Roles of Na-K-2Cl and Na-Cl cotransporters and ROMK potassium channels in urinary concentrating mechanism

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Hebert, Steven C. Roles of Na-K-Cl and Na-Cl cotransporters and ROMK potassium channels in urinary concentrating mechanism. Am. J. Physiol. 275 (Renal Physiol. 44): F325–F327, 1998.—The cloning of the apical transport proteins involved in NaCl absorption in the thick ascending limb of Henle and the distal convoluted tubule has ushered in a new era that promises to provide molecular details of the urinary concentrating and diluting processes. We now have at hand tools to examine the regulation of these proteins that can affect the concentrating and diluting capacity.

distal convoluted tubule; Bartter’s syndrome; thick ascending limb of Henle

THE SEPARATION of NaCl from water in the thick ascending limb of Henle (TAL) and the distal convoluted tubule (DCT) is crucial to urinary concentration and dilution. This is accomplished by the active reabsorption of NaCl in these relatively watertight epithelia. Major advances have been made over the past few years in our understanding of NaCl transport mechanisms in these nephron segments. This has been fueled by the molecular identification of the Na-K-2Cl cotransporter (BSC1; see Refs. 2, 13) and K⁺ secretory channels (ROMK; see Refs. 8, 20) expressed in the TAL and the Na-Cl transporter (TSC1; see Refs. 2, 3) expressed in the DCT (see Fig. 1A).

The TAL and DCT reabsorb about 10–15% and 5–7%, respectively, of the filtered NaCl. The NaCl reabsorption mechanisms shown in Fig. 1 for TAL and DCT are secondary active transport processes that depend on low intracellular Na⁺ activities maintained by active extrusion of Na⁺ from the cell by the basolateral Na⁺-K⁺-ATPase (Na⁺ pump). K⁺ that enters the TAL cell by Na-K-2Cl cotransport recycles back to the tubular urine through potassium channels (4–6). This latter process has two major consequences: 1) it replenishes the urinary K⁺ that would otherwise be lost through absorption by the Na-K-2Cl cotransporter, ensuring a virtually inexhaustible supply of tubular K⁺ necessary for Na⁺ reabsorption; and 2) it also results in a lumen-positive transepithelial voltage that provides the driving force for paracellular transport of one-half of the total Na⁺ reabsorption (7).

1 This report is the second in a series of minireviews, which are based on a symposium on the urinary concentrating mechanism, held at Experimental Biology ’97 in New Orleans, LA.

The Na-K-2Cl and Na-Cl cotransporters, together with the recently cloned K⁺-Cl cotransporters, comprise a newly identified family of proteins, i.e., the electroneutral cation-chloride cotransporters. Polