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The voltage-gated K^+ channel subunit Kv1.1 links kidney and brain

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Analysis of Mendelian Mg^{2+} wasting disorders helps us to unravel the mechanisms of Mg^{2+} homeostasis. In this issue of the *JCI*, Glaudemans and colleagues show that mutations in voltage-gated K^+ channel subtype 1.1 (Kv1.1) cause autosomal dominant hypomagnesemia in humans (see the related article beginning on page 936). Interestingly, other mutations in the same protein cause the neurological disease episodic ataxia type 1. The authors show, using cells with heterologous expression of the wild-type and mutant channels, that the mutant channel is dysfunctional and speculate that Mg^{2+} wasting results from changes in apical membrane voltage along the nephron. Mechanisms by which the apical voltage is generated and how Kv1.1 fits within this context are discussed herein.

Rare Mendelian diseases are windows into both physiology and pathogenesis. Examples include the rare Mg^{2+} wasting disorders that form the basis for most of our current understanding of renal Mg^{2+} transport. Several proteins that mediate Mg^{2+} transport, both around and through cells, have now been identified and cloned, using positional cloning approaches. Secondary dysfunction of these proteins may also contribute to hypomagnesemia in the critically ill, where the incidence has been estimated as 20%–60% and has been associated with excess mortality (1). Hypomagnesemia is often drug related, with diuretics, calcineurin inhibitors, and antineoplastic agents (e.g., cisplatin and cetuximab) common offenders (2). The study of Mendelian disorders of Mg^{2+} homeostasis has also led to the identification of novel and sometimes unexpected regulatory pathways that impact transport pathways secondarily.

Eighty percent of plasma Mg^{2+} is ultrafilterable by glomeruli. Whereas the majority of every other ion studied to date is reabsorbed along the proximal tubule, proximal Mg^{2+} reabsorption constitutes only 10%–15% of the filtered load. In contrast, the thick ascending limb (TAL) reabsorbs approximately 70% of filtered Mg^{2+} and clearly plays a central role in regulating Mg^{2+} excretion. What surprised many investigators, however, was that most disorders of Mg^{2+} balance result from dysfunction along the distal convoluted tubule (DCT), a short nephron segment that, just a few years ago, was believed to play only a minor role in Mg^{2+} homeostasis (3). The DCT is now recognized as important not only for Mg^{2+} balance, but also for the control of Na^+, K^+, and Ca^{2+} levels (4). In this issue of the *JCI*, Glaudemans and colleagues report that missense mutations in K^+ voltage-gated channel, Shaker-related subfamily, member 1 (*KCNA1*), which encodes voltage-gated K^+ channel subtype 1.1 (Kv1.1) expressed by DCT cells, causes autosomal dominant hypomagnesemia in humans (5). Surprisingly, other mutations in the same gene cause episodic ataxia type 1 (EA1) (6), a neurological syndrome in which hypomagnesemia has not been reported. In the present study, the investigators showed that Kv1.1 localizes to the apical membrane of DCT cells, where the transient receptor potential cation channel, subfamily M, member 6 (TRPM6) controls Mg^{2+} entry, driven by its electrochemical potential. Expression studies showed that the mutated Kv1.1 protein, while having no direct effect on TRPM6, exhibited reduced

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Nonstandard abbreviations used: CNT, connecting tubule; DCT; distal convoluted tubule; ENaC, epithelial Na^+ channel; KCNA1, K^+ voltage-gated channel, Shaker-related subfamily, member 1; Kv1.1, voltage-gated K^+ channel subtype 1.1; ROMK, renal outer medullary K^+ channel; TAL, thick ascending limb; TRPM6, transient receptor potential cation channel, subfamily M, member 6.

