# 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 ClK-1, ClC-K2 and ClC-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,[1] 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 ClC-K channels

The 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,[2,3] are almost exclusively expressed in kidneys. Uchida et al.[2] first demonstrated that ClC-K1 is highly expressed in the medulla and is up-regulated by dehydration. Immunohistochemical studies using an antibody that recognized both ClC-K1 and ClC-K2 allowed identification of the two ClC-K channels in basolateral membranes of thick ascending limb (TAL) cells, distal convoluted tubules (DCT) and in some cells of

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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 (*Clcnk1*$^{-/-}$). The expression of rClC-K1 in *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 ClC-K1. The fact that ClC-K channels expressed in *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

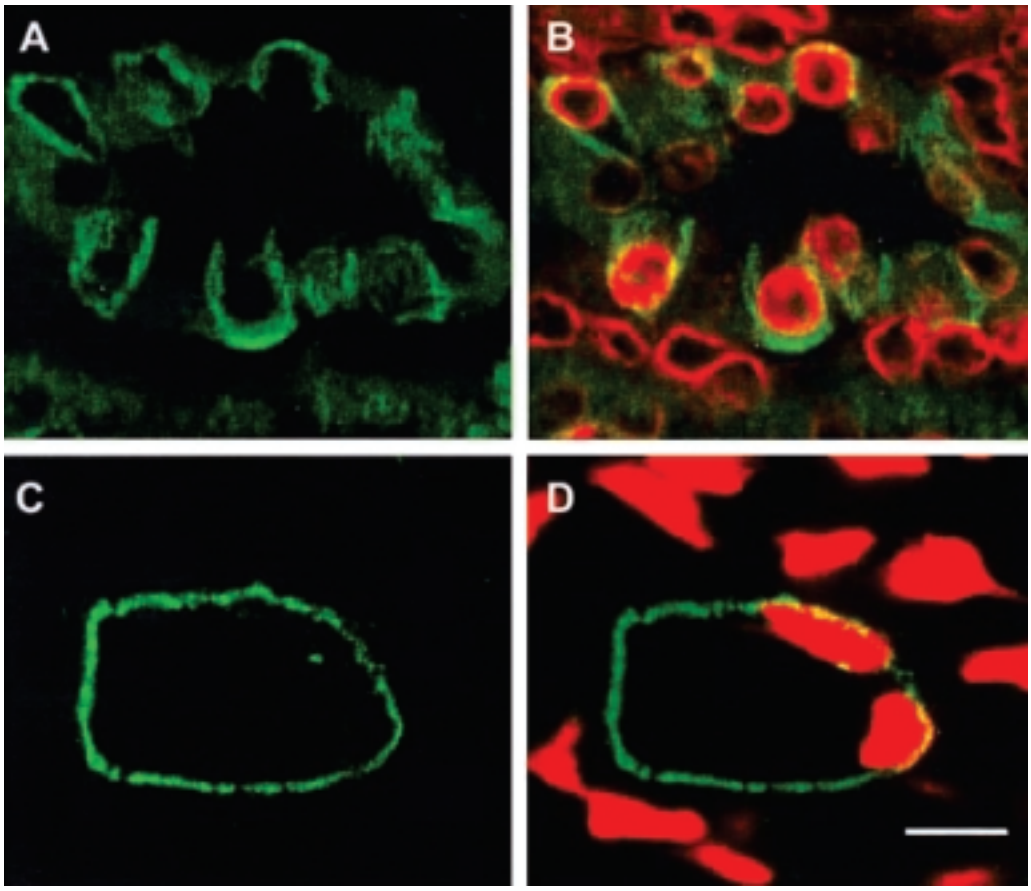


**Fig. 1** Immunolocalization of ClC-K channels (in green) in basolateral membranes of distal collecting duct cells (A, B) and medullary TAL cells (C, D). Nuclei (in red) are stained with iodine propidium. Bar = 10 μm. (from Reference 4)

adequate NaCl reabsorption. Overall, these findings have provided lines of evidence that the ClC-K2 chloride channel plays a critical role in basolateral exit of chloride in TAL and DCT.

