the function of PCLN-1 is unclear since the investigators have not been able to demonstrate Mg^{2+} transport by the recombinant protein. The suggestion is that each/many claudin(s) may selectively regulate paracellular permeability to various ions (Simon *et al.*, 1999; Van Itallie *et al.*, 2001).

Many hormones, such as parathyroid hormone (Kuntziger *et al.* 1974; Shareghi & Agus 1982a), calcitonin (Quamme 1980), glucagon (Bailly & Amiel 1982), arginine vasopressin (de Rouffignac *et al.* 1983), insulin (Mandon *et al.* 1993), and aldosterone (Suki *et al.* 1968), increase in Mg^{2+}reabsorption by the cTAL. The actions of these hormones are mediated by different pathways affecting lumenal voltage and paracellular structure.

The extracellular Ca^{2+} sensing receptor (CaSR), which also binds Mg^{2+}, at physiological concentrations, may play a role in Mg^{2+} transport in the cTAL, especially when plasma Mg^{2+} concentration is high (Hebert 1996; Di Stefano *et al.* 1997). In this condition, activation of CaSR decreases NaCl absorption

(inhibition of NKCC2), thereby decreasing the lunempositive voltage, the primary driving force for Ca^{2+} and Mg^{2+} transport in the cTAL. Potassium depletion (Eknoyan *et al.* 1970; Gutsche *et al.* 1984; Luke *et al.* 1978) and hypophosphatemia (Coburn & Massry, 1970; Wong *et al.* 1980a) decrease Mg^{2+} reabsorption in the cTAL. Although changes in transcellular voltage and/or paracellular structure are likely, the Mg^{2+} transport mechanisms are unknown.

Distal convoluted tubule (DCT)

The DCT reabsorbs 5–10% of filtered Mg^{2+}. This tubule segment seems to play a primary role in determining final urinary Mg^{2+} excretion since there is no evidence for significant Mg^{2+} absorption beyond the DCT. Mg^{2+} transport in isolated DCT segments has not been extensively studied primarily because these segments are very difficult to isolate. Quamme and Dai, using Madin-Darby canine kidney (MDCK) cells and mouse DCT cells, have reported that apical Mg^{2+} entry is through specific and regulated pathways that are not shared with Ca^{2+}, in MDCK-cells (Quamme & Dai 1990). Mg^{2+} entry is stimulated by hyperpolarization (Dai *et al.* 1997c), and they have suggested that cellular Mg^{2+} entry in the DCT is mediated by a Mg^{2+} channel (Quamme 1997). Reilly and Ellison recently postulated an alternative explanation for Mg^{2+} movement across the DCT (Reilly & Ellison 2000). Although they do not demonstrate Mg^{2+} reabsorption under normal conditions in the DCT, they suggest that it occurs through PCLN-1 in the DCT in the presence of loop diuretics. Additionally, they suggest that Mg^{2+} is secreted through PCLN-1 in Gitelman's syndrome, known to result in hypokalemic metabolic alkalosis, hypomagnesemia, and hypocalciuria. These explanations do not seem likely because Mg^{2+} secretion in microperfused DCT has not been detected (Quamme 1997).

Although the mechanisms of Mg^{2+} entry in the DCT are not known, the most likely mechanism is through an active transcellular pathway. Figure 4 shows a schematic model of Mg^{2+} absorption in the DCT where we suggest that current observations are most compatible with Mg^{2+} transport by a Na^+/Mg^{2+} exchanger (NMX) at the lumen. The driving force for such an exchanger is presumably provided from active NaCl reabsorption through the Na^+-Cl^- co-transporter (NCC) and the amiloride-sensitive Na^+ channel (ENaC). Mutations in NCC cause Gitelman's syndrome (Simon *et al.* 1996c). Since active NaCl reabsorption is obstructed by this mutation, NMX function would be diminished or abolished, leading to Mg^{2+} wasting. When comparing Bartter's syndrome, patients with Gitelman's syndrome have more severe hypomagnesemia. The reason may the lack of any Mg^{2+} reabsorption beyond the DCT.