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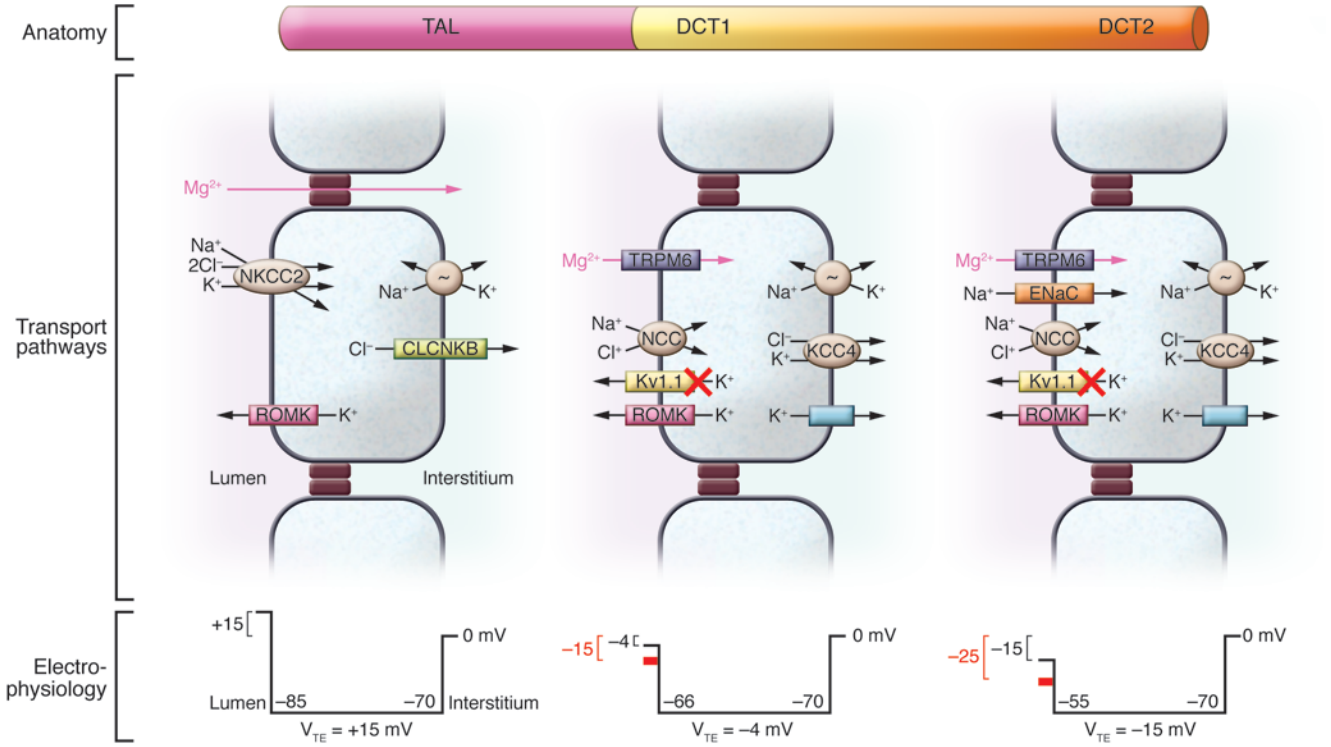

Figure 1
Schematic diagram of the anatomy, molecular pathways, and electrophysiology of Mg^{2+} transport along the distal nephron. Top panel: Nephron segmentation, including the TAL and the DCT subsegments DCT1 and DCT2. Note that the transition from DCT1 to DCT2 is gradual. Middle panel: Transport pathways in these segments include Na^+/K^+-ATPase (~), the furosemide-sensitive Na^+K^+2Cl cotransporter (NKCC2), the Cl^- channel (CLCNKB), the epithelial Na^+ channel (ENaC), and the thiazide-sensitive NaCl cotransporter (NCC). Mg^{2+} transport along the TAL is primarily paracellular. Along the DCT, however, it is mediated by TRPM6. Two K^+ channels are present in the apical membrane: ROMK and, as shown by Glaudemans et al. in this issue of the *JCI* (5), Kv1.1 (see text for further details). K^+ and Cl^- can exit DCT cells via a coupled K^+/Cl^- cotransporter (KCC4) or a discrete K^+ channel. Lower panel: Membrane voltages along each segment, in millivolts (mV). The basolateral voltage is similar in each cell type. V_{TE}, transepithelial voltage. As postulated by Glaudemans et al. (5), a defective Kv1.1 (indicated by red X) should depolarize the apical membrane (red bars) along the DCT, leading to hyperpolarization of the transepithelial voltage.