ClC-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.[12] In addition, *Clcnk1*$^{-/-}$ mice failed to concentrate urea and NaCl in their renal medulla.[13] 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.[14] Mutations in the human CLCN5 gene have been found to occur in Dent's disease,[15] an X-linked hereditary hypercalciuric nephrolithiasis causing low molecular weight proteinuria, hypercalciuria, nephrolithiasis, progressive renal insufficiency and, in some cases, rickets.[16] 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.[17] 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.[18-20] Recently Picollo and Pusch[21] have shown that ClC-5 and ClC-4, another intracellular chloride channel, are able to carry substantial amounts of proton across plasma membranes when activated by positive voltages. Scheel et al.[22] reported at the same time that these two channels act as electrogenic $Cl^-/H^+$ exchangers. These two studies demonstrated that ClC-5 (like ClC-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[14] raised the possibility that ClC-5 could be transiently expressed in the plasma membrane during the recycling of endocytic vesicles. Schwake et al.[23] 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,[24,25] and have shown that this motif is important in the endocytosis of ClC-5.

Wang et al.[26] reported that ClC-5 knockout mice exhibit a phenotype similar to that of Dent's disease. Piwon et al.[27] also 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,[28] 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.[29]

**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.[30] 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.[31-34] 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

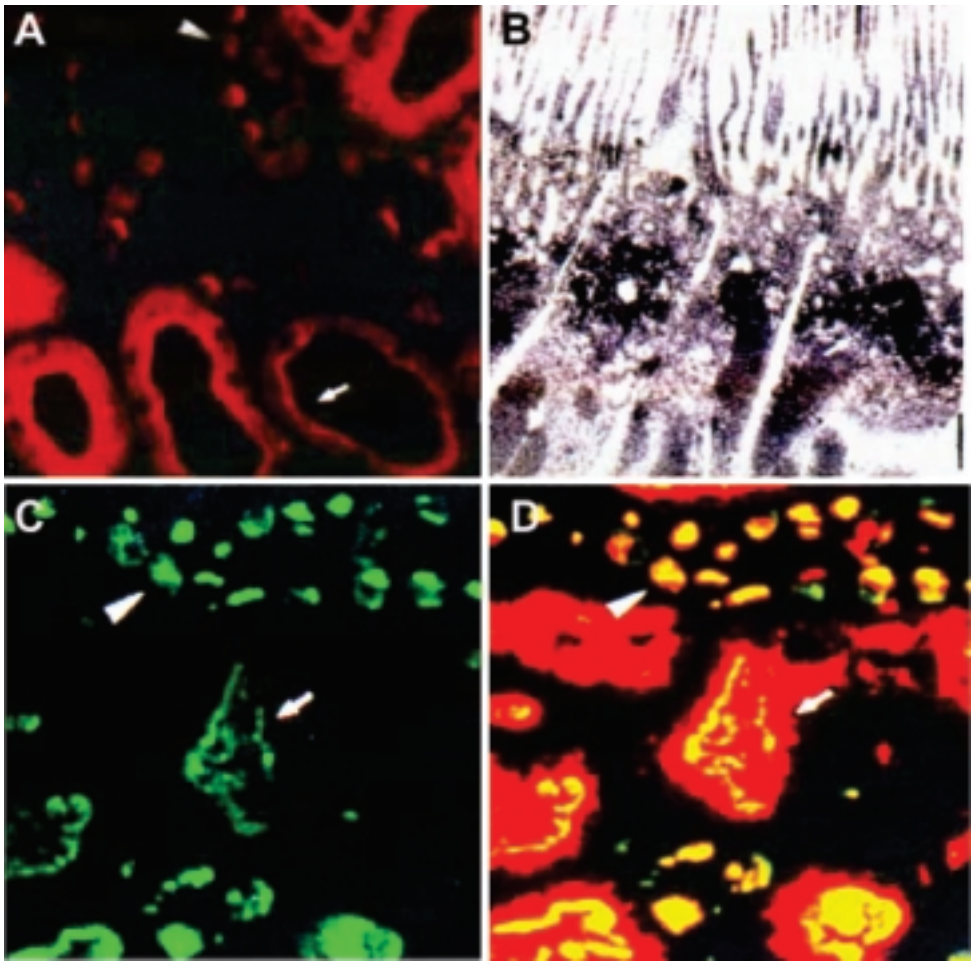


**Fig. 2** (A) Immunolocalization of ClC-5 (in red) in proximal tubule cells (arrow) and in some, but not all, collecting duct cells (arrowhead). (B) Ultrastructural localization of CLC-5 below the apical brush border membrane of proximal tubule cells. (C) (D). Colocalization of ClC-5 (in green) and vesicular $H^+$-ATPase (in red) in proximal tubule cells (arrows) and collecting duct cells (arrowheads). (unpublished observations)

