Expression and Function of CLC and Cystic Fibrosis Transmembrane Conductance Regulator Chloride Channels in Renal Epithelial Tubule Cells: Pathophysiological Implications

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Cl⁻ channels play important roles in the regulation of a variety of functions, including electrical excitability, cell volume regulation, transepithelial transport and acidification of cellular organelles. They are expressed in plasma membranes or reside in intracellular organelles. A large number of Cl⁻ channels with different functions have been identified. Some of them are highly expressed in the kidney. They include members of the CLC Cl⁻ channel family: ClC-K1 (or ClC-Ka), ClC-K2 (or ClC-Kb) and ClC-5. The identification of mutations responsible for human inherited diseases (Bartter syndrome for ClC-Kb and Dent’s disease for ClC-5) and studies on knockout mice models have evidenced the physiological importance of these CLC Cl⁻ channels, permitting better understanding on their functions in renal tubule epithelial cells. The cystic fibrosis transmembrane conductance regulator (CFTR) Cl⁻ channel, also expressed in renal tubule epithelial cells, is involved in the transepithelial transport of Cl⁻ in the distal nephron. This short review focuses on intrarenal distribution, subcellular localization and function of the CIK-1, CIK-2 and CIK-5 Cl⁻ channels in renal tubule epithelial cells, and the role of the CFTR Cl⁻ channel in chloride fluxes elicited by vasopressin in the distal nephron. (Chang Gung Med J 2007;30:17-25)

Key words: kidney, chloride channel, CFTR, proximal tubule cells, collecting duct cells.

Immunohistochemical studies, ion transport and electrophysiological studies, together with the identification of mutations in human patients, and the development and study of knockout mice models, have led to a better understanding of the functions of chloride channels expressed in kidney epithelial cells. This review will focus on the intrarenal distribution, subcellular localization and function of some of the chloride channels belonging to the CLC family, and the role of the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel in chloride fluxes elicited by vasopressin in the distal nephron.

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

The two closely homologous CIC chloride channels, referred to as rCIC-K1 and rCIC-K2 in rats and as hCIC-Ka and hCIC-Kb in humans, are almost exclusively expressed in kidneys. Uchida et al. first demonstrated that CIC-K1 is highly expressed in the medulla and is up-regulated by dehydration. Immunohistochemical studies using an antibody that recognized both CIC-K1 and CIC-K2 allowed identification of the two CIC-K channels in basolateral membranes of thick ascending limb (TAL) cells, distal convoluted tubules (DCT) and in some cells of...
the collecting duct, presumably intercalated cells\(^{3,4}\) (Fig. 1). More recently Kobayashi et al.\(^{5}\) confirmed these results and showed that the ClC-K2 chloride channel is expressed in the basolateral membranes of collecting duct type A intercalated cells in the kidneys of ClC-K1 knockout mice (\textit{Clcnk1}\(^{-/-}\)). The expression of rClC-K1 in \textit{Xenopus} oocytes yielded pH independent-voltage currents activated by extracellular calcium.\(^{6}\) The chloride currents produced by hClC-K2 have different characteristics from those generated by CIC-K1. The fact that ClC-K channels expressed in \textit{Xenopus} oocytes generated no or very low chloride currents led to the suggestion that accessory protein(s) could be necessary for the functional expression of ClC-K channels.

A better understanding of the function these ClC chloride channels came from the identification of mutations responsible for various forms of salt wasting syndromes. Simon et al.\(^{7}\) first reported mutations in the human chloride channel CLCNKB, equivalent to hClC-Kb, as a cause of Bartter syndrome type III. Other mutations have also been identified in patients exhibiting pure Bartter syndrome or mixed Bartter-Gitelman syndrome.\(^{8,9}\) More recently, mutations in barttin, a protein not related to any known transporter or channel, have been identified in a severe variant form of Bartter syndrome (BSND) associated with sensorineural deafness.\(^{10}\) Barttin acts as an essential subunit of the ClC-Ka and ClC-Kb chloride channels and colocalizes with them in the basolateral membranes of renal tubule cells and K\(^+\)-secreting cells from the inner ear.\(^{11}\) Moreover, functional analyses of BSND-associated point mutations also revealed impaired ClC-K activation by barttin. These findings demonstrate that the activation of the ClC-K2 by the accessory protein barttin is required for
adequate NaCl reabsorption. Overall, these findings have provided lines of evidence that the CIC-K2 chloride channel plays a critical role in basolateral exit of chloride in TAL and DCT.