Many hormones, including parathyroid hormone (Burnatowska *et al.* 1977), calcitonin (Poujeol *et al.* 1980), glucagon (Dai *et al.* 1998a), arginine vasopressin (Dai *et al.* 1998a), insulin (Dai *et al.* 1999), and aldosterone (Dai *et al.* 1998b) increase Mg^{2+} reabsorption in the DCT similarly to their action on the cTAL. Essentially, the action of the hormones is to increase active NaCl reabsorption through a cyclic AMP-dependent pathways. Potassium depletion (Dai *et al.* 1997a), hypophosphatemia (Dai *et al.* 1997b), and administration of aminoglycosides, e.g., gentamicin, tobramycin, streptomycin, and neomycin (Kang *et al.* 2000) decrease Mg^{2+} reabsorption in the DCT, but the mechanisms are unknown. These conditions would obstruct a Mg^{2+}transporter or diminish active NaCl reabsorption.

Renal disease and treatment associated with Mg^{2+} transport

Dai and coworkers have recently reviewed several disease states associated with altered Mg^{2+} homeostasis in the distal nephron (Dai *et al.* 2001). A summary and update of that data follows.

Diuretics

Both amiloride and its congeners (inhibitors of ENaC) and chlorothiazides (inhibitors of NCC) can alter Mg^{2+} balance in the distal nephron. Amiloride appears to stimulate Mg^{2+} uptake in DCT cells through a nifedipine-sensitive pathway (Dai *et al.* 1997c). Though the mechanism of the inhibition remains unclear, Cefaratti, Romani and Scarpa demonstrated blockade of a Ca^{2+}-Mg^{2+} exchange mechanism in liver plasma membranes (Cefaratti *et al.* 1998). In contrast the mechanism of chlorothiazide inhibition seems to require an integrated cellular response likely mediated by NCC.

Familial Mg^{2+} disorders

As previously discussed, mutations in NCC (SLC12A3), NKCC2, ROMK1, ClCNKB, and PCLN1 all give rise

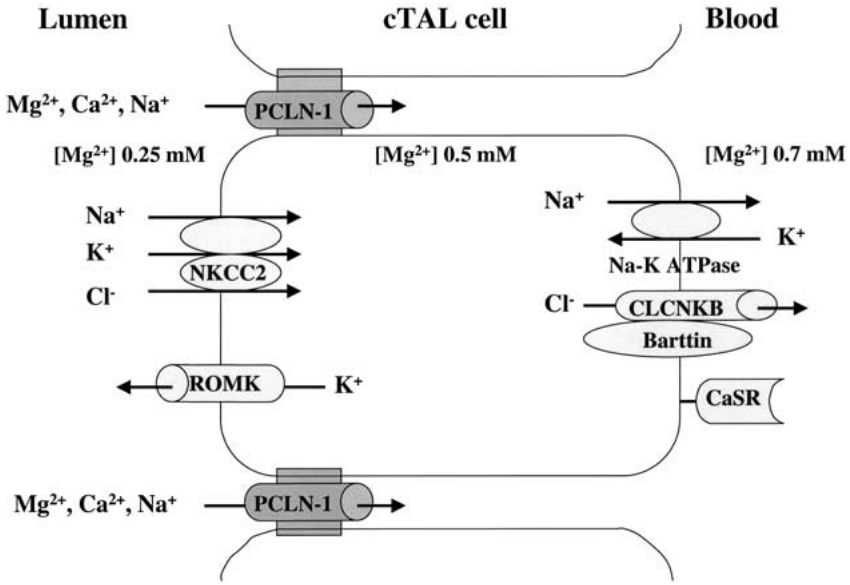


Fig. 3. Schematic model of Mg^{2+} absorption in cortical thick ascending limb of Henle (cTAL). The bulk of Mg^{2+} transport occurs in the TAL. As indicated in the text and diagram, the transport appears exclusively paracellular via PCLN-1. This paracellular transport is believed to be driven by the other indicated transporters responsible for transepithelial transport and which maintain the transepithelial, lumen positive, potential.

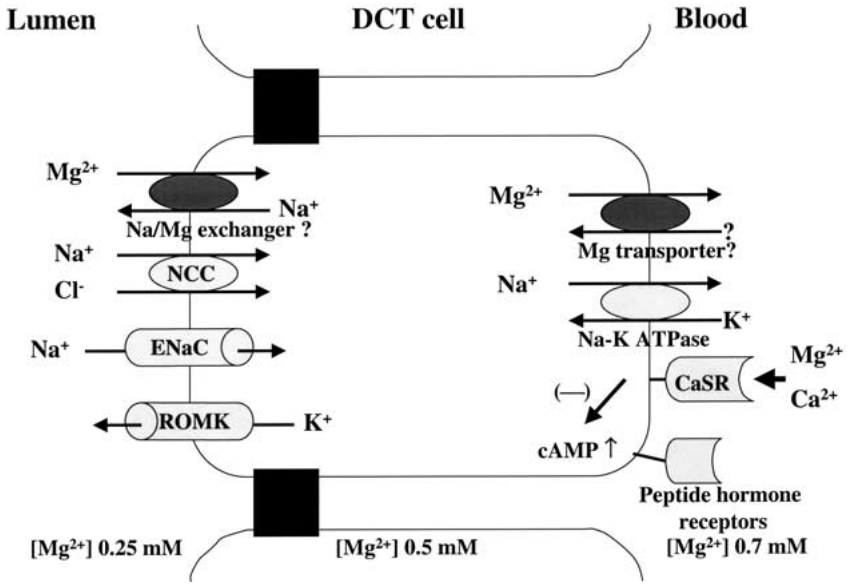


Fig. 4. Schematic model of Mg^{2+} absorption in distal convoluted tubule (DCT). Mg^{2+} transport in the DCT is the final titration of Mg^{2+} and is therefore highly regulated. Though the diagram does not depict this regulation, hormonal and agonist regulation of the indicated transporters all appear to affect Mg^{2+} homeostasis.

to a Mg^{2+} disorder phenotype though none of these proteins, with the probable exception of PCLN1, are directly involved in cellular Mg^{2+} transport. Rather, the effects of a defective cellular transport process on the complex integration of cellular ion homeostasis results in altered Mg^{2+} homeostasis.

More directly related to Mg^{2+} transport is the CaSR (Brown *et al.* 1993; Riccardi *et al.* 1995) that also senses Mg^{2+} (Bapty *et al.* 1998; Hebert & Brown 1996; Riccardi *et al.* 1995). Mutations of the CaSR show both activating and inactivating properties (Hebert & Brown 1996). Specifically, inactivating mutations of the CaSR are manifest as familial hypocalciuric hypercalcemia or neonatal severe hyperparathyroidism (Chou *et al.* 1995; Pollak *et al.* 1993, 1994a, b). Inactivating CaSR mutations lead to inappropriate Ca^{2+} and Mg^{2+} handling by the distal tubule (Hebert 1996). Activating mutations of the CaSR are associated with the autosomal dominant form of hypoparathyroidism, presenting as an isolated hypocalcemic hypoparathyroidism (Okazaki *et al.* 1999; Pearce *et al.* 1996b). These activating mutations are associated with subclinical but detectable hypomagnesemia (Okazaki *et al.* 1999). Indeed, expression of the CaSR in HEK-293 cells directly demonstrates that activation or inactivation of CaSR by mutations is the cause of the pathophysiology (Bai *et al.* 1996; Pearce *et al.* 1996a).

Other Mg^{2+} genetic links

There are several forms of altered Mg^{2+} homeostasis for which individual protein targets have not been identified or are not clearly linked to Mg^{2+} transport: (a) hypomagnesemic with secondary hypocalcemia (OMIM #602014, HOMG1) and (b) autosomal dominant, late-onset, isolated hypomagnesemia (OMIM #154020, HOMG2). HOMG1 is autosomal recessive and is genetically linked to 9q12-22.2 (Walder *et al.* 1997). These patients respond to a 20-fold increase in dietary Mg^{2+} intake, implicating intestinal Mg^{2+} malabsorption or renal Mg^{2+} wasting. Either possibility suggests a defective Mg^{2+} transport system. However, neither a cDNA nor a gene are currently linked to this syndrome. In contrast, H092 was previously genetically linked to 11q23, and Meij and associates recently cloned and identified mutations in a gamma subunit of the Na^{+}-K^{+} pump (FXYD2) which result in the primary hypomagnesemia (Meij *et al.* 2000). These mutations affect proper membrane trafficking. The diminished cellular Na^{+} gradient in the distal tubule is hypothesized to lead to Mg^{2+} wasting. Recessive forms of isolated hypomagnesemia appear to all result from PCLN1 mutations (see OMIM#248250). The genetics of Mg^{2+} disorders is described in depth in the article by Meij et al. in this issue.

