Modulation of CIC-K Channel Function by the Accessory Subunit Barttin

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The human ClC-Ka/ClC-Kb and rodent ClC-K1/ClC-K2 channels are the pore-forming channel proteins participating in electrolyte transport across the thick (ClC-Kb, ClC-K2) and thin (ClC-Ka, ClC-K1) ascending limbs of Henle’s Loop; further nephron segments, such as distal convoluted tubule and collecting duct (ClC-K2, ClC-Kb); and the marginal cells of the stria vascularis in the inner ear (ClC-Ka, ClC-K1 and ClC-Kb, ClC-K2).1–4 When expressed alone, ClC-K1 generates only small Cl− currents, whereas ClC-K2, ClC-Ka and ClC-Kb do not generate any appreciable currents. The channels require barttin as accessory subunit to become fully functional.

Null mouse models as well as various genetic disorders highlight the physiologic and pathophysiologic importance of CIC-K/barttin channel complexes for renal salt and water reabsorption as well as endolymph secretion in the inner ear. Knockout of ClC-K1 leads to renal diabetes insipidus with diuresis despite increased antidiuretic hormone levels, a defect resulting from failure to upregulate medullary osmolarity adequately during dehydration.5 Naturally occurring loss-of-function mutations of CIC-Ka have hitherto not been described.2 Loss-of-function mutations of CIC-Kb lead to renal salt wasting (Barter syndrome type III).3,6,7 The simultaneous impairment of distal tubular Cl− transport may result in clinical features resembling Gitelman syndrome.7 Loss-of-function mutations of both ClC-Ka and ClC-Kb leads to severe renal salt loss in conjunction with sensorineural deafness.9 A common variant of the gene (CLCNKB) encoding CIC-Kb leading to excessive activity of CIC-Kb has been associated with increased BP in some populations9,10 but not in others.11 Common variants of the gene (CLCKA) encoding ClC-Ka have similarly been associated with salt-sensitive hypertension.12 Complete loss-of-function mutations of barttin leads to severe renal salt wasting (Barter syndrome type IV) and sensorineural deafness.13,14 Partial loss-of-function mutations of barttin may result in nonsyndromal hearing loss.15 ClC-K/barttin channels are under control of multiple signal cascades. ClC-K/barttin is inhibited by acid pH and stimulated by extracellular calcium.13,16 The ClC-K/barttin channel complexes may be targeted by the ubiquitin ligase Nedd4-2, which presumably ubiquitinates the channel proteins, thus labeling them for proteosomal degradation.17 Nedd4-2 is phosphorylated by serum and glucocorticoid-inducible kinase 1.2,17 Phosphorylated Nedd4-2 is bound to the scaffolding protein 14-3-3, which prevents its interaction with the channel proteins. Accordingly, serum and glucocorticoid-inducible kinase 1 upregulates CIC-Ka.17,18 Dehydration upregulates ClC-K1 expression, and salt depletion upregulates ClC-K2 expression. Furosemide has differing effects on CIC-K1 and CIC-K2. It upregulates CIC-K2 but downregulates ClC-K1 expression.3 ClC-K1 expression is markedly downregulated and urinary concentrating ability impaired in animals lacking tissue angiotensin-converting enzyme.19 CIC-K2 is downregulated by (uro)guanylin.20 After excision of channel-containing membrane patches, the ClC-K/barttin channels close. Apparently, some cellular component, such as cytoskeleton, is required to maintain the channels in the open configuration.21

Barttin exerts multiple effects in CIC-K channel function. It modifies CIC-K protein stability, subcellular distribution, and voltage-dependent gating.13,16,21 In this issue of JASN, Fischer et al.21 used heterologous expression in mammalian cells and patch-clamp to study the biophysical effects of barttin on rat ClC-K1 and human ClC-Ka. They compared the currents generated by expression of CIC-K1 with or without additional barttin expression. CIC-K channels are double-barreled channels with two identical protopores that are governed by individual or common gating. In the absence of barttin, Fischer et al.21 observed two gating processes with distinct kinetics and voltage dependence. A fast gating process, activated by membrane hyperpolarization, was shown to open and close individual CIC-K1 protopores. In contrast, a slow gating step, stimulated by membrane depolarization, regulated the common gating of the two protopores. Notably, the protopores displayed fast gating, even though CIC-K channels lack the “gating” glutamate at the amino-terminal end of the F-helix, which is considered to underlie the fast protopore gating in other CIC channels.21 Upon co-transfection with barttin, the channels displayed only one gating process that resembled macroscopic fast gating of the same channel in the absence of barttin. In the presence of barttin, the open probability of the slow gate lost voltage dependence and remained constitutively open. Accordingly, barttin increased channel activity at physiologic potentials. Human ClC-Ka...
was functional only after coexpression with barttin. The authors conclude that barttin constitutively opens the common slow gate of ClC-K/barttin channels.

The article by Fischer et al.\textsuperscript{21} provides novel insight into the biophysical properties of the ClC-K channels and their modification by the accessory subunit barttin. The observations not only are relevant for the function of ClC-K channels but also significantly expand on our knowledge on the function of ClC channels in general. ClC chloride channels are voltage-gated channels, but the physiologic significance of these gating processes has been largely unclear. The study by Fischer et al.\textsuperscript{21} establishes that barttin modifies the function of ClC-K channels and optimizes epithelial transport by modulating gating. Barttin also reveals the first biological function of cooperative gating of ClC channels. The peculiar double-barreled structure of ClC channels and the existence of individual and cooperative gating processes have attracted a lot of interest in recent years. Fischer et al.\textsuperscript{21} now demonstrate that modification of epithelial transport by barttin is achieved through changed cooperative gating. ClC channel gating is dysfunctional in various genetic diseases, and novel therapeutic approaches addressing ClC channels might be helpful in treating those diseases. The identification of barttin-binding sites could be a first step for a rational design in the development of ClC activators or blockers.

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**DISCLOSURES**

None.

**REFERENCES**


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