K^+ permeability (conductance), compared with the wild-type channel. The investigators therefore postulate that disease-causing defective K^+ channels depolarize the apical membrane of DCT cells, reducing the electrical driving force favoring Mg^{2+} entry, leading to renal Mg^{2+} loss.

Mg^{2+} transport and transepithelial voltage

Years before ion transport proteins expressed by DCT and TAL were identified at the molecular level, micropuncture and microperfusion studies provided important details about the electrophysiology of these nephron segments. The lumen is electrically positive along the TAL, with respect to the interstitium (Figure 1), whereas it is electrically negative along the last portion of the DCT (Figure 1) and remains negative along the connecting tubule (CNT) and collecting duct (not shown). The transepithelial voltage drives ion movement throughout the nephron. Along the TAL, the lumen positive voltage drives Ca^{2+}, Mg^{2+}, and, at least along part of the segment, Na^+ absorption across paracellular pathways; along the CNT and collecting duct, the lumen negative voltage drives K^+ secretion, primarily via the renal outer medullary K^+ channel (ROMK) (7). Along part of the DCT, the transepithelial voltage is near zero (Figure 1); the DCT, therefore, acts like an insulator, permitting two segments with voltages oriented in opposite directions to coexist. Yet the DCT is not a uniform segment but instead comprises distinct early (DCT1) and late (DCT2) regions (8). The transepithelial voltage is likely near zero along the DCT1 (9), but along the DCT2 it is oriented such that the lumen voltage is negative.

Because Ca^{2+} and Mg^{2+} are divalent cations, their sensitivity to transepithelial voltage is twice that of Na^+. Along the TAL, where the tight junctions are permeable to cations (mediated largely by claudins 16 and 19), voltage drives Ca^{2+} and Mg^{2+} strongly in the absorptive direction (10). As noted, however, reabsorption of Mg^{2+} continues along the DCT (both the DCT1 and DCT2 subsegments), where the transepithelial voltage becomes an impediment to absorption. To complicate matters further, the magnitude of the transepithelial voltage along the DCT2 *increases* whenever circulating levels of the hormone aldosterone rise (11), as during depletion of the extracellular fluid volume. Clearly, if there were substantial paracellular permeability for divalent cations along these segments, Mg^{2+} would not be reabsorbed; although the initial report suggested that claudin 16 (formerly paracellin-1) is expressed by the DCT (12), expression levels must be very low, because substantial paracellular transport does not occur.

Mg^{2+} and K^+ channels in the distal nephron

The conundrum for Mg^{2+} (and Ca^{2+}) (i.e., how to achieve reabsorption against an electrochemical gradient) is solved by a change in absorptive route, from paracellular to transcellular (Figure 1). This difference corresponds to the appearance of transient receptor potential channels that mediate Mg^{2+} uptake by DCT cells. In the case of Mg^{2+}, TRPM6, perhaps in association with TRPM7, plays this role (13); in the DCT, however, the voltage that drives ion movement is *not* the transepithelial voltage, but the voltage across the apical membrane (Figure 1), which is oriented with the cell interior being negative, with respect to apical fluid.

