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When EGF is offside, magnesium is wasted

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Our understanding of magnesium (Mg^{2+}) regulation has recently been catapulted forward by the discovery of several disease loci for monogenic disorders of Mg^{2+} homeostasis. In this issue of the *JCI*, Groenestege et al. report that their study of a rare inherited Mg^{2+} wasting disorder in consanguineous kindred shows that EGF acts as an autocrine/paracrine magnesiotropic hormone (see the related article beginning on page 2260). EGF stimulates Mg^{2+} reabsorption in the renal distal convoluted tubule (DCT) via engagement of its receptor on the basolateral membrane of DCT cells and activation of the Mg^{2+} channel TRPM6 (transient receptor potential cation channel, subfamily M, member 6) in the apical membrane. These authors show that a point mutation in pro-EGF retains EGF secretion to the apical but not the basolateral membrane, disrupting this cascade and causing renal Mg^{2+} wasting. This work is another seminal example of the power of the study of monogenic disorders in the quest to understand human physiology.

Magnesium (Mg^{2+}) is a critical cofactor in many enzymatic reactions and as such participates in all cellular functions. It is the second most common intracellular ion and the fourth most abundant cation in the body, and plasma and cellular Mg^{2+} concentrations are both tightly controlled. The renal regulation of Mg^{2+} excretion can range from 100% reabsorption of the filtered load (0% excretion) to excretion of greater than 100% of the filtered load (renal secretion) under experimental conditions (1, 2). This extraordinary homeostatic feat performed by the kidney still evades our comprehension after several decades of investigation. The initial advances in our understanding of Mg^{2+} handling stemmed from clearance, micropuncture, microcatheterization, and microperfusion studies. While these experiments furnished the key foundations of understanding the regulation of Mg^{2+} balance, this process is surprisingly poorly understood at the cellular and molecular levels, largely due to a lack of good surrogate cell model systems and a slow rate of emergence and hence paucity of cDNAs and specific reagents for Mg^{2+} homeostatic proteins. Almost all of the seminal progress in enlightening our understanding of the molecular mechanisms of Mg^{2+} handling arose from identification of disease loci of rare human monogenic Mg^{2+} disorders (3–10). The discovery reported by Groenestege et al. in this issue of the *JCI* (11) appends a new page to this catalog of pedagogical disorders. These authors show that EGF is an autocrine/paracrine magnesiotropic hormone that regulates renal Mg^{2+} reabsorption by regulating the activity of the Mg^{2+}-permeable channel TRPM6 (transient receptor potential cation channel, subfamily M, member 6). They go on to demonstrate that a point mutation in pro-EGF that disrupts sorting of the protein to the basolateral membrane of distal convoluted tubule (DCT) cells in kidney nephrons and thus release of EGF to the basolateral space or inhibition of the EGFR by anti-EGFR antibodies led to suppressed activity of TRPM6 and renal Mg^{2+} wasting in humans.

Nonstandard abbreviations used: DCT, distal convoluted tubule; HSH, hypomagnesemia with secondary hypocalcemia; IRH, isolated recessive renal hypomagnesemia; NCC, NaCl cotransporter; TRPM6, transient receptor potential cation channel, subfamily M, member 6.

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Mg^{2+} homeostasis

The systemic balance of Mg^{2+} and its intracellular concentration are determined by intestinal absorption and renal excretion. The main site of intestinal Mg^{2+} absorption is the small bowel, with some additional absorption in the large bowel. Renal handling commences with glomerular filtration of the non–protein bound plasma fraction (free and complex) followed by passive absorption through the paracellular pathway in the proximal tubule and the thick ascending loop of Henle and active transcellular absorption by the DCT (Figure 1) (12). The molecular mechanism of these processes remained elusive for many years until identification of disease genes underlying hereditary Mg^{2+} homeostatic disorders. Analysis of the mutations leading to familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) disclosed that passive Mg^{2+} absorption by the thick ascending

