Divalent cation transport by the distal nephron: insights from Bartter’s and Gitelman’s syndromes

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Ellison, David H. Divalent cation transport by the distal nephron: insights from Bartter’s and Gitelman’s syndromes. Am J Physiol Renal Physiol 279: F616–F625, 2000.—Elucidation of the gene defects responsible for many disorders of renal fluid and electrolyte homeostasis has provided new insights into normal and abnormal physiology. Identifying the causes of Gitelman’s and Bartter’s syndromes has greatly enhanced our understanding of ion transport by thick ascending limb and distal convoluted tubule cells. Despite this information, several phenotypic features of these diseases remain confusing, even in the face of molecular insight. Paramount among these are disorders of divalent cation homeostasis. Bartter’s syndrome is caused by dysfunction of thick ascending limb cells. It is associated with calcium wasting, but magnesium wasting is usually mild. Loop diuretics, which inhibit ion transport by thick ascending limb cells, markedly increase urinary excretion of both calcium and magnesium. In contrast, Gitelman’s syndrome is caused by dysfunction of the distal convoluted tubule. Hypocalciuria and hypomagnesemia are universal parts of this disorder. Yet although thiazide diuretics, which inhibit ion transport by distal convoluted tubule cells, reduce urinary calcium excretion, they have minimal effects on urinary magnesium excretion, when given acutely. This review proposes mechanisms that may account for the differences between the effects of diuretic drugs and the phenotypic features of Gitelman’s and Bartter’s syndromes. These mechanisms are based on recent insights from another inherited disease of ion transport, inherited magnesium wasting, and from a review of the chronic effects of diuretic drugs in animals and people.
metanide-sensitive Na-K-2Cl cotransporter (NKCC2 or BSC1) in Bartter’s syndrome would be predicted to cause salt wasting and extracellular fluid volume depletion, secondary hyperaldosteronism, and hypokalemia. All of these phenotypic features are observed in these clinical syndromes. Yet, despite a clear understanding of the molecular causes of these disorders, the pathogenesis of other phenotypic features continues to confuse. This review will merge recent insights from molecular genetics and molecular anatomy with insights from more traditional disciplines in an attempt to clarify the physiological basis of Gitelman’s and Bartter’s syndromes. Because other reviews have described the pathogenesis of extracellular fluid volume depletion and hypokalemia (40, 57, 85, 95), this review will emphasize alterations in divalent ion homeostasis, because this has remained a subject of special confusion.

Patients with both Bartter’s and Gitelman’s syndromes typically present with hypokalemic metabolic alkalosis and a normal to low blood pressure (94). For many years, this common phenotype led to confusion about the discrete nature of these distinct disorders. Several investigators suggested that distinct phenotypes of Bartter’s syndrome could be discerned (78, 102). Bettinelli (3) clarified the nature of the two distinct syndromes. One is now called Bartter’s syndrome and includes hypokalemic alkalosis and normal to low blood pressure. These patients often present at a young age and may have polyuria. Many have hypercalciuria, which can cause nephrocalcinosis. The other phenotype is now called Gitelman’s syndrome. It also includes hypokalemic alkalosis with normal to low blood pressure, but these patients tend to present at an older age and the disease is often milder. The phenotypic signature of Gitelman’s syndrome, however, is hypocalciuria and hypomagnesemia. Bartter’s syndrome has been linked to mutations in three ion transport proteins, but all of the recognized causes affect a final common pathway that participates in ion transport by thick ascending limb cells (88, 90–92, 96, 104). The first genetic cause to be identified involved mutation in the apical form of NKCC2 (90). Later, mutations in a K channel (ROMK) (91) and in a basolateral Cl channel (CCLNKB) (88) were shown to be linked to the disease in some patients. On the basis of our understanding of mechanisms of NaCl transport by thick ascending limb cells, dysfunction of any of these proteins would be predicted to impair transepithelial NaCl transport, leading to salt wasting and extracellular fluid volume contraction. Several other phenotypic features can now be explained on the basis of knowledge of the molecular pathogenesis. First, it is clear that macula densa cells, cells that control renin secretion (7, 49, 110), express the transport proteins that are mutated in patients with Bartter’s syndrome (70). Inasmuch as entry of Na and Cl into macula densa cells inhibits renin secretion (99), the genetic absence of Na and Cl transport pathways in macula densa cells probably accounts for the extreme elevations in plasma renin activity that occur in this disorder and for the juxtaglomerular hypertrophy with which this disease is associated (37). Second, the hypokalemia that occurs in patients with Bartter’s syndrome probably results from both a decrease in K reabsorption along the thick ascending limb and an increase in K secretion by cells of the late distal tubule and collecting duct, owing to the combination of high serum aldosterone and high distal flow rate (109).

