How Bartter’s and Gitelman’s Syndromes, and Dent’s Disease Have Provided Important Insights into the Function of Three Renal Chloride Channels: CIC-Ka/b and CIC-5

Marie Briet  Rosa Vargas-Poussou  Stephane Lourdel  Pascal Houillier  Anne Blanchard

Département de Physiologie, Hôpital Européen Georges Pompidou, Assistance Publique-Hôpitaux de Paris; INSERM U.356, IFR 58, and Rene Descartes University and School of Medicine, Paris, France

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Abstract
Chloride channels are expressed in almost all cell membranes and are potentially involved in a wide variety of functions. The kidney expresses 8 of the 9 chloride channels of the CIC family that have been cloned so far to date in mammals. This review focuses on the pathophysiology of two renal disorders that have contributed recently to our understanding of the physiological role of chloride channels belonging to the CIC family. First are the related syndromes of Bartter’s and Gitelman’s, in which inactivating mutations of the genes encoding either of the CIC-Ks, or their regulatory β-subunit barttin, have shown the important contribution of these chloride channels to renal tubular sodium and chloride (NaCl) transport along the loop of Henle and distal tubule. Second is the renal Fanconi syndrome known as Dent’s disease, in which CIC-5 disruption has revealed the key role of this endosomal chloride channel in the megalin-mediated endocytic pathway in the proximal tubule. The underlying pathophysiology of this inherited disorder demonstrates how CIC-5 is directly or indirectly required for the reabsorption of filtered low-molecular-weight proteins and bioactive peptides, also expression of membrane transporters, and clearance of calcium-based stone-forming crystals.

Introduction
In mammals the CIC channel family consists of 9 CLC gene products classified on the base of their homologies into three subfamilies. The first subfamily comprises plasma membrane channels, whereas the proteins encoded by the other two subfamilies are thought to be located (mainly) in intracellular membranes [1]. The cloning of the genes encoding these channels has allowed detailed studies of their structure and function, but has failed to establish their physiological role(s). Two members of the CLC gene family are expressed predominantly in the human kidney: CIC-Ka and CIC-Kb. These channels are ex...
pressed on the basolateral membrane of the thin ascending limb (ClC-Ka only), thick limb (TAL) and distal convoluted tubule (DCT) cells, as well as intercalated cells (ClC-Ka and -Kb) of the collecting duct (CD). In mice, disruption of the CLCNK1 gene (the mouse ortholog of ClC-Ka) leads to a form of nephrogenic diabetes insipidus, demonstrating the crucial role of ClC-K1 in NaCl reabsorption in the mouse thin ascending limb and its contribution to the urinary concentrating mechanism [2].

The aim of this review is to highlight how recent clinical data have contributed to our understanding of the physiological function of ClC-Ka and ClC-Kb, as well as the description of a new ClC channel, ClC-5, which is involved in endosomal endocytosis and intracellular trafficking.

**Evidence for Physiological Involvement of ClC-Ka and -Kb NaCl Reabsorption along the Renal Tubule: Lessons from Bartter’s and Gitelman’s Syndromes**

Bartter’s syndrome is a heterogeneous group of renal tubular disorders that can be classified into three distinct phenotypes: the ‘antenatal’ and ‘classic’ Bartter’s syndromes, in which there is renal salt wasting, polyuria and hypercalciuria, and Gitelman’s syndrome, in which there is renal salt wasting and normal or low urinary calcium excretion and marked hypomagnesemia, but no polyuria [for a review, see 3]. Antenatal Bartter’s syndrome is sometimes also referred to as the hyperprostaglandin E syndrome, and is the most severe form of the disease. The salt and water losses lead to antenatal polyhydramnios, premature birth, life-threatening episodes of salt and water depletion in the neonatal period, and failure to thrive. An antenatal variant associated with deafness is particularly severe and most affected patients develop early onset renal failure. Classic Bartter’s syndrome occurs in infancy or childhood, when it presents with dehydration, hypokalemia, failure to thrive, and hyper- or normocalciuria; rarely, renal failure and nephrocalcinosis occur.

Gitelman’s syndrome is also characterized by hypokalemia, although the symptoms and signs are usually milder, consisting of failure to thrive in childhood and/or transient muscle weakness and spasms, but also acute epileptic seizures in adulthood. The later onset of Gitelman’s and the presence of hypomagnesemia and hypocalciuria (especially) are considered hallmarks of the disease, but are not always present. Based on the early observation of an impaired natriuretic response to the loop diuretic bumetamide in Bartter’s syndrome and the thiazide diuretic hydrochlorothiazide in Gitelman’s syndrome, the site of the defect in NaCl transport was thought to be the TAL and DCT, respectively. Although cell models of NaCl transport along the TAL and DCT had already been well characterized, and the likely transport processes affected in Bartter’s and Gitelman’s syndromes known, their actual identity remained elusive. However, based on a molecular genetic approach, Bartter’s and Gitelman’s syndromes have now been linked to at least seven gene defects, corresponding to five Bartter’s and two Gitelman’s subtypes; although involvement of as yet unidentified genes is still possible [3].

Antenatal Bartter’s syndrome is most often due to inactivating mutations of the SLC12A1 gene encoding the Na⁺,K⁺-2Cl⁻ co-transporter NKCC2 (type 1) or in the KCNJ1 gene encoding the apical K channel ROMK (type 2) (fig. 1). Classic Bartter’s is usually due to mutations in the CLCNKB gene encoding for ClC-Kb (type 3), providing the first evidence for the contribution of this basolateral chloride channel to NaCl transport along the TAL. More recently, the antenatal Bartter’s subtype associated with deafness has been shown to be due to mutations in the BSND gene, which encodes a novel protein barttin (type 4) [4]. This accessory protein co-localizes with ClC-Ka/b proteins in kidney epithelial cells, as well as in the inner ear. Subsequent functional studies established that barttin is the regulatory β-subunit required for normal trafficking and function of ClC-Ka and -Kb channels [5]. Finally, a last subtype of classic Bartter’s syndrome (type 5) has been attributed to a gain-of-function mutation in the CASR gene that encodes the calcium receptor located on the basolateral membrane of TAL cells. In this form of Bartter’s syndrome, constitutive activation of the calcium receptor exerts an inhibitory effect on NaCl reabsorption, as well as on divalent cation transport, along the TAL.

The first subtype of Gitelman’s syndrome is due to mutations in the gene SLC12A3 encoding the NaCl (NCCT) co-transporter on the apical membrane of DCT cells. More recently, a second subtype has been described due to mutations in the CLCNKB gene, demonstrating that ClC-Kb is also involved in basolateral exit of Cl⁻ from DCT cells [6, 7]. Various phenotypes ranging from severe antenatal Bartter’s to Gitelman’s syndromes have been reported in related subjects bearing the same homozygous mutation in CLCNKB gene [3], but none of these patients were deaf, in contrast to patients with mutations in the BSND gene. These data suggest that an alternative
basolateral pathway for Cl⁻ exit might compensate for ClC-Kb inactivation in the inner ear and (more variably) in the kidney. At least three pieces of evidence suggest that ClC-Ka may contribute to such compensation: (1) ClC-Ka expression overlaps with that of ClC-Kb in TAL and DCT, as well as in the inner ear [1]; (2) the severity of the phenotype observed in patients bearing mutations in the BSND gene suggests that the compensatory mechanism for ClC-Kb loss requires functional barttin, as does ClC-Kb itself, and (3) a phenotype resembling a barttin defect has been reported in a child with combined loss of function of ClC-Ka and ClC-Kb due to a digenic defect in their closely adjacent genes, CLCNKA and CLCNKB on chromosome 1p36 [8].

Data obtained in patients with Bartter/Gitelman syndrome related to a ClC-Kb defect prompted some groups to look for activating mutations or polymorphisms in CLCNKB as a genetic factor in hypertension. A possible genetic predisposition due to a T481S CLCNKB polymorphism proposed by Jeck et al. has recently been reviewed and criticized [9]. Moreover, the linkage of this polymorphism to hypertension was not confirmed in a similar study in Japan [10].

**Dent’s Disease and the Crucial Role of CIC-5 in Endosomal Function**

Dent’s disease includes a heterogeneous group of X-linked disorders in which hemizygous males develop abnormalities that include low-molecular-weight proteinuria, hypercalciuria with nephrocalcinosis/nephrolithiasis, and in many cases renal failure. Proximal tubule solute wasting is variable, causing an incomplete renal Fanconi syndrome with hypophosphatemic rickets, proximal tubular acidosis, glucosuria, and aminoaciduria. Carrier women usually present with a milder phenotype (due to lyonisation), but rarely develop nephrocalcinosis or chronic renal failure [11]. In the past decade, positional cloning in affected families successfully identified the primary defect in a subgroup of patients: mutation of a novel gene CLCN5, encoding a chloride channel with unknown function, CIC-5 [12]. More recently, in a subgroup of patients with the Dent’s phenotype, but with a normal CLCN5 genotype, Hoopes et al. [13] found mutations in the gene for Lowe’s syndrome, OCRL1. Unlike patients with typical Lowe’s syndrome, mental retardation was absent or mild, and none of the patients had metabolic acidosis or ocular abnormalities on slit-lamp examination. This milder phenotype was not related to...
less severe changes in protein expression or enzyme activity, as both were significantly reduced or absent. Moreover, in this study a small group of patients with the Dent’s phenotype were negative for mutations in CLCN5 and OCRL1, suggesting that at least one other gene may cause this phenotype.