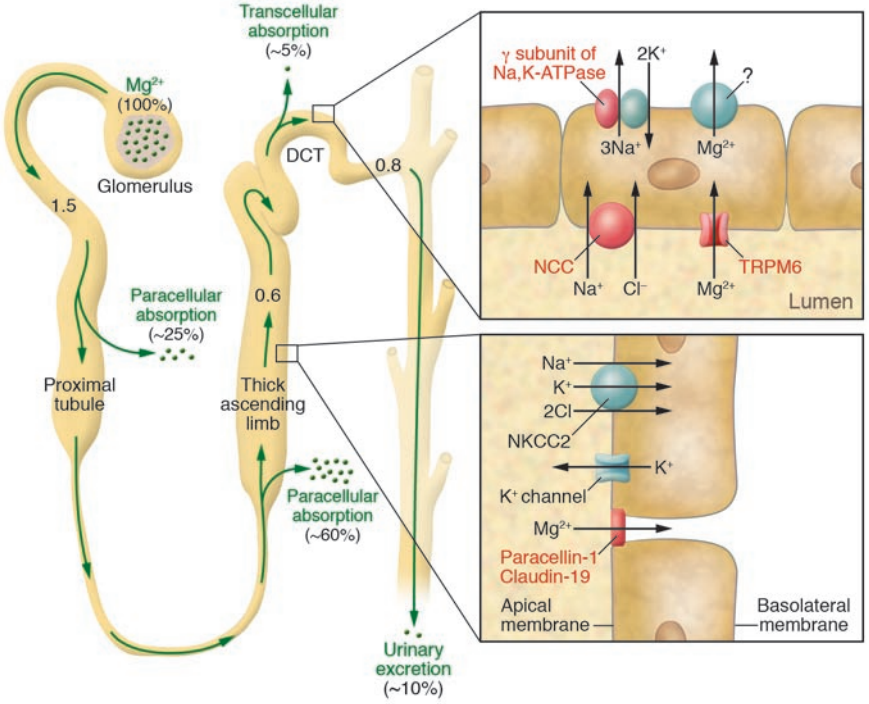

Figure 1

Renal Mg^{2+} handling. Non–protein bound Mg^{2+} is filtered freely at the glomerulus, and the approximate percentages of filtered Mg^{2+} absorbed at different locations are shown. Under most physiologic conditions, about 10% of filtered Mg^{2+} is excreted. The final regulatory segment, the DCT, controls approximately 5% of filtered Mg^{2+}. Mg^{2+} is transported by both the paracellular and transcellular pathways. Four monogenic diseases that lead to renal Mg^{2+} wasting as a result of mutations in the genes coding for the proteins shown in red have been described to date. Mutations in paracellin-1 and claudin-19 are involved in familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC). Mutations in TRPM6 are involved in HSH. Mutations in NaCl cotransporter are involved in Gitelman syndrome, and mutations in the γ subunit of Na,K-ATPase are involved in autosomal dominant renal hypomagnesemia with hypocalciuria (ADRHH). The question mark indicates unknown pathways; the numbers 1.5, 0.6, and 0.8 indicate Mg^{2+} concentrations in moles in the lumen of the respective segment. NKCC2, Na,K-2Cl^- cotransporter.

limb is mediated by a tight-junction Mg^{2+} pathway, which includes paracellin-1 and claudin-19 (Figure 1) (3–5). Loss-of-function mutations in these proteins lead to a form of combined urinary Mg^{2+} and Ca^{2+} wasting (3–5). The DCT is responsible for only 5–10% of the filtered Mg^{2+}, but this critical section fine-tunes Mg^{2+} reabsorption to determine the final urinary Mg^{2+} concentration and thus is key to the regulation of Mg^{2+} homeostasis (12, 13). Magnesuria (physiologic or pathophysiologic) that exceeds 15% of the filtered load likely involves segments proximal to the DCT.