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[34] because CFTR can act as a down regulator of ENaC.[37-39] To better understand the function of CFTR in the distal nephron, Bens et al.[35] 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 *cftr*$^{m1unc}$ [(CFTR(-/-)] mice that lack CFTR-mediated, cyclic AMP-dependent chloride secretion in the colon, airways and exocrine pancreas cells.[40,41] 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

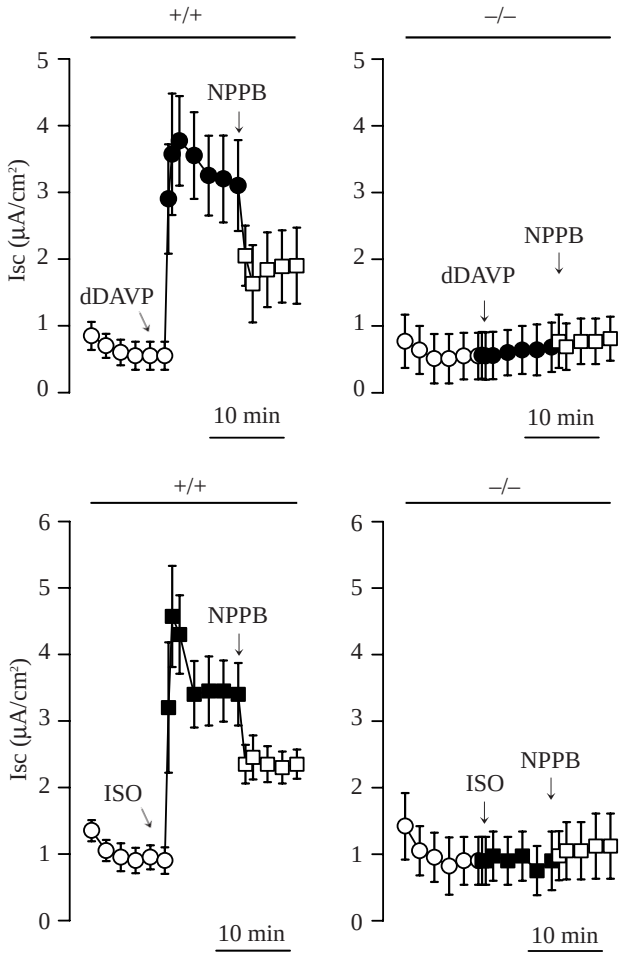


**Fig. 3** Effect of apical substitution of $Na^+$ on short-circuit current (Isc) elicited by dDAVP or isoproterenol (ISO). Isc was measured on confluent cultures of CCDs dissected from the kidneys of wild-type (+/+) or CFTR knockout (-/-) mice. Note the lack of Isc increase caused by dDAVP or ISO in CFTR (-/-) CCDs. Values are given as means ± SE (standard error). (from Reference 35)

form of hypertension,[42] which results from excessive sodium absorption in the distal nephron due to mutations by deletion or modification of a conserved PY motif (PPPxY) in the cytoplasmic COOH termini of β- and γ-ENaC subunits.[43,44] Previous heterologous expression studies on *Xenopus* oocytes and cultured cells have demonstrated that the ENaC mutations that cause Liddle's syndrome induce an increase in both the number and activity of ENaC channels, and in the retention of the channels at the cell surface.[45,46] These studies pointed out the decisive role of the 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[25] and/or endocytosis of the channels *via* interaction with clathrin-coated pits.[47] Pradervand et al.[48] have generated a mouse model of Liddle's syndrome (L/L mice) using targeted gene replacement to introduce a $R_{566stop}$ 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[49,50] (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.[51,52] The fact that mutations in the PY motif of ENaC can alter the retrieval of the channels at the cell surface[53] 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[54,55] showed that ENaC mutants do maintain their ability to respond to vasopressin. Hopf et al.[56] 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.[50] 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