Cardiac disorders

About 20% of the cardiac output goes to the kidneys, translating to ~180 l/day of renal blood flow. Renal filtration and nephron ion transport are dependent on this blood flow. That is, increased renal blood flow increases glomerular filtration and nephron ion transport, while decreased flow has the opposite effect (Alpern *et al.* 1983; Alpern & Rector 1996). Thus, ailments of the cardiovascular system which can affect cardiac output and systemic blood flow, can also adversely affect the kidneys.

The benefits of Mg^{2+} supplementation in ischemic heart disease or heart failure are well known. However, it is not known whether low intracellular free Mg^{2+} is a causal factor in such myocardial dysfunction. Griffiths reported that a low intracellular Mg^{2+} concentration ($[Mg^{2+}]_i$) can itself cause significant cardiomyocyte dysfunction in absence of any contributing disease state(Griffiths 2000). And, hypomagnesemia can occur as chronic or acute manifestation of physiological changes, pathological conditions, or pharmacological interventions (Dai *et al.* 2001). Furthermore, it is reported that variation in the contractile properties associated with change in extracellular Mg^{2+} may be effected by alteration in Ca^{2+} transients (Nair & Nair 2000). Kh *et al.* indicated that magnesium supplementation prevents blood pressure elevation in deoxycorticosterone acetate induced hypertension in rats, an effect associated with inhibition of platelet calcium uptake and decreased intracellular free calcium concentration(Kh *et al.* 2000). Decreased intracellular Mg^{2+} concentration may be involved in the pathogenesis of primary hypertension. Thus, there is an intimate interplay between the kidney and the heart for maintaining Mg^{2+} homeostasis in mammals.

Ions influencing Mg^{2+} homeostasis

As indicated in the previous discussion and in the review by Romani and Maguire in this issue, several ions influence Mg^{2+} homeostasis: Ca^{2+}, Mg^{2+}, PO_4^{3-}, and H^{+} (pH). Additionally, water balance, i.e., volume homeostasis, also appears to regulate renal Mg^{2+} transport.

A. Mg^{2+} Transport Systems: *Known molecular entities*

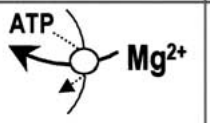
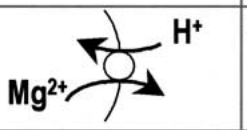
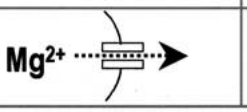
	Name	cDNA	$[Mg^{2+}]_i$	Process	Inhibitor	Tissue
A	CorA	CorA	↑, ↓		Cobalt-hexaammine	Bacteria Archaea **(NO mammalian homolog)**
B	Mgt	MgtA/B MgtE	↑			Bacteria **(NO mammalian homolog)**
C	Mg^{2+}/ H^+ exchanger	AtMHX ?	↓		?	Arabidopsis -functionally in kidney, liver, and heart
D	Paracellin	PCLN-1	?		?	Kidney: cTAL

Fig. 5. Known/Hypothesized Mg^{2+} Transport Systems. A. Documented and cloned Mg^{2+} transport systems. B. Hypothesized Mg^{2+} transporters based on physiological experiments. No cDNAs have been reported that encode the proposed ion transport function.