In the DCT, active transcellular Mg^{2+} transport requires passive Mg^{2+} entry across the luminal membrane, Mg^{2+} flow from the apical to the basal pole, and active extrusion across the basolateral membrane (Figure 1). Of these processes, only the pathway that mediates Mg^{2+} influx across the luminal membrane has been elucidated. Here again, an understanding of the cause of the condition hypomagnesemia with secondary hypocalcemia (HSH) disclosed the luminal Mg^{2+} entry pathway. HSH is a rare autosomal-recessive disease typified by low serum Mg^{2+} levels and high urinary fractional Mg^{2+} excretion and is caused by nonsense or missense mutations of TRPM6, a member of the transient receptor potential channel family (6, 7, 13, 14). Subsequent studies showed that TRPM6 is a Mg^{2+}-permeable channel that is expressed in the luminal membrane of the intestinal epithelium and the DCT (8, 15). Inactivating mutations of TRPM6 thus causes the pernicious combination of impaired gut absorption of Mg^{2+} and renal wasting. How Mg^{2+} traverses the cytoplasm from the apical to the basal poles and how it exits the cell across the basolateral membrane are not known. If diligence and luck prevail, there will be discoveries of more monogenic diseases on the horizon to help unveil the identity of the proteins involved in this process.

There are other monogenic diseases in this nephron segment that do not directly involve Mg^{2+}-transporting proteins. Inactivating mutations of the NaCl cotransporter causes Gitelman syndrome (9), which is characterized by hypomagnesuria with inappropriate renal wasting. It is presently unclear how defective apical NaCl entry can lead to Mg^{2+} wasting in the DCT. The disorder autosomal dominant renal hypomagnesemia with hypocalciuria (ADRHH) is caused by a dominant negative mutation of the γ subunit of the Na,K-ATPase, which causes mistargeting of the protein that is expressed mainly in the kidney with high levels in the DCT (10). At present, there exist only speculations as to how this mutation leads to severe renal Mg^{2+} wasting.

EGF is a magnesiotropic hormone

In their study in this issue of the *JCI*, Groenestege et al. (11) analyzed yet another form of hereditary hypomagnesemia, isolated recessive renal hypomagnesemia (IRH), a syndrome with classical symptoms of hypomagnesemia frequently presenting in infancy and childhood due to renal Mg^{2+} wasting, and found that the disease is caused by a P1070L mutation in the cytoplasmic domain of pro-EGF. Pro-EGF is a type 1 membrane protein expressed at high levels in the luminal and low levels in the basolateral membranes of the DCT (Figure 2). Pro-EGF is cleaved by a series of extracellular proteases to generate the active EGF in the luminal and basolateral spaces. Whether luminal EGF has a biologic effect is unclear since there is a paucity of luminal EGFRs under normal conditions. Groenestege et al. show that the P1070L mutation in EGF specifically prevents secretion of EGF to the basolateral space with no effect on secretion to the luminal space in polarized Madin-Darby canine kidney (MDCK) cells. Aberrant secretion of EGF may be the result of aberrant targeting of pro-EGF^{P1070L} to the basolateral membrane or aberrant processing by proteases. Although P1070 may be part of a basolateral targeting motif of PXXP, expression of pro-EGF^{P1070L} in human embryonic kidney (HEK) cells also affects EGF formation, leaving open the possibility that the mutation may affect pro-EGF processing.

How mistargeting and/or processing of pro-EGF causes IRH became clear when Groenestege et al. (11) found that EGF markedly increases TRPM6 activity. This