To date, Gitelman’s syndrome appears to be molecularly homogeneous. The only identified cause of this disorder involves mutation in the NCC (59, 66, 98, 103). We have shown that many of the mutations that cause Gitelman’s syndrome generate proteins that are functionally inactive when expressed in *Xenopus laevis* oocytes (55). Most of the mutant proteins are processed (or folded) abnormally, probably activating the “quality control” system of the endoplasmic reticulum. These misprocessed proteins appear to be degraded without reaching the plasma membrane. Dysfunction of this transport protein would be expected to lead to salt wasting, depletion of the extracellular fluid volume, and stimulation of the renin-angiotensin-aldosterone system. This would be predicted to cause hypokalemia by stimulating K secretion along the distal tubule and collecting duct. As would be expected on the basis of the comparative transport capacities of the loop of Henle and distal tubule, Bartter’s syndrome is a more severe disorder than is Gitelman’s syndrome. Bartter’s syndrome typically presents at a younger age, often as failure to thrive, whereas Gitelman’s syndrome is often only mildly symptomatic (4, 5, 98). Yet, hypokalemia may be severe in patients with Gitelman’s syndrome. Although increases in circulating aldosterone, induced by extracellular fluid volume contraction, contribute to the K wasting, the elevation of aldosterone is usually milder than in Bartter’s syndrome and other factors may contribute. First, hypomagnesemia predisposes to hypokalemia; correction of hypomagnesemia was shown to reduce or correct renal potassium wasting in patients with Gitelman’s syndrome (48). Second, the low luminal calcium concentration along the distal convoluted tubule (DCT) that occurs in Gitelman’s syndrome may predispose patients to potassium wasting, because luminal calcium blocks Na channels and inhibits K secretion by the distal tubule (47, 71).

**CALCium HOMEOSTASIS**

A major phenotypic difference between Bartter’s and Gitelman’s syndromes involves urinary calcium excretion. The hypercalciuria of Bartter’s syndrome is believed to result largely from dysfunction of thick ascending limb cells. Figure 1 summarizes sites of calcium, magnesium, and sodium reabsorption along the nephron in normal individuals. As shown in Fig. 2, calcium absorption along the loop of Henle is largely passive, paracellular, and driven by the lumen-positive transepithelial voltage that is generated by Na-K-2Cl cotransport and luminal K recycling (38, 39). When Na-K-2Cl cotransport is reduced or blocked by loop diuretics or genetic abnormality, the lumen-positive voltage declines or approaches zero (10). For this rea-
son, calcium reabsorption declines (11). Although this mechanism undoubtedly contributes to calcium wasting, another cause may also contribute. As will be discussed in more detail below, rates of Na and calcium transport by distal tubules tend to correlate inversely (16–19, 33). Because dysfunction of the thick ascending limb increases distal NaCl delivery (32, 46), and because distal NaCl transport is load dependent (31, 50), NaCl transport by DCT cells will be increased in patients with Bartter’s syndrome (see Fig. 2). This situation resembles that observed during chronic loop diuretic administration (32, 100). Increased cellular Na and Cl entry raises the intracellular Cl activity of DCT cells, which would be predicted to deplorlize them, inhibiting the apical calcium channel (36, 43). This would be predicted to impair distal calcium reabsorption, providing a distal contribution to calcium wasting and nephrolithiasis.

In contrast to patients with Bartter’s syndrome, patients with Gitelman’s syndrome invariably demonstrate hypocalciuria (89). The hypocalciuria resembles the clinically useful effect of DCT diuretics (thiazides and others) to reduce urinary calcium excretion (35). The mechanisms of hypocalciuria in Gitelman’s syndrome are now reasonably well established (see Fig. 3). First, mild contraction of the extracellular fluid volume will increase calcium reabsorption along the proximal tubule (18). Second, the reduction in NaCl entry into DCT cells stimulates transepithelial calcium transport. When apical Na and Cl entry into DCT cells is inhibited, because of either diuretic treatment or genetic disease, the intracellular Cl concentration declines. As noted above, a lower intracellular Cl activity hyperpolarizes the cell. Friedman and colleagues (36) showed that hyperpolarization activates a distinctive calcium channel that is expressed by DCT cells. Thus hyperpolarization increases apical calcium entry. Proteins cloned by Bindels and colleagues (42) (ECaC) and Peng and colleagues (72a) (CaT2) may be apical calcium channels of the distal nephron (43). Direct measurements of calcium current through these channels, however, have not been reported, and it is not known whether their open probability is affected by membrane voltage. In contrast, a distal tubule calcium channel studied by Matsunaga and colleagues (67) does demonstrate increased open probability during membrane hyperpolarization. Another difference between the cloned channels and the channels studied by Matsunaga and colleagues involves sensitivity to dihydropiridines. Dihydropiridines have little effect on ECaC and CaT2 (42), whereas they are strongly inhibitory of the distal tubule channels described by Matsunaga and colleagues (67).