CIC-5 is expressed mainly in the kidney in endosomes of proximal tubular cells and to a lesser extent medullary thick ascending limb cells and intercalated cells of the collecting duct [14]. To study the pathogenesis of Dent’s disease due to CIC-5 inactivation, several models have been reported (knockout or knockdown mouse models and cell lines) that reproduce at least some of the features of the human disease [15–17]. These models revealed that low-molecular-weight proteinuria is due to a defect in early endosomal function, which impairs the internalization and degradation of low-molecular-weight proteins by proximal tubule cells. Piwon et al. [17] first demonstrated that iodinated β2-microglobulin injected into the bloodstream was lost in the urine of CLCN5+/– male mice in much larger quantities than in wild-type mice. CIC-5 affects endocytosis in a cell-specific manner, as is evident in heterozygous (CLCN5+/–) females. Due to random X-inactivation, these females are chimeras, in which proximal tubule cells either express the wild-type or mutant gene product. These same authors showed that cells lacking normal CIC-5 take up less protein than their neighboring wild-type cells [17]. Because CIC-5 co-localizes with H+-ATPase, the central hypothesis to explain the endocytosis defect is that CIC-5 may provide an electrical shunt for electrogentic H+ secretion into early endosomes, and that loss of function of CIC-5 would impair endosomal acidification, a crucial step in normal endosomal function [for a review, see 18, 19]. However, this hypothesis is now under scrutiny. Evidence for an alteration in endosomal acidification [20] is still lacking [19, 21] and the acidification defect, if present, may not be the only defect in endocytosis that is related to CIC-5 loss of function. Christensen et al. [21] have shown that CIC-5 inactivation inhibits (primarily) apical protein endocytosis in proximal tubule cells by causing a trafficking defect of megalin and cubulin, though in the absence of any ultra-structural alterations in the apical endocytic apparatus (see later). By contrast, drugs that abolish vacuolar acidification do not affect the rate of endocytic uptake, but do inhibit recycling, or arrest transfer from endosomes to lysosomes [19]. This uncertainty is reinforced by recent data from two groups showing that CIC-5 does not function as a Cl− channel, as was originally thought, but as an electrogenic Cl−/H+ exchanger [22, 23]. An exchanger could still regulate endosomal pH, as the initial endosomal [Cl−] should be high (and similar to extracellular fluid) relative to intracellular [Cl−], and so promote H+ entry and endosomal acidification (even in the absence of an H+-ATPase).

The work of Hryciw et al. [24] demonstrated that CIC-5 is likely to contribute to the protein-protein interactions required for receptor-mediated endocytosis (i.e., from internalization of the ligand-receptor complex in clathrin-coated pits to degradation of the ligand in lysosomes after dissociation from its receptor in ‘late endosomes’). They showed that CIC-5 interacts with coflin to alter the actin cytoskeleton in the vicinity of the endocytic complex. Thus, CIC-5 expressed at the plasma membrane might be a rate-limiting step in protein uptake by proximal tubules. They also showed that uptake of albumin by proximal tubule cells requires Nedd-4, which by ubiquinatizing CIC-5 may shuttle it from plasma membrane to endosomes.

The defect in endocytosis in Dent’s disease does not readily explain the hypercalciuria [25]. Studies in patients with this disease are difficult, because it is rare, many patients when diagnosed already have chronic renal failure and often those with normal renal function are too young for study. However, two observations suggest that the primary defect is in the kidney: in humans, CIC-5 is expressed almost exclusively in the kidney and hypercalciuria did not recur in 2 patients who had renal transplants. Two renal defects may explain the hypercalciuria: a decrease in tubular reabsorption of calcium and/or a primary increase in 1,25-dihydroxyvitamin D (calcitriol) production. A decrease in renal tubular calcium reabsorption would increase urinary calcium excretion, lowering serum calcium concentration and stimulating PTH secretion and calcitriol synthesis. Reinhart et al. [26] studied 6 affected males and documented an absorptive (intestinal) hypercalciuria in 5. However, in this study the presence of fasting hypercalciuria in 3 patients also suggested an abnormality in tubular handling of calcium. Our own data in 40 unrelated kindred indicate that the calcitriol concentration is disproportionately elevated relative to the PTH level, which is in the low-normal range, and associated with both fasting and non-fasting hypercalciuria (unpublished observations). The findings in CICN5 knockout mice are inconsistent. In two models hypercalciuria and nephrocalcinosis occur [15, 16], but not in the model of Jentsch and co-workers [17]. Two studies have demonstrated that urinary calcium excretion depends on dietary calcium intake [15, 27]. However, more detailed physiological studies have demonstrated a renal loss of calcium, but they could not detect an increase in intesti-
nal absorption of calcium, despite elevated calcitriol levels [27]. The mechanism of the renal calcium loss is still unclear. A defect in Ca\textsuperscript{2+} transport by renal tubule cells seems unlikely, because ClC-5 is not expressed at the major sites of transepithelial calcium reabsorption, i.e., the cortical TAL, DCT and connecting segment. Hypercalciuria might be due to a defect in Na\textsuperscript{+} reabsorption in a nephron segment where Ca\textsuperscript{2+} reabsorption is linked to Na\textsuperscript{+} reabsorption, such as in proximal tubule [25]. Further studies of segmental nephron function in vivo and in vitro are necessary to understand the basis of this variability in calciuria in Dent’s disease and its relationship to ClC-5.