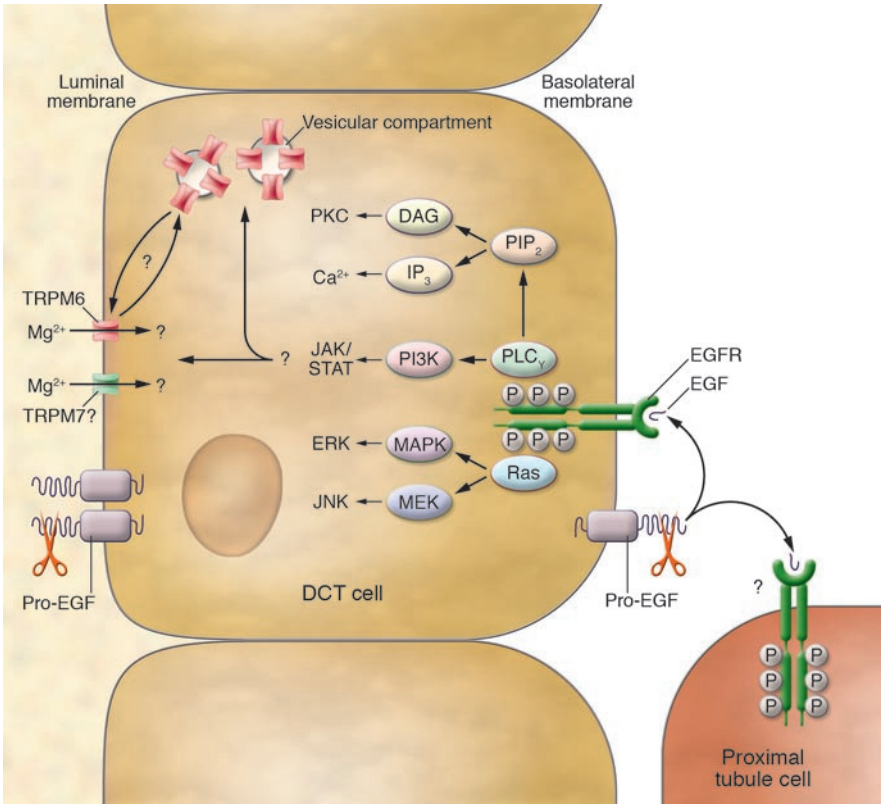

Figure 2

Model of the autocrine/paracrine action of EGF in the DCT cell and potential mechanisms by which EGF can regulate TRPM6 activity. Mg^{2+} influx across the luminal membrane is mediated by TRPM6 and may require the ubiquitous TRPM7. In this issue of the *JCI*, Groenestege et al. (11) report that EGF is a magnesiotropic hormone that regulates renal Mg^{2+} reabsorption by stimulating the EGFR, which then increases the activity of TRPM6. Aberrant targeting of pro-EGF to the basolateral membrane by the P1070L mutation results in reduced EGF production at the basolateral membrane, reduced activation of EGFR, reduced TRPM6 activity, and, consequently, Mg^{2+} wasting. Future studies should reveal which of the pathways activated by EGF mediates activation of TRPM6 and the mechanism by which TRPM6 activity is increased. As indicated by the long arrows, activation of EGFR by EGF and its tyrosine phosphorylation may directly activate TRPM6 and/or TRPM7 channel activity or may regulate insertion or retrieval or TRPM6 present in intracellular vesicular compartments. Because of the proximity of the DCT and proximal tubule, EGF generated by the DCT may activate EGFRs at the proximal tubule and therefore affect Mg^{2+} handling by this nephron segment, which reabsorbs 25% of filtered Mg^{2+}. DAG, diacylglycerol; IP_3, inositol-1,4,5 trisphosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; PLCγ, phospholipase Cγ; P, phosphate.

led the authors to propose the physiologic model in which baseline activity of basolateral EGFR activation is required for TRPM6 activity and Mg^{2+} entry. This model is compatible with the hypomagnesemia previously observed in cancer patients treated with the anti-EGFR antibody Cetuximab (11, 16, 17). To support that notion, the authors showed that Cetuximab also antagonizes the stimulation of TRPM6 activity by EGF in cultured cells.

Future directions

The novel finding that EGF acts as an autocrine/paracrine magnesiotropic hormone (11) opens the way to a better understanding of active Mg^{2+} reabsorption and its regulation. This first step raises many questions that are likely to be addressed in the coming years (Figure 2). A fundamental question is that of how EGF regulates TRPM6 activity. In principal, EGF can rapidly change TRPM6 expression in the plasma membrane of the DCT and/or EGF may directly gate TRPM6 channel activity. EGF can activate several signaling pathways, among which are the MAPK pathway, phospholipase Cγ (PLCγ) to activate PKC and increase intracellular Ca^{2+} levels, and the PI3K pathway. It will be of interest to determine which of these signaling pathways mediates activation of TRPM6 by EGF, as many of these signaling cascades are linked to the control of protein trafficking. Parathyroid hormone, calcitonin, glucagons, and vasopressin affect Mg^{2+} reabsorption by the DCT (18). Since these hormones activate some of the same pathways that are activated by EGF, the question is whether these hormones can regulate TRPM6 activity in parallel or in tandem to the autocrine/paracrine EGF system. The EGF axis is a target for therapy in autosomal dominant polycystic kidney disease (19). Does blanket blockade of EGF in the DCT have consequences in Mg^{2+} handling? EGF stimulates proximal tubule phosphate transport via basolateral receptors (20). Proteolytic release of EGF from the DCT is likely to result in contact between EGF and the proximal tubule in its immediate vicinity. Do patients with IRH have subtle phosphaturia? Finally, knowledge of the upstream regulator of DCT Mg^{2+} reabsorption opens up a host of possible therapeutic targets to manipulate renal Mg^{2+} handling.