For the increase in apical calcium entry to result in increased transepithelial calcium transport, calcium movement from lumen to cell must be balanced by calcium movement from cell to interstitium and blood. The increase in cellular calcium consequent to increased luminal calcium entry will stimulate calcium efflux via the basolateral Na/Ca exchanger and the Ca-ATPase (84), but other factors contribute as well. First, inhibition or absence of apical NaCl entry reduces the intracellular Na activity. This stimulates 3Na/Ca exchange. Second, the hyperpolarization, noted above stimulates Na/Ca exchange because this transport protein operates in an electrogenic mode, carrying 3Na ions into the cell for each calcium ion extruded.

**MAGNESIUM HOMEOSTASIS**

Whereas the pathogenesis of calcium disorders in Bartter’s and Gitelman’s syndromes appears relatively
clear, the pathogenesis of magnesium disorders is more confusing. Gitelman’s syndrome is associated with severe hypomagnesemia, whereas Bartter’s syndrome is not (89). This is surprising because more magnesium is reabsorbed along the loop of Henle than along the distal tubule (80, 85) and because DCT diuretics induce less magnesium wasting, when given acutely, than do loop diuretics (25, 26, 29). Recent insights from molecular genetics, molecular anatomy, and a review of earlier physiological studies, however, shed light on this issue and present the potential to resolve some conflicts.

Simon and colleagues (97) recently used positional cloning to identify a cause of inherited magnesium wasting. They found that mutations in a novel protein, called paracellin-1, cause the disease. Immunocytochemical studies and studies using nephron-segment RT-PCR indicate that paracellin-1 is expressed predominantly in tight junctions along the thick ascending limb. Furthermore, the molecular characteristics of this protein suggest that it may be a paracellular conductive pathway for magnesium. Several insights come directly from this observation. First, it provides a molecular explanation for the previously established behavior of magnesium along the loop of Henle, where transport is driven by the lumen-positive transepithelial voltage (80). Loop diuretics such as furosemide increase urinary magnesium excretion by reducing the lumen-positive voltage and thereby the electrical gradient favoring reabsorption. This identifies a molecular component that may contribute to the magnesium wasting that sometimes accompanies Bartter’s syndrome.
As mentioned, however, magnesium wasting is much more severe in Gitelman's syndrome than in Bartter's syndrome. Scheinman and colleagues (85) suggested that the magnesium wasting of Gitelman's syndrome is "determined by the balance of hormonal effects and intracellular potassium stores in the distal convoluted tubule" (85). They suggested that higher aldosterone concentrations in Bartter's patients may attenuate magnesium wasting, whereas the effects of hypokalemia predominate in patients with Gitelman's syndrome. That hypokalemia is central to the pathogenesis of magnesium wasting in Gitelman's patients is disputed by results of gene-knockout experiments. Schultheis and colleagues (87) showed that NCC-knockout mice develop hypocalciuria and hypomagnesemia. Surprisingly, these mice are normokalemic. Although the reason that NCC knockout does not cause hypokalemia in mice (whereas Gitelman's syndrome is associated with hypokalemia in humans) is not clear, the results clearly indicate that hypokalemia is not required for magnesium wasting to occur in animals that lack NCC activity. This motivates the search for other mechanisms.

One piece of data that has become available recently involves potential mechanisms of magnesium transport along the DCT. Interestingly, whereas paracellin-1 was shown to be expressed along the thick ascending limb, where its function is clear, it was also reported to be expressed along the DCT (97) (see Fig. 4). The function of paracellin-1 in this segment was not addressed by the authors, but this pattern of paracellin-1 expression is consistent with the low rate of magnesium transport that normally occurs along the DCT (80). The transepithelial voltage along the DCT is normally very low. It is near 0 mV at the proximal end and becomes only slightly lumen negative at its distal end (83). Because the luminal magnesium concentration is normally slightly below interstitial in fluid entering the distal tubule [TF/UF = 0.6 (82), where TF/UF is the tubule fluid-to-ultrafilterable ratio], and because the electrical gradient is near to zero at this site, little paracellular magnesium transport should occur, even though the paracellular pathway is potentially magnesium permeable. Calcium may also traverse paracellin-1, on the basis of the observation that patients with congenital magnesium wasting are also hypercalciuric (77). This pathway, however, is not the major route of transepithelial calcium transport along the DCT because this segment rapidly absorbs calcium even though the electrochemical gradient favors calcium secretion (20). This results from rapid calcium reabsorption via the apical ECaC (34, 42).