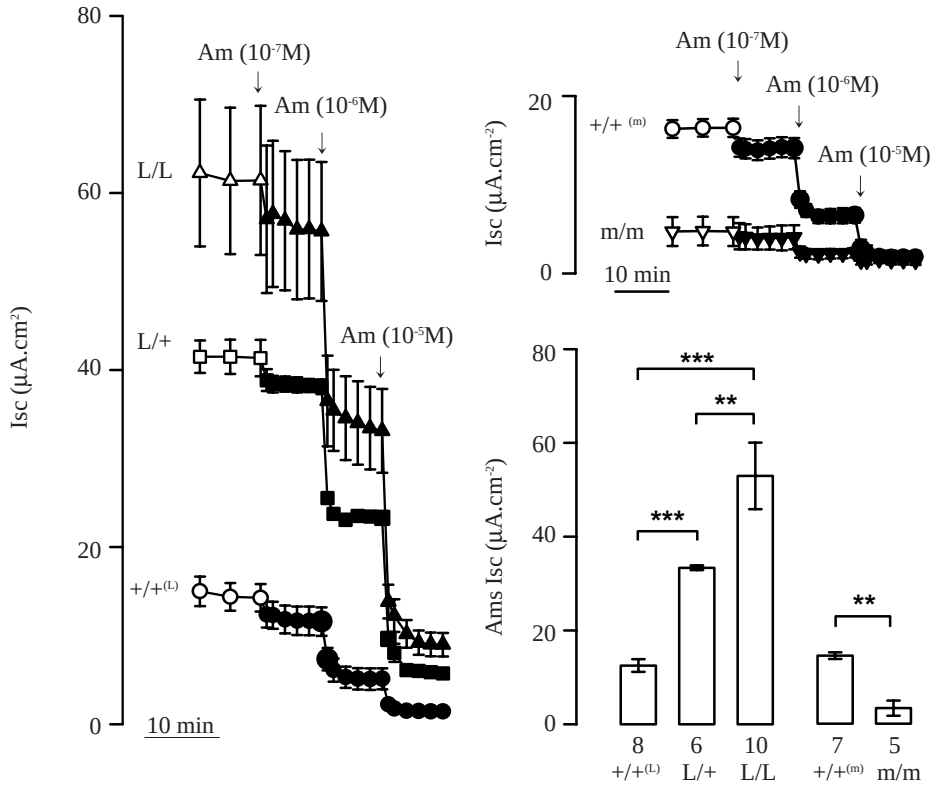


**Fig. 4** Short-circuit current (Isc) recordings before and after sequential apical additions of increasing concentrations of amiloride in confluent cultures of CCD dissected from the kidneys of heterozygous (L/+) and homozygous (L/L) Liddle mice, and wild-type (+/+) counterparts. As controls, Isc was 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)

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 CCD layers (Fig. 5). These findings demonstrate that vasopressin stimulates greater apical CFTR chloride conductance in the renal CCD cells of mice with Liddle's syndrome than in wild-type mice. These findings combine to support the idea that the CFTR chloride channel could also contribute to the enhanced NaCl absorption that occurs in the distal nephron of Liddle's syndrome patients.

