Diversity within the CLC chloride channel family involved in inherited diseases: from plasma membranes to acidic organelles

Alain Vandewalle

Faculté de Médecine, Xavier Bichat, Paris, France

Keywords: CLC chloride channels

Introduction

Since the first voltage-gated ClC-0 chloride channel was discovered by Jentsch et al. [1] in the marine ray Torpedo marmorata, nine chloride channels belonging to the CLC family have been identified in mammals [2]. These ClC channels exhibit marked differences in their tissue and cellular distribution. All ClC channels, except the ClC-1 channel [2], are expressed in mammalian kidneys. They have important functions, and mutations of some of them are responsible for inherited diseases. Furthermore, the development of ClC knockout mice models has produced a great deal of new information about the function(s) of these ClC channels and their pathological impact.

Expression and function of the kidney-specific ClC-K channels

Two closely homologous ClC chloride channels, referred to as rClC-K1 and rClC-K2 in rats and as hClC-Ka and hClC-Kb in humans [3,4], are almost exclusively expressed in kidney tissue. Uchida et al. [3] first demonstrated that ClC-K1 is expressed in the renal medulla and is stimulated by dehydration. The ClC-K1 protein is mainly, if not exclusively, located in the basolateral membranes of the thick ascending limb (TAL) of rat kidneys [4,5], whereas ClC-K2 is more broadly distributed in basolateral membranes from TAL, distal tubule cells, and α-intercalated cells [5,6]. Waldegger and Jentsch [7] have shown that the expression of the rat ClC-K1 in Xenopus oocytes yielded pH independent-voltage currents activated by extracellular calcium. Because the function of rClC-K2 and hClC-Kb as anion channels was still being debated, these authors used a chimeric approach to show that the chloride currents produced by hClC-Kb had different characteristics from those generated by ClC-K1 [7]. The data from these studies raised important questions about the molecular organization of these two membrane ClC-K channels which led to the hypothesis that accessory proteins and/or second messengers may be necessary for the functional expression of ClC-K channels.

Simon et al. [8] first identified mutations in the human chloride channel gene CLCNKB, equivalent to hClC-Kb, as a cause of Bartter type III syndrome. Other mutations in CLCNKB have also been identified in patients exhibiting either a pure Bartter syndrome [9] or a mixed Bartter-Gitelman syndrome [10]. In contrast, no mutation in the gene encoding for hClC-Ka has so far been reported in patients exhibiting Bartter syndrome.

The fact that ClC-K1 is expressed in TAL and is stimulated by dehydration suggests that it is involved in urinary concentration–dilution mechanisms. ClC-K1 knockout mice (Clcnk1<sup>-/-</sup>) develop nephrogenic diabetes insipidus characterized by severe water diuresis, which is insensitive to dD-arginine vasopressin [11]. Moreover, both NaCl and urea accumulation are impaired in the renal medulla of Clcnk1<sup>-/-</sup> mice [12]. These findings strongly suggest that disruption of the ClC-K1 gene impairs not just one, but several interacting transporters and membrane ion channels involved in the regulation of the countercurrent system. On the other hand, it is highly probable that in TAL ClC-K2 is responsible for the transcellular exit of chloride stimulated by cAMP agonists.

Expression and function of the ClC-5 channel in Dent’s disease

ClC-5 is highly expressed in the kidney [13]. Mutations in the gene encoding for ClC-5 are responsible for Dent’s disease [14], an X-linked recessive nephrolithiasis.
characterized by microproteinuria with hypercalciuria, hypophosphataemia and, in some cases, rickets or osteomalacia [15]. In contrast to patients suffering from most other types of renal insufficiency, patients with Dent’s diseases exhibit high plasma levels of 1.25(OH) vitamin D3 and normal or low parathyroid hormone (PTH) [15]. The clinical features of the disease suggest that CIC-5 is not a typical plasma membrane chloride channel, but rather an intracellular channel. Günther et al. [16] reported that CIC-5 is intracellularly located in endocytically active proximal tubule cells and x-intercalated cells of rat kidney. These authors also showed that CIC-5 is colocalized with vacuolar proton ATPase and with endocytosed β2-microglobulin in intact proximal tubule cells and with β2-microglobulin in COS-7 cells transfected with CIC-5. These findings have provided the first evidence that the intracellular CIC-5 chloride channel provides an electrical shunt for the electrogenic proton pump which is required for the acidification of the organelles involved in the endocytosis of low molecular weight proteins. Other groups have reported similar findings in rat, mouse and human kidneys [16–19]. The fact that CIC-5 cRNA generates chloride currents in Xenopus oocytes [13] raises the possibility that intracellular CIC-5 could be transiently expressed in the plasma membrane during the recycling of endocytotic vesicles. Schwake et al. [20] have identified a motif in the carboxy-terminal region of CIC-5, similar to a PY motif known to play a crucial role in the internalization and degradation of the epithelial sodium channel (ENaC) by ubiquitin-protein ligases [21,22], and have shown that this motif is important in the endocytosis of CIC-5 [20].