The possible presence of paracellin-1 along the DCT suggests that paracellular magnesium transport might occur along this segment. Earlier studies do provide evidence that magnesium secretion can occur along the distal tubule and collecting duct. The tubule fluid-to-ultrafilterable ratio of magnesium has been observed to rise along the length of the accessible distal tubule (8, 9, 79, 81, 82). Furthermore, several studies suggest that magnesium secretion may occur along the collecting duct, at least under certain conditions (8, 56, 58). Although this secretion may traverse paracellin-1, it may traverse other pathways as well. Regardless of the route by which magnesium secretion may occur, these data suggest an alternative hypothesis for the profound magnesium wasting of Gitelman's syndrome. To generate such an hypothesis, however, it is useful to consider additional anatomic and molecular information that has become available recently. During the past several years, the molecular anatomy of the mammalian distal tubule has been clarified (see Fig. 4 and Ref. 83). Shortly beyond the region of the macula densa, cortical thick ascending limb cells change abruptly to become DCT cells. Further distally, at a point >50% of the distance from the macula densa to the cortical collecting duct (CCD), the epithelium changes again to comprise connecting tubule (CNT) cells (24). Finally, just before the junction with another nephron, the epithelium changes again to comprise...
CCD (also called “principal”) cells (52). In rabbits, each of these transitions is abrupt, but in rodents and humans the transitions from DCT to CNT and CNT to CCD are gradual. This anatomic arrangement can now be juxtaposed with the molecular organization of transport pathways along the distal tubule. In all species studied to date, including rat (69, 75, 111), mouse (63), rabbit (1, 106), and human (69), the NCC is expressed predominantly, if not exclusively, by DCT cells. The β- and γ-subunits of the endothelial Na channel, ENaC, are expressed conversely by CNT and CCD cells (68). In rat, mouse, and probably human, however, the DCT comprises two distinct subsegments, DCT1 and DCT2 (62, 63, 69). The DCT1 expresses the NCC as its predominant apical Na entry pathway [it may also express type 2 Na/H exchanger (12)]. The DCT2 expresses both the NCC and the epithelial Na channel, ENaC, at its apical membrane (30, 62, 63, 69). The DCT2 also expresses 11β-hydroxysteroid dehydrogenase, the mineralocorticoid receptor (6), and the Na/Ca exchanger (69).

Another piece of important information comes from observations about effects of aldosterone on magnesium handling. States of aldosterone excess are frequently associated with magnesium wasting, and states of aldosterone deficiency, such as Addison’s disease, are associated with hypermagnesemia (45). Experiments designed to discern an acute effect of aldosterone on magnesium excretion have been contradictory. Most of the effects of aldosterone administration on magnesium excretion have been attributable to secondary extracellular fluid volume expansion (64, 65). Yet the aldosterone antagonist spironolactone has consistently shown the ability to reduce urinary magnesium excretion (2, 28, 101), an effect that can be dissociated from changes in filtered load (68). In a series of patients with Gitelman’s syndrome, spironolactone reduced fractional magnesium excretion from 6.5 to 3.0% (15). Spironolactone has also been shown to increase serum magnesium and reduce urinary magnesium in normal individuals (68), in patients with primary aldosteronism (45), in patients with cirrhotic ascites (101), and in patients with hypertension. In view of the fact that spironolactone reduces urinary magnesium excretion in the setting of expanded or contracted extracellular fluid volume, it seems likely that aldosterone has direct effects on magnesium transport. The observation that acute aldosterone infusion has very little effect on renal magnesium handling and yet spironolactone reduces urinary magnesium excretion is one of several paradoxes concerning magnesium homeostasis. These are presented in Table 1 and must be resolved to understand mechanisms of renal magnesium homeostasis.