Glaudemans et al. (5) could not detect a direct effect of mutant Kv1.1 on TRPM6 in a heterologous expression system and therefore postulate that Mg^{2+} wasting is indirect, a result of depolarization of the apical membrane of DCT cells (Figure 1; see also Figure 4 in ref. 5). This provocative hypothesis is consistent with their data, which show that cells transfected with wild-type Kv1.1 display a transmembrane voltage near –50 mV, whereas cells transfected with mutant Kv1.1 do not. It also, however, raises intriguing questions for future study. Figure 4 in the Glaudemans et al. study (5) shows apical pathways for Mg^{2+} and K^+ in a model DCT cell. Figure 1 herein incorporates several additional pathways into a model of the TAL and DCT and emphasizes some complicating features. First, a second K^+ channel, ROMK, is expressed throughout the distal tubule (14). Second, the major apical depolarizing current in DCT2 (and CNT) is carried by Na^+ through the epithelial Na^+ channel (ENaC). Third, the voltage across any membrane will be determined largely by ionic concentration differences, factored by the relative conductance to each ion. In most models of DCT and CNT function, the K^+ conductance is generated predominantly by ROMK (7).

The model postulated by Glaudemans et al. to account for Mg^{2+} wasting in the disease setting (see Figure 4 in ref. 5) suggests that a dysfunctional Kv1.1 causes apical depolarization (note the red bars in Figure 1). This assumes that Kv1.1 contributes importantly to apical conductance, at steady state, together with ENaC and ROMK and implies that its dysfunction causes, in addition to apical depolarization, an increase in the magnitude of transepithelial voltage. Because ROMK is expressed by DCT cells (14), apical depolarization would enhance K^+ secretion, which should generate a phenotype similar to that of Gitelman syndrome, with K^+ and Mg^{2+} wasting; this is not typical for patients with autosomal dominant hypomagnesemia. The expression studies reported by Glaudemans et al. used nonpolarized cells that do not express most of the solute transport proteins of the distal nephron, so the membrane depolarization hypothesis could not be tested directly. Future work could perhaps address this issue by knocking a mutant *KCNA1* gene into mice. Another puzzling issue for future study is the observation that distinct mutations in nearby amino acid residues of *KCNA1* generate phenotypes that reflect dysfunction in two different organs (brain and kidney). One possible reason is that Kv1 subunits are (probably) never made into homomeric channels. They partner with other Kv1 subunits to constitute the functional tetrameric channel. In brain, Kv1 subunits coassemble with Kv1.2 and Kv1.4. In addition, they also coassemble with a variety of β subunits, and the complement of partners, both pore-forming α subunits and auxiliary β subunits, dictates the functional characteristics of the channels (15). Therefore, the composition of channels containing Kv1.1 in kidney and brain may well be different, and in the context of these tissue-specific heteromeric assemblies, mutations even at close locations within the protein exert their effects in a tissue-specific manner, giving rise to distinct physiological abnormalities.

Rare diseases and common syndromes

Inherited Mg^{2+} wasting is rare, but the current results (5) help to explain hypomagnesemia in several relatively common clinical situations. Patients with heart failure commonly suffer from Mg^{2+} wasting (16), perhaps owing to aldosterone excess and chronic loop diuretic use. Both factors depolarize the apical membrane of DCT2 cells just as in the postulated model of Kv1.1 dysfunction. Cisplatin treatment also depolarizes the apical membrane, thereby enhancing Mg^{2+} and K^+ excretion (17). Conversely, drugs that block aldosterone or ENaC represent the only effective approach to inhibiting renal Mg^{2+} wasting in most clinical situations (18). These drugs enhance the driving force favoring Mg^{2+} reabsorption, by hyperpolarizing the apical membrane of DCT cells. Discoveries such as the one reported in this issue by Glaudemans et al. will continue to illuminate pathophysiology; they also emphasize that mechanisms of ion transport are shared in kidney and brain, and in epithelial and nonepithelial cells.

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Myoglobin tames tumor growth and spread

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Tumor growth is accompanied by tissue hypoxia, but does this reduced oxygen availability promote further tumor expansion, resulting in a vicious cycle? In this issue of the *JCI*, Galluzzo et al. report that increasing oxygen tension in tumor cells by ectopically expressing the oxygen-binding hemoprotein myoglobin indeed affects tumorigenesis (see the related article beginning on page 865). Tumors derived from cells transfected with myoglobin grew more slowly, were less hypoxic, and were less metastatic. These results will spur further mechanistic inquiry into the role of hypoxia in tumor expansion.