Magnesium

As observed with most ions, there is a negative feedback system to 'self-regulate' ion homeostasis. Not surprisingly, hypermagnesemia, i.e., high blood Mg^{2+}, increases proximal tubule Mg^{2+} absorption (Wen *et al.* 1970b). Somewhat paradoxically, this elevated Mg^{2+} level also leads to reduced cTAL Mg^{2+} absorption. This latter phenomenon likely results from decreased Mg^{2+} delivery to the cTAL as a result of the increased PT absorption. However, the limiting effect in the TAL during hypermagnesemia is basolateral rather than lumenal membrane transport (Quamme & Dirks 1980). Recent evidence indicates that the CaSR may regulate this process in the cTAL (Hebert & Brown 1996). Conversely, hypomagnesemia, i.e., low blood Mg^{2+}, decreases PT Mg^{2+} absorption while increasing cTAL Mg^{2+} absorption. The mechanisms, and proposed mechanisms, are discussed above in *Renal disease and treatment associated with Mg^{2+} transport.*

Calcium

Hypercalcemia results increased Ca^{2+} excretion and Mg^{2+} excretion, though Mg^{2+} excretion rates are often higher (Suki 2000). This effect seems located in the cTAL (Quamme 1982). Hypocalcemia decreases Mg^{2+} excretion (Quamme & Dirks 1980). Again, the CaSR of the cTAL is thought to mediate this regulation (Hebert & Brown 1996; Suki 2000).

Phosphate

The major body store of Ca^{2+} and Mg^{2+} is in bone as phosphate salts. Phosphate depletion results in hypermagnesuria and hypercalciuria (Kreusser *et al.* 1978; Sachtjen *et al.* 1979). A study in dogs indicates that the transport defect is likely in the cTAL and DCT (Wong *et al.* 1980b), perhaps also associated with the CaSR. This process can be hormonally reversed with PTH or by phosphate supplementation. Phosphate depletion seems to directly effect DCT epithelia because acute phosphate depletion reduces intracellular Mg^{2+} in 30–60 min (Dai *et al.* 1997b).

Acid-base status

The solubility of divalent cation salts is strongly influenced by solution pH. In general, Ca^{2+} and Mg^{2+} are more soluble as the solution pH decreases.

Acidosis.
Metabolic acidosis is most often associated with decreased renal Mg^{2+} absorption (Lennon & Piering 1970). Blood pH is normally 7.35–7.45. Acidosis means that blood pH is lower than normal, e.g., 7.0–7.3. Thus, more ionic Mg^{2+} is in solution, in this

B. Mg^{2+} Transport Systems : *No molecular information*

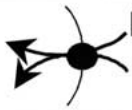
	Name	cDNA	$[Mg^{2+}]_i$	Process	Inhibitor	Tissue
E	Na^+/Mg^{2+} exchanger	?	↓		(amiloride) imipramine	Invertebrates / Vertebrates Heart, liver, kidney
F	Mg^{2+}/ Ca^{2+} exchanger	?	↓		amiloride imipramine	Heart ? kidney- DT, CCD liver
G	Mg^{2+}- Anion- cotransporter	?	↑		DIDS	RBCs, gut heart

Fig. 5. Continued.

example in the blood. From pure solution chemistry this may be 'perceived' by the nephron as a systemic increase in free blood Mg^{2+} thereby reducing Mg^{2+} absorption. Both acute and chronic acidosis generally lead to Mg^{2+} loss in the urine (Dai *et al.* 2001; Lennon & Piering 1970). This loss is partially reversed by bicarbonate infusion (Suki 1991). Not surprisingly, the decreased absorption again appears associated with the cTAL and DCT implicating a role for the CaSR. It is also noteworthy that improperly controlled diabetes mellitus can result in a diabetic ketoacidosis thereby resulting in hypomagnesemia and renal Mg^{2+} wasting (Dai *et al.* 2001; Husmann *et al.* 1997; Kelepouris & Agus 1998).

Alkalosis.

In contrast to acidosis, alkalosis (blood pH 7.5–7.7) is most often associated with increased Mg^{2+} absorption (Dai *et al.* 2001; Suki 1991). Wong and associates found that this increased absorption occurred in the presence of furosemide indicating Mg^{2+} absorption prior to the cTAL (Wong *et al.* 1986). Though increased blood bicarbonate appeared to facilitate this absorption (Suki 1991), Dai and coworkers found that mouse DCT Mg^{2+} transport was sensitive to the bathing pH rather than increased bicarbonate in particular (Dai *et al.* 1997c).