Another question regarding the target effector of EGF is whether EGF can activate the TRPM6 homolog TRPM7 both alone and when it is coexpressed with TRPM6 (Figure 2). TRPM7 is ubiquitously expressed, functions as a Mg^{2+} channel, is exquisitely sensitive to intracellular Mg^{2+} and Mg^{2+}-ATP, and is suggested to be the cellular Mg^{2+} sensor (21). TRPM7 forms heteromultimers with TRPM6 (6, 14, 22), and the channel properties of the resulting TRPM6/TRPM7 heteromultimer are different from those of homodimers. Moreover, a missense mutation in TRPM6 suppresses TRPM7 activity (14). It is possible that the physiologically relevant Mg^{2+} influx channel is a TRPM6/TRPM7 heteromultimer although this notion remains controversial (13). Determining regulation of TRPM7 and TRPM6/TRPM7 channels by EGF may begin to clarify whether TRPM7 participates in Mg^{2+} reabsorption.

After many decades of work, both biologists and clinicians are still laboring at a rather early stage in understanding mammalian Mg^{2+} homeostasis. Mg^{2+} deficiency in humans is probably more prevalent than recognized and has been linked to cardiovascular disease, diabetes, hypertension, and inflammation. The epidemiology of Mg^{2+} deficiency and the basis for the correlative relationship between this condition and other diseases mandate elucidation and

confirmation by vigorous scientific studies. The demonstration of autocrine/paracrine regulation of TRPM6 by EGF adds a new chapter in the journey toward this goal.

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"AMPing up" our understanding of the hypothalamic control of energy balance

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AMP-activated protein kinase (AMPK) has emerged as a metabolic "fuel gauge," which oscillates between anabolic and catabolic processes that ultimately influence energy balance. A study in this issue of the *JCI* by Claret et al. now extends the role of AMPK in medial basal hypothalamic neurons (see the related article beginning on page 2325). These findings maintain AMPK signaling as a common cellular mechanism in proopiomelanocortin and neuropeptide Y/agouti-related protein neurons and links hypothalamic AMPK to coordinated energy and glucose homeostasis.

As we live in the midst of rising rates of obesity, diabetes, and associated comorbidities, intense interest exists in increasing the understanding of the cellular and molecular mechanisms by which nutrients and metabolic cues modulate neuronal activity and how neurons may ultimately regulate energy homeostasis. Key targets of such cues are neurons that reside in the medial basal hypothalamus. The prototypical "sensing" cells are proopiomelanocortin (POMC) and neuropeptide Y/agouti-related protein (NPY/AgRP) neurons in the arcuate nucleus of the hypothalamus. A wealth of data has demonstrated the inherent ability of these neurons to respond to changing levels of a number of signals including insulin, leptin, and glucose. The ability of these (and other) neurons to sense and integrate coordinated responses to changing levels of metabolic signals is thought to contribute to the control of energy balance (1–5). On the other hand, it is becoming apparent that dysregulation of this regulatory system contributes to the pathophysiology of obesity, diabetes, and other components of the metabolic syndrome (6–8).

In addition to identifying the key sensing neurons, we now are beginning to

Nonstandard abbreviations used: AgRP, agouti-related protein; AMPK, AMP-activated protein kinase; NPY, neuropeptide Y; POMC, proopiomelanocortin.

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