## 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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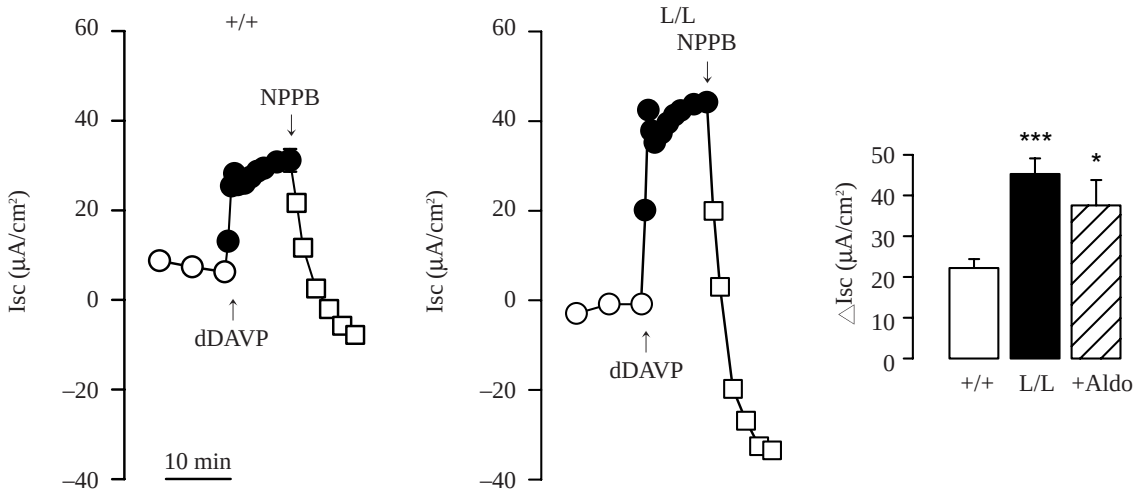


**Fig. 5** Effects of basolateral membrane permeabilization on dDAVP-stimulated Isc. Isc was measured in sets of confluent cultures of wild-type (+/+) and L/L CCD cells grown on filter after the basolateral membrane had been permeabilized by adding nystatin to the basal side of the filter and a $Cl^-$ (basal medium: 149 mM; apical medium: 14.9 mM) gradient imposed. After stabilization of the traces, cells were sequentially incubated with basal dDAVP for 10 min and then with apical NPPB for an additional 10 min. Bars represent the relative $\Delta$Isc increase caused by dDAVP. Values are given as means $\pm$ SE (standard error). (from Reference 50)

## REFERENCES

1. Jentsch TJ, Friedrich T, Schriever A, Yamada H. The CLC chloride channel family. Pflugers Arch 1999;437:783-95.
2. Uchida S, Sasaki S, Furukawa T, Hiraoka M, Imai T, Hirata Y, Marumo F. Molecular cloning of a chloride channel that is regulated by dehydration and expressed predominantly in kidney medulla. J Biol Chem 1993;268:3821-4.
3. Kieferle S, Fong P, Bens M, Vandewalle A, Jentsch TJ. Two highly homologous members of the ClC chloride channel family in both rat and human kidney. Proc Natl Acad Sci USA 1994;91:6943-7.
4. Vandewalle A, Cluzeaud F, Bens M, Kieferle S, Steinmeyer K, Jentsch TJ. Localization and induction by dehydration of ClC-K chloride channels in the rat kidney. Am J Physiol 1997;272:F678-88.
5. Kobayashi K, Uchida S, Mizutani S, Sasaki S, Marumo F. Intrarenal and cellular localization of CLC-K2 protein in the mouse kidney. J Am Soc Nephrol 2001;12:1327-34.
6. Waldegger S, Jentsch TJ. Functional and structural analysis of ClC-K chloride channels involved in renal disease. J Biol Chem 2000;275:24527-33.
7. Simon DB, Bindra RS, Mansfield TA, Nelson-Williams C, Mendonca E, Stone R, Schurman S, Nayir A, Alpay H, Bakkaloglu A, Rodriguez-Soriano J, Morales JM, Sanjad SA, Taylor CM, Pilz D, Brem A, Trachtman H, Griswold W, Richard GA, John E, Lifton RP. Mutations in the chloride channel gene, CLCNKB, cause Bartter's syndrome type III. Nat Genet 1997;17:171-8.
8. Konrad M, Vollmer M, Lemmink HH, van den Heuvel LP, Jeck N, Vargas-Poussou R, Lakings A, Ruf R, Deschenes G, Antignac C, Guay-Woodford L, Knoers NV, Seyberth HW, Feldmann D, Hildebrandt F. Mutations in the chloride channel gene CLCNKB as a cause of classic Bartter syndrome. J Am Soc Nephrol 2000;11:1449-59.
9. Jeck N, Konrad M, Peters M, Weber S, Bonzel KE, Seyberth HW. Mutations in the chloride channel gene, CLCNKB, leading to a mixed Bartter-Gitelman phenotype. Pediatr Res 2000;48:754-8.
10. Birkenhager R, Otto E, Schurmann MJ, Vollmer M, Ruf EM, Maier-Lutz I, Beekmann F, Fekete A, Omran H, Feldmann D, Milford DV, Jeck N, Konrad M, Landau D, Knoers NV, Antignac C, Sudbrak R, Kispert A, Hildebrandt F. Mutation of BSND causes Bartter syndrome with sensorineural deafness and kidney failure. Nat Genet 2001;29:310-4.
11. Estevez R, Boettger T, Stein V, Birkenhager R, Otto E, Hildebrandt F, Jentsch TJ. Barttin is a $Cl^-$ channel beta-subunit crucial for renal $Cl^-$ reabsorption and inner ear $K^+$ secretion. Nature 2001;414:558-61.
12. Matsumura Y, Uchida S, Kondo Y, Miyazaki H, Ko SB, Hayama A, Morimoto T, Liu W, Arisawa M, Sasaki S, Marumo F. Overt nephrogenic diabetes insipidus in mice lacking the CLC-K1 chloride channel. Nat Genet 199;21:95-8.
13. Akizuki N, Uchida S, Sasaki S, Marumo F. Impaired solute accumulation in inner medulla of Clcnk1-/- mice kidney. Am J Physiol Renal Physiol 2001;280:F79-87.