CIC-K1, abundantly expressed in medullary TAL cells, is stimulated by dehydration and is involved in urinary concentration-dilution mechanisms. Clcnk1−/− mice develop nephrogenic diabetes insipidus insensitive to dD-arginine vasopressin, a vasopressin analog that specifically binds to the V2 receptor. In addition, Clcnk1−/− mice failed to concentrate urea and NaCl in their renal medulla. These findings indicate that ClC-K1 plays a critical role in the regulation of the urinary concentration-dilution mechanisms that take place in the renal medulla.

Function of the ClC-5 chloride channel
The ClC-5 chloride channel encoded by the CLCN5 gene is also highly expressed in the kidney. Mutations in the human CLCN5 gene have been found to occur in Dent’s disease, an X-linked hereditary hypercalciuric nephrolithiasis causing low molecular weight proteinuria, hypercalciuria, nephrolithiasis, progressive renal insufficiency and, in some cases, rickets. The clinical features of the disease suggested that ClC-5 was not a typical plasma membrane chloride channel but rather an intracellular channel. Gunther et al. first reported that ClC-5 is expressed in endocytically active proximal tubule cells and α-intercalated cells of the rat kidney (Fig. 2). These authors also showed that ClC-5 colocalized with endocytosed β2-microglobulin in intact proximal tubule cells and colocalized with the small GTPase rab5a expressed in early endosomes. These findings suggest that the intracellular ClC-5 chloride channel can provide an electrical shunt for the electrogenic proton pump, which is required for the acidification of vesicular organelles involved in the endocytosis of low molecular weight proteins. Other groups have reported similar intracellular localization of ClC-5 in rat, mouse and human kidneys. Recently Picollo and Pusch have shown that ClC-5 and CIC-4, another intracellular chloride channel, are able to carry substantial amounts of proton across plasma membranes when activated by positive voltages. Scheel et al. reported at the same time that these two channels act as electrogenic Cl−/H+ exchangers. These two studies demonstrated that CIC-5 (like CIC-4) plays an active role in endosomal acidification and also in regulating Cl− concentration in endosomal compartments.

The fact that ClC-5 expressed in Xenopus oocyte generates chloride currents raised the possibility that ClC-5 could be transiently expressed in the plasma membrane during the recycling of endocytic vesicles. Schwake et al. have identified a motif in the COOH terminus region of ClC-5, similar to the PY motif known to play a crucial role in the internalization and degradation of the epithelial sodium channel (ENaC) by ubiquitin-protein ligases and have shown that this motif is important in the endocytosis of ClC-5.

Wang et al. reported that ClC-5 knockout mice exhibit a phenotype similar to that of Dent’s disease. Piwon et al. generated ClC-5 knockout mice and showed that the disruption of the mouse ClC-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 and caused abnormalities in the internalization rate of the sodium-phosphate (NaPi-2) cotransporter leading to a rise in intraluminal concentration of parathormone. These important findings have confirmed the critical role of ClC-5 in proximal endocytosis of low molecular weight proteins. ClC-5 was also shown to be highly expressed in other epithelial cell types, particularly enterocytes, where it is colocalized with proton pumps, and rab4 and rab5a, two small GTPases expressed in endosomes and involved in the transcytosis of polymeric immunoglobulin receptors.

Function of CFTR in the distal nephron
The role of the CFTR in the kidney remained unclear because there was no major disruption of renal function in cystic fibrosis (CF) patients. The CFTR protein has been detected in most segments of the renal tubule, although its exact cellular and/or membranous localization remains to be identified. CFTR-like chloride currents and/or CFTR mRNA have been reported in a variety of cultured cells from the DCT, cortical collecting duct (CCD) and inner medullary collecting duct. It is now generally agreed that CFTR is mainly expressed at the apical surface of distal and collecting duct cells, and there is increasing indirect evidence that renal chloride secretion, a minor process under most physiological
circumstances, is one function of CFTR in the terminal parts of the renal tubule.

It has been suggested that cAMP may stimulate CFTR-like chloride channels and inhibit ENaC in cultured mouse M-1 CCD cells because CFTR can act as a down regulator of ENaC. To better understand the function of CFTR in the distal nephron, Bens et al. have analyzed the effects of dD-arginine vasopressin on chloride currents in confluent cultures of isolated CCD dissected from the kidneys of wild-type CFTR (+/+ ) and cfr−/− mice [(CFTR(−/−)] that lack CFTR-mediated, cyclic AMP-dependent chloride secretion in the colon, airways and exocrine pancreas cells. Ion fluxes were measured by the short-circuit method in the presence or absence of specific inhibitors of ENaC (benzamyl amiloride or amiloride) and anion channels [5-nitro-2-(3-phenylpropylamino)-benzoic acid, NPPB], and under particular apical ion substitution conditions (Fig. 3). Such analyses demonstrated that dDAVP stimulated the sodium absorption mediated by ENaC in both CFTR (+/+ ) and (−/−) CCDs to a very similar degree, suggesting that CFTR does act as a regulator of ENaC activity in the renal collecting duct and that CFTR mediates the cAMP-stimulated component of secreted chloride measured under short-circuit conditions in the mouse CCD.