Mice with reduced CIC-5 expression, using an antisense ribozyme transgene, were slightly hypercalciuric, but did not exhibit proteinuria or phosphaturia [23]. In contrast, Wang et al. [24] reported that CIC-5 knockout mice exhibited a phenotype similar to that of the Dent’s disease with polyuria, proteinuria associated with impaired proximal tubule reabsorption of proteins, aminoaciduria, glycosuria, hypercalciuria and renal calcium deposits. Piwon et al. [25] also showed that disruption of the mouse CIC-5 (Clcn5) gene not only caused proteinuria, but also reduced the expression of megalin, which is involved in the endocytosis and activation of 25(OH) vitamin D3 [26] and caused slight abnormalities in the internalization rate of the sodium-phosphate (NaPi-2) cotransporter, leading to a rise in the intraluminal concentration of PTH and the activation of its apical receptors. This experimental evidence has provided confirmation of the crucial role of CIC-5 in proximal tubule endocytosis, and has also indicated that hyperphosphaturia and hypercalciuria are indirect consequences of the defect of apical endocytosis of PTH and 25(OH) vitamin D3. CIC-5 is expressed in other, non-renal epithelial cell types, including enterocytes, where it is colocalized with proton pumps and small GTPases rab4 and rab5a in endosomes involved in the transcytosis of polymeric immunoglobulin receptors [27].

**Expression and function of the other CIC channels**

CIC-2 is a broadly expressed membrane chloride channel [2]. This channel is expressed early during development and may play a role during kidney maturation [28]. Other studies have shown that it may also play a role in cell volume regulation [2]. Its localization in apical membranes of lung epithelia and in junctional complexes of enterocytes [29] led to the suggestion that CIC-2 may compensate for the secretion of chloride mediated by CFTR which is defective in cystic fibrosis. Rather unexpectedly, CIC-2-deficient mice developed severe degeneration of the retina and the testis, leading to selective male infertility [30].

The functions of CIC-3 and CIC-4, which are broadly expressed in various tissues including brain and kidney [2], have as yet not been fully elucidated. Like CIC-5, CIC-3 is expressed in intercalated cells [31]. Stobrawa et al. [32] have shown that mouse CIC-3 (Clcn3) is present in endosomes and in the synaptic vesicles of neurons, and that Clcn3+/− knockout mice exhibit selective degeneration of the retina and hippocampus.

The function of CIC-6 is unknown, but a recent study of the mouse CIC-7 gene in knockout mice (Cln7−/−) revealed that this chloride channel resides in the late endosomal and lysosomal compartments of osteoclasts. As a result, Cln7−/− mice developed severe osteopetrosis, characterized by retarded growth, abnormal body posture, and degeneration of the retina [33].

**Conclusions**

Electrophysiological and immunohistochemical studies, together with the identification of mutations in human patients and studies of knockout mice, have led to a better understanding of the function of most of the CIC chloride channels. CIC-5 and CIC-7, and perhaps CIC-3 as well, play important roles in endocytic processes. It is highly probable that these channels have very similar functions in various cell types of different origins. However, the intrarenal localization of some of them, such as CIC-2 and CIC-4, still remains to be unambiguously demonstrated. The fine cellular and subcellular localization of these chloride channels will certainly provide further information about their role in the kidney.

**References**

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