14. Steinmeyer K, Schwappach B, Bens M, Vandewalle A, Jentsch TJ. Cloning and functional expression of rat CLC-5, a chloride channel related to kidney disease. J Biol Chem 1995;270:31172-7.
15. Lloyd SE, Pearce SH, Fisher SE, Steinmeyer K, Schwappach B, Scheinman SJ, Harding B, Bolino A, Devoto M, Goodyer P, Rigden SP, Wrong O, Jentsch TJ, Craig IW, Thakker RV. A common molecular basis for three inherited kidney stone diseases. Nature 1996;379:445-9.
16. Scheinman SJ. X-linked hypercalciuric nephrolithiasis: clinical syndromes and chloride channel mutations. Kidney Int 1998;53:3-17.
17. Gunther W, Luchow A, Cluzeaud F, Vandewalle A, Jentsch TJ. ClC-5, the chloride channel mutated in Dent's disease, colocalizes with the proton pump in endocytotically active kidney cells. Proc Natl Acad Sci USA 1998;95:8075-80.
18. Luyckx VA, Goda FO, Mount DB, Nishio T, Hall A, Hebert SC, Hammond TG, Yu AS. Intrarenal and subcellular localization of rat CLC5. Am J Physiol 1998;275:F761-9.
19. Sakamoto H, Sado Y, Naito I, Kwon TH, Inoue S, Endo K, Kawasaki M, Uchida S, Nielsen S, Sasaki S, Marumo F. Cellular and subcellular immunolocalization of ClC-5 channel in mouse kidney: colocalization with $H^+$-ATPase. Am J Physiol 1999;277:F957-65.
20. Devuyst O, Christie PT, Courtoy PJ, Beauwens R, Thakker RV. Intra-renal and subcellular distribution of the human chloride channel, CLC-5, reveals a pathophysiological basis for Dent's disease. Hum Mol Genet 1999;8:247-57.
21. Picollo A, Pusch M. Chloride/proton antiporter activity of mammalian CLC proteins ClC-4 and ClC-5. Nature 2005;436:420-3.
22. Scheel O, Zdebik AA, Lourdel S, Jentsch TJ. Voltage-dependent electrogenic chloride/proton exchange by endosomal CLC proteins. Nature 2005;436:424-7.
23. Schwake M, Friedrich T, Jentsch TJ. An internalization signal in ClC-5, an endosomal $Cl^-$ channel mutated in dent's disease. J Biol Chem 2001;276:12049-54.
24. Staub O, Dho S, Henry P, Correa J, Ishikawa T, McGlade J, Rotin D. WW domains of Nedd4 bind to the proline-rich PY motifs in the epithelial $Na^+$ channel deleted in Liddle's syndrome. EMBO J 1996;15:2371-80.
25. Kamynina E, Debonneville C, Bens M, Vandewalle A, Staub O. A novel mouse Nedd4 protein suppresses the activity of the epithelial $Na^+$ channel. FASEB J 2001;15:204-14.
26. Wang SS, Devuyst O, Courtoy PJ, Wang XT, Wang H, Wang Y, Thakker RV, Guggino S, Guggino WB. Mice lacking renal chloride channel, CLC-5, are a model for Dent's disease, a nephrolithiasis disorder associated with defective receptor-mediated endocytosis. Hum Mol Genet 2000;9:2937-45.
27. Piwon N, Gunther W, Schwake M, Bosl MR, Jentsch TJ. ClC-5 $Cl^-$ channel disruption impairs endocytosis in a mouse model for Dent's disease. Nature 2000;408:369-73.