Liddle’s syndrome is a dominant autosomal
form of hypertension, which results from excessive sodium absorption in the distal nephron due to mutations by deletion or modification of a conserved PY motif in the degradation of ENaC at the cell surface via its ubiquitination as a result of binding to the WW domain of the ubiquitin ligase Nedd4-2 and/or endocytosis of the channels via interaction with clathrin-coated pits. Pradervand et al. have generated a mouse model of Liddle’s syndrome (L/L mice) using targeted gene replacement to introduce a R566stop mutation, found in the original pedigree described by Liddle, into the mouse Scnn1b (β-ENaC) gene. In vivo and in vitro studies revealed that intact CCD cells dissected from the kidneys of these L/L mice exhibit higher transepithelial potential differences, and that in primary cultures they produce greater amiloride-sensitive equivalent short-circuit currents, reflecting sodium absorption mediated by ENaC, than wild-type CCD cells (Fig. 4). These findings suggest that the Liddle mutation may lead to constitutive hyperactivity of ENaC in the renal CCD.

Vasopressin and cAMP agonists also stimulate Na absorption in the target cells by increasing the density of ENaC at the cell surface. The fact that mutations in the PY motif of ENaC can alter the retrieval of the channels at the cell surface in transfected rat thyroid cells led to the suggestion that the ENaC mutants responsible for Liddle’s syndrome may be less sensitive or even totally insensitive to cAMP agonists. However, other studies of transfected renal CCD cells and dissected CCDs from Liddle-syndrome mice showed that ENaC mutants do maintain their ability to respond to vasopressin. Hopf et al. reported that Na current from ENaC mutants carrying Liddle’s syndrome mutations can be down-regulated by CFTR when co-expressed in Xenopus oocytes. This raises the question as to whether the cAMP-regulated CFTR chloride channel is involved in the regulation of NaCl absorption in the renal CCDs of Liddle-syndrome mice. Chang et al. analyzed the effects of dDAVP on ENaC and CFTR activities in renal CCDs from Liddle-syndrome mice. These authors showed that short-term incubation with dDAVP did not produce any further increase in the excessive sodium absorption occurring in the renal CCD cells from Liddle-syndrome mice but did hyperstimulate CFTR-dependent chloride currents in the apical membrane. This implies that the hyperactivity of the β-ENaC subunit responsible for the Liddle’s syndrome phenotype is associated with hyperactive cAMP-stimulated CFTR chloride currents. Short-circuit experiments performed after the

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**Fig. 3** Effect of apical substitution of Na on short-circuit current (Isc) elicited by dDAVP or isoproterenol (ISO). Isc was measured on confluent cultures of CCDs dissected from the kidneys of wild-type (+/+) or CFTR knockout (-/-) mice. Note the lack of Isc increase caused by dDAVP or ISO in CFTR (-/-) CCDs. Values are given as means ± SE (standard error). (from Reference 35)
basal membrane had been permeabilized with nystatin and a basal-to-apical chloride gradient had been imposed demonstrated that dDAVP also stimulated larger chloride currents across L/L and L/+ CCD layers than wild-type (+/+ ) counterparts. As controls, Isc was also measured on confluent cultures of CCD dissected from the kidneys of m/m mice almost completely lacking the β-ENaC subunit. The bars represent the amiloride-sensitive component of Isc reflecting sodium absorption mediated by ENaC. Values are given as means ± SE (standard error). (from Reference 50)

**Fig. 4** Short-circuit current (Isc) recordings before and after sequential apical additions of increasing concentrations of amiloride in confluent cultures of CCD dissected from the kidneys of heterozygous (L/+) and homozygous (L/L) Liddle mice, and wild-type (+/+ ) counterparts. As controls, Isc was also measured on confluent cultures of CCD dissected from the kidneys of m/m mice almost completely lacking the β-ENaC subunit. The bars represent the amiloride-sensitive component of Isc reflecting sodium absorption mediated by ENaC. Values are given as means ± SE (standard error). (from Reference 50)

Conclusions

The identification of mutations in genes encoding chloride channels, together with the development of knockout mice and the refinement of electrophysiological techniques, has led to a better understanding of the function and regulation of anion channels in a variety of tissues, including renal tubule epithelial cells. It has allowed the demonstration of the diversity of functions of these different chloride channels expressed in the kidney. There is no doubt that some of these chloride channels are involved in a large variety of other renal pathophysiological processes, which certainly deserve future studies.

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