Water balance

Isotonic volume expansion leads to increased renal excretion Na^+, Ca^{2+} and Mg^{2+}, and therefore decreased absorption (Massry *et al.* 1967b; Shareghi & Agus 1982b). Conversely, hypotonic volume expansion increases distal Mg^{2+} absorption implicating an effect on TAL transepithelial voltage (Shareghi & Agus 1982b). Antidiuretic hormone (ADH) is best known for increasing water absorption in the distal nephron segments. ADH also effects other nephron segments. The action of ADH to stimulate NKCC2 via cyclic AMP (Imbert-Teboul *et al.* 1978) is hypothesized to facilitate this process by increasing the cTAL lumen positive voltage (Hall & Varney 1980; Hebert *et al.* 1981; Sasaki & Imai 1980). The increased renal Mg^{2+} excretion due to isotonic volume expansion thus seems to be controlled at the cTAL. Since the DCT fine-tunes the final 5–10% of Mg^{2+} absorption (Figure 1), increased Mg^{2+} from decreased cTAL Mg^{2+} absorption can overwhelm the DCT transport mechanisms. ADH also increases Mg^{2+} uptake in cultured DCT cells (Dai *et al.* 1998a).

Molecular entities

To date, Mg^{2+} transporter genes or cDNAs (Figure 5A) have been cloned only in bacteria (Hmiel *et al.* 1989; Snavely *et al.* 1989) and a plant (Shaul *et al.* 1999). Mammalian and teleost Mg^{2+} transport activities have been characterized. As previously indicated, PCLN-1 mutations are genetically linked to hypomagnesemia originating in the cTAL (Simon *et al.* 1999). Though a role for PCLN-1/claudin-16 in the paracellular permeability of Mg^{2+} was hypothesized, these investigators were not able to directly

demonstrate Mg^{2+} transport. Thus, no direct evidence is currently available showing cloning or characterization of Mg^{2+} transporter cDNAs or proteins from mammalian tissues (Figure 5B).

Are there other proteins that can affect cellular Mg^{2+} transport? Recently, Tashiro and coworkers have reported that the Na^{+}/Ca^{2+} antiporter NCX can transport Mg^{2+} and may therefore play a role in Mg^{2+} extrusion (Tashiro *et al.* 2000); however, since Mg^{2+} concentrations in these experiments were 10-fold greater than normal, it is unclear whether NCX physiologically acts to transport Mg^{2+}. Figure 5 shows Mg^{2+} transporters and channels inferred from phenotypic characterization.

Conclusion

Recent research has certainly expanded our knowledge of Mg^{2+} transport in the kidney. However, detailed knowledge and understanding of Mg^{2+} homeostasis in the body must await identification of specific Mg^{2+} transporters and channels in the kidney. Such information will undoubtedly provide new concepts of renal Mg^{2+} handling.

Though there are not yet molecular entities identified that directly mediate Mg^{2+} transport, renal Mg^{2+} transport studies focuses on human pathophysiology to guide our understanding. In fact, there are at least 10 distinct, inherited human diseases associated with renal Mg^{2+} handling referenced in the Online Mendelian Inheritance in Man (OMIM) database[a]. Several of these OMIM citations (numbers 602014 and 154020) have yet to be linked to specific genes. Clearly, an active area of investigation is genetic linkage of these disorders with affected kindreds to identify the gene and cDNA associated with the pathophysiology.

Mg^{2+} buffering, homeostasis and transport has emerged as directed and highly regulated as observed with most mono- and divalent inorganic ions handled by the kidney. New measurement techniques are now being applied to Mg^{2+} transport research, e.g., fluorescence, electrophysiology and DNA array technology. In particular, DNA array techniques will allow investigators to treat cells and/or organisms with physiologic manipulations that have similar Mg^{2+} transport outcomes. Comparison of such samples will allow investigators to determine if similar genes are involved in the physiologic response. Finally, this entire process of gene and transporter discovery will be greatly aided by the draft of the human genome now available, as well as the mouse and rat genomes soon to follow.

[a]OMIM is located at http://www3.ncbi.nlm.nih.gov/omim

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