28. Nykjaer A, Dragun D, Walther D, Vorum H, Jacobsen C, Herz J, Melsen F, Christensen EI, Willnow TE. An endocytic pathway essential for renal uptake and activation of the steroid 25-(OH) vitamin D3. Cell 1999;96:507-15.
29. Vandewalle A, Cluzeaud F, Peng KC, Bens M, Luchow A, Gunther W, Jentsch TJ. Tissue distribution and subcellular localization of the ClC-5 chloride channel in rat intestine. Am J Physiol Cell Physiol 2001;280:C373-81.
30. Stanton BA. Cystic fibrosis transmembrane conductance regulator (CFTR) and renal function. Wien Klin Wochenschr 1997;109:457-64.
31. Rubera I, Tauc M, Bidet M, Poujeol C, Cuiller B, Watrin A, Touret N, Poujeol P. Chloride currents in primary cultures of rabbit proximal and distal convoluted tubules. Am J Physiol 1998;275:F651-63.
32. Husted RF, Volk KA, Sigmund RD, Stokes JB. Anion secretion by the inner medullary collecting duct. Evidence for involvement of the cystic fibrosis transmembrane conductance regulator. J Clin Invest 1995;95:644-50.
33. Vandorpe D, Kizer N, Ciampollilo F, Moyer B, Karlson K, Guggino WB, Stanton BA. CFTR mediates electrogenic chloride secretion in mouse inner medullary collecting duct (mIMCD-K2) cells. Am J Physiol 1995;269: C683-9.
34. Letz B, Korbmacher C. cAMP stimulates CFTR-like Cl-channels and inhibits amiloride-sensitive $Na^+$ channels in mouse CCD cells. Am J Physiol 1997;272:C657-66.
35. Bens M, Van Huyen JP, Cluzeaud F, Teulon J, Vandewalle A. CFTR disruption impairs cAMP-dependent Cl- secretion in primary cultures of mouse cortical collecting ducts. Am J Physiol Renal Physiol 2001;281:F434-42.
36. Van Huyen JP, Bens M, Teulon J, Vandewalle A. Vasopressin-stimulated chloride transport in transimmortalized mouse cell lines derived from the distal convoluted tubule and cortical and inner medullary collecting ducts. Nephrol Dial Transplant 2001;16:238-45.
37. Stutts MJ, Canessa CM, Olsen JC, Hamrick M, Cohn JA, Rossier BC, Boucher RC. CFTR as a cAMP-dependent regulator of sodium channels. Science 1995;269:847-50.
38. Stutts MJ, Rossier BC, Boucher RC. Cystic fibrosis transmembrane conductance regulator inverts protein kinase A-mediated regulation of epithelial sodium single channel kinetics. J Biol Chem 1997;272:14037-40.
39. Briel M, Greger R, Kunzelmann K. $Cl^-$ transport by cystic fibrosis transmembrane conductance regulator (CFTR) contributes to the inhibition of epithelial $Na^+$ channels (ENaCs) in Xenopus oocytes co-expressing CFTR and ENaC. J Physiol 1998;508:825-36.
40. Snouwaert JN, Brigman KK, Latour AM, Malouf NN, Boucher RC, Smithies O, Koller BH. An animal model for cystic fibrosis made by gene targeting. Science 1992;257:1083-8.
41. Clarke LL, Grubb BR, Yankaskas JR, Cotton CU, McKenzie A, Boucher RC. Relationship of a non-cystic