A hypothesis that may explain the phenotypic variation in Dent’s disease has been put proposed by Piwon et al. [17]. PTH and vitamin D binding protein (VDBP) are normally filtered by the glomerulus and taken up from the tubule lumen via the megalin-mediated endocytotic route (fig. 2). Thus, urinary excretion of intact PTH and VDBP is increased in patients with Dent’s disease [28], as well as in CLCN5 knockout mice [17]. Intact bioactive PTH may then act from the lumen to stimulate internalization of the apical NaPi-2 co-transporter, so reducing phosphate absorption. Because the apical PTH receptor expressed along the proximal tubule is coupled to phospholipase C, which exerts an inhibitory effect on 1\textsubscript{α}-hydroxylase activity, phosphate depletion, rather than luminal PTH (as the authors had suggested), may explain the increase in calcitriol synthesis [25]. A combination of these effects could occur in individual patients to produce 25-hydroxyvitamin D deficiency (due renal losses of its carrier protein VDBP) and phosphate depletion, which favors bone disease, and stimulation of 1\textsubscript{α}-hydroxylase activity, which enhances intestinal calcium absorption and together with a renal leak of calcium cause hypercalciuria.

It is generally thought that nephrocalcinosis contributes to progressive renal impairment in Dent’s disease. However, the pathophysiology of nephrocalcinosis is unclear and its presence and severity are not consistently
related to renal failure or degree hypercalciuria. In fact, many patients with idiopathic hypercalciuria have similar rates of urinary calcium excretion, but do not develop nephrocalcinosis. Sayer et al. [29] have reported in vitro findings suggesting that nephrocalcinosis in Dent’s disease may be due to a combination of hypercalciuria and a defect in the handling of calcium oxalate and phosphate (which is more usual in Dent’s disease) crystals in the medullary collecting duct. They showed that transient expression of antisense ClCN5 mRNA in an inner medullary collecting duct cell line reduced the ability of these cells to bind calcium oxalate or calcium phosphate crystals and favored the formation of crystal agglomerates.

To date, the aim of most treatments given to patients with Dent’s disease is to reduce hypercalciuria (e.g., by thiazide diuretics) in the hope of limiting the progression of nephrocalcinosis and possibly renal failure. However, as already mentioned, the link between nephrocalcinosis and renal failure has never been conclusively established; moreover, in other forms of nephrocalcinosis, progressive loss of renal function is unusual. Low-molecular-weight proteinuria might be a cause of the decline in renal function in Dent’s disease. Proteinuria is evident early on and can precede renal failure by 10–20 years [11]. Cutille et al. [30] studied the proteins excreted in the urine of patients with Dent’s disease using mass spectroscopy and found abnormally high levels of proteins linked to progressive renal injury, including cytokines, apolipoproteins and complement components. They suggested that the cubilin-megalin receptor complex may have a greater affinity for carrier proteins and potentially toxic proteins of plasma origin than for other classes of proteins and so normally ‘protect’ the tubule (especially more distal segments) from such potentially bioactive peptides. However, this concept is at odds with a recent suggestion that toxicity in glomerular proteinuria is related to an increase in proximal tubular endocytosis of filtered proteins, and that a potentially beneficial effect of HMG-CoA reductase inhibitors in proteinuric renal disease is related to their inhibition of endocytosis [31], which may be associated with tubular proteinuria. It is becoming apparent that studying Dent’s disease and other forms of renal Fanconi syndrome may provide novel insights into the renal tubular handling of proteins and ultimately the pathophysiology of proteinuric renal disease.

References