The information discussed above can be used to construct a hypothesis to explain the magnesium wasting of Gitelman’s syndrome that resolves some of the paradoxes listed in Table 1. Gitelman’s syndrome is caused by dysfunction of the NCC (87). Dysfunction of the NCC in DCT2 cells converts them from cells that transport Na predominantly coupled to chloride in an electoneutral manner to cells that transport Na predominantly alone, via electrogenic pathways (via ENaC). If this is the case, then one might predict that DCT diuretics would increase the magnitude of the transepithelial voltage in the distal tubule. Surprisingly, when DCT diuretics have been perfused into DCT segments in vivo, the transepithelial voltage has not increased (20). Under similar conditions, luminal perfusion of DCT diuretics does not increase K secretion (108). Yet, these drugs clearly cause K wasting when used chronically. The reason that chronic systemic DCT diuretics administration causes potassium and magnesium wasting is probably related to the interaction between transport inhibition and aldosterone. As noted above, cells of the DCT are aldosterone responsive (6, 105). Yet, aldosterone normally stimulates the NCC in DCT cells (13, 51, 105). This leads to an increase in Na transport but does not change the transepithelial voltage (41). In contrast, when electroneutral apical entry pathways are knocked out or dysfunctional, aldosterone would be expected to increase the magnitude of the lumen-negative transepithelial voltage, because some DCT cells express ENaC (61–63, 83). Thus knocking out or blocking the NCC generates an aldosterone-sensitive segment that absorbs Na in an electrogenic manner and may express paracellin-1 (see Fig. 3). The resulting large, lumen-negative transepithelial voltage should strongly favor magnesium (and potassium) secretion.

Table 1. Paradoxes of magnesium homeostasis in Gitelman’s and Bartter’s syndromes

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<th>Acute Changes</th>
<th>Chronic Effects</th>
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<tr>
<td>Effects of diuretics in vivo</td>
<td>Loop diuretics increase magnesium excretion, whereas DCT diuretics have little effect.</td>
<td>DCT diuretics cause significant magnesium wasting.</td>
</tr>
<tr>
<td>Effects of aldosterone</td>
<td>Aldosterone has a modest effect on magnesium excretion, primarily owing to changes in extracellular fluid volume.</td>
<td>Spironolactone consistently reduces magnesium wasting, independently of extracellular fluid volume.</td>
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<tr>
<td>Comparison of cells and whole animals</td>
<td>DCT diuretics and amiloride both stimulate magnesium uptake into MDCT cells. Aldosterone also predisposes to increased magnesium uptake.</td>
<td>In vivo, DCT diuretics cause magnesium wasting, whereas amiloride and spironolactone reduce magnesium excretion.</td>
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DCT, distal convoluted tubule; MDCT, mouse DCT.
This hypothesis predicts that DCT diuretics should also lead to magnesium wasting. Although data on acute effects of DCT diuretics on magnesium excretion tend to suggest little effect (25), their chronic use is clearly associated with changes in magnesium balance. In the Multiple Risk Factor Intervention Trial, DCT diuretics were shown to have a small but measurable effect on plasma magnesium (53), a finding that usually indicates severe magnesium depletion. In other studies, DCT diuretics led to small but reproducible reductions in serum magnesium concentration (44). Recall, however, that in this, as in most recent trials of the antihypertensive effect of DCT diuretics, the dose was limited and given once per day. Such low doses do not deplete the extracellular fluid volume enough to strongly stimulate aldosterone secretion. Like the side effect hypokalemia, the side effect hypomagnesemia is strongly dose related (72).

Recent molecular information also helps to resolve another paradox of magnesium homeostasis (Table 1). Magnesium uptake into mouse DCT cells grown in culture was shown to be enhanced by thiazide diuretics (21) and by amiloride (22). In animals and humans, these drugs have opposite effects on renal magnesium handling (15, 44). Furthermore, although aldosterone alone does not affect magnesium transport in mouse DCT cells, it potentiates the effects of diuretic hormone and glucagon to stimulate magnesium uptake (23). In humans, this hormone tends to cause magnesium wasting, as discussed above. One explanation for these discrepancies is that the mouse DCT cells used to study magnesium uptake are not polarized and do not have tight junctions (74). Thus studies of magnesium uptake into these cells may not mimic effects of physiological perturbations in vivo, where paracellular effects may predominate and paracel lin-1 expression plays a vital role. This is in contrast to effects on calcium transport by the DCT, where transcellular transport pathways clearly predominate. Thus the effects of perturbations on calcium transport by DCT cells in vitro closely mimic effects observed in vivo (33).

Many of the concepts reviewed in this paper remain speculative. Even if magnesium secretion does occur along the distal tubule and collecting duct, it may traverse pathways that are independent of paracel lin-1. Yet, on the basis of recent molecular insights, it has become increasingly possible to resolve the paradoxes cited above and to construct hypotheses that do fit available data. Understanding magnesium and calcium homeostasis is of more than scientific interest. Disorders of magnesium balance may contribute importantly to patient morbidity in congestive heart failure and even hypertension. In fact, interventions that improve magnesium balance in patients with congestive heart failure (spironolactone) reduce cardiac ec topy (2) and prolong life (73).

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