Tumorigenesis involves not only cell autonomous genetic alterations that result in activation of oncogenes and loss of tumor suppressor genes but also adaptation of neoplastic cells to the tumor microenvironment. Although the activation of oncogenes and their products MYC, AKT, PI3K, and RAS as well as the loss of p53 and VHL tumor suppressors have been linked to altered tumor metabolism, it has become apparent that the hypoxic tumor microenvironment, which activates the low oxygen–sensing HIF transcription factors, also plays a key role in tumor metabolism and tumorigenesis (1). It is well documented that new blood vessels recruited into a growing tumor mass are disorganized (2), often culminating in vascular "dead-ends" rather than providing the canonical microvasculature characteristic of normal tissue, in which arterioles are connected to venules via a capillary bed. Hence, tumors endure significant hypoxia that is distributed heterogeneously within a tumor mass, and areas of tumor hypoxia could fluctuate with time (3). In this regard, whether tumor hypoxia itself contributes to tumor progression has been a matter of debate rather than of experimentation.

Transduction of myoglobin to improve tumor oxygenation

In this issue of the *JCI,* Galluzzo et al. (4) present an innovative and elegant approach to addressing the question of whether hypoxia is a side effect of or a key player in tumor progression. The authors used lentiviral vector–mediated transduction to achieve expression of myoglobin (Mb) in human lung carcinoma cells as a genetic tool to prevent tumor hypoxia. Like its molecular relative hemoglobin, Mb — a cytosolic hemoprotein present in skeletal and heart muscle (5) — reversibly binds O_2 and thus facilitates O_2 transport from the blood to mitochondria during periods of increased metabolic activity or serves as an O_2 reservoir under hypoxic conditions (Figure 1). Transduced cancer cells expressing Mb showed no signs of altered cellular proliferation in vitro, but, surprisingly, in vivo xenograft growth was severely diminished after injection of transduced cells into mice. The data reported by Galluzzo and coworkers are quite impressive: presence of Mb resulted in a 5-fold decrease in tumor expansion compared with controls. Furthermore, the expression of Mb suppressed both local and distal metastatic spread, led to enhanced cancer cell differentiation, and reduced the degree of abnormal vascularization. The authors also report that the expression of HIF-1α, which is generally accepted as a master regulator of the cellular hypoxic response, was downregulated in Mb-expressing cancer cells. The data suggest that the beneficial effects of the presence of Mb within cancer cells are the result of improved O_2 delivery to the tumor, which "calms" the tumor's craving to expand.

Globins, oxygen, and beyond

Beyond the initial finding of Mb's O_2-binding properties — set into a physiological context especially by the seminal work of Beatrice and Jonathan Wittenberg — within the last decade, the field of globin biology was invigorated, particularly by the generation of gene-deficient mutants but also by the discovery of two new members of the globin family, cytoglobin (Cb) and neuroglobin (reviewed in refs. 5–8). Based on this recent work, the role of Mb in muscle physiology has been reassessed and its scope of function has been considerably extended beyond oxygen storage and delivery to also include a role as an important scavenger of NO and ROS, signaling molecules involved in cellular oxidative stress (Figure 1). Appreciating the diversity of Mb's properties, Galluzzo et al. (4) used mutated forms of Mb unable to bind O_2 to demonstrate that the inhibition of tumor progression is primarily caused by improved O_2 delivery. However, it should be kept in mind that structural alterations at Mb's heme-binding pocket will also alter the in vivo kinetics for all other events taking place at this site, including radical reactions. It is, therefore, tempting to speculate that these newly uncovered properties of Mb may

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Nonstandard abbreviations used: Cb, cytoglobin; Mb, myoglobin.

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