fibrosis transmembrane conductance regulator-mediated chloride conductance to organ-level disease in Cftr(-/-) mice. Proc Natl Acad Sci USA 1994;91:479-83.
42. Liddle GW, Bledsoe T, Coppage WS Jr. A familial renal disorder simulating primary aldosteronism but with negligible aldosterone secretion. Trans Assoc Am Physicians 1963;76:199-213.
43. Shimkets RA, Warnock DG, Bositis CM, Nelson-Williams C, Hansson JH, Schambelan M, Gill JR Jr, Ulrick S, Milora RV, Findling JW, Canessa CM, Rossier BC, Lifton RP. Liddle's syndrome: heritable human hypertension caused by mutations in the beta subunit of the epithelial sodium channel. Cell 1994;79:407-14.
44. Hansson JH, Nelson-Williams C, Suzuki H, Schild L, Shimkets R, Lu Y, Canessa C, Iwasaki T, Rossier B, Lifton RP. Hypertension caused by the truncated epithelial sodium channel gamma subunit: genetic heterogeneity of Liddle syndrome. Nat Genet 1995;11:76-82.
45. Snyder PM, Price MP, McDonald FJ, Adams CM, Volk KA, Zeiher BG, Stokes JB, Welsh MJ. Mechanism by which Liddle's syndrome mutations increase activity of a human epithelial Na+ channel. Cell 1995;83:969-78.
46. Schild L, Lu Y, Gautschi I, Schneeberger E, Lifton RP, Rossier BC. Identification of a PY motif in the epithelial Na channel subunits as a target sequence for mutations causing channel activation found in Liddle syndrome. EMBO J 1996;15:2381-7.
47. Shimkets RA, Lifton RP, Canessa CM. The activity of the epithelial sodium channel is regulated by clathrin-mediated endocytosis. J Biol Chem 1997;272:25537-41.
48. Pradervand S, Wang Q, Burnier M, Beermann F, Horisberger JD, Hummler E, Rossier BC. A mouse model for Liddle's syndrome. J Am Soc Nephrol 1999;10:2527-33.
49. Pradervand S, Vandewalle A, Bens M, Gautschi I, Loffing J, Hummler E, Schild L, Rossier BC. Dysfunction of the epithelial sodium channel expressed in the kidney of a mouse model for Liddle syndrome. J Am Soc Nephrol 2003;14:2219-28.
50. Chang CT, Bens M, Hummler E, Boulkroun S, Schild L, Teulon J, Rossier BC, Vandewalle A. Vasopressin-stimulated CFTR Cl- currents are increased in the renal collecting duct cells of a mouse model of Liddle's syndrome. J Physiol 2005;562:271-84.
51. Marunaka Y, Eaton DC. Effects of vasopressin and cAMP on single amiloride-blockable Na channels. Am J Physiol 1991;260:C1071-84.
52. Morris RG, Schafer JA. cAMP increases density of ENaC subunits in the apical membrane of MDCK cells in direct proportion to amiloride-sensitive $Na^{(+)}$ transport. J Gen Physiol 202;120:71-85.
53. Snyder PM. Liddle's syndrome mutations disrupt cAMP-mediated translocation of the epithelial $Na^{(+)}$ channel to the cell surface. J Clin Invest 2000;105:45-53.
54. Auberson M, Hoffmann-Pochon N, Vandewalle A, Kellenberger S, Schild L. Epithelial $Na^+$ channel mutants causing Liddle's syndrome retain ability to respond to aldosterone and vasopressin. Am J Physiol Renal Physiol 2003;285:F459-71.
55. Dahlmann A, Pradervand S, Hummler E, Rossier BC, Frindt G, Palmer LG. Mineralocorticoid regulation of epithelial $Na^+$ channels is maintained in a mouse model of Liddle's syndrome. Am J Physiol Renal Physiol 2003;285:F-310-8.
56. Hopf A, Schreiber R, Mall M, Greger R, Kunzelmann K. Cystic fibrosis transmembrane conductance regulator inhibits epithelial $Na^+$ channels carrying Liddle's syndrome mutations. J Biol Chem 1999;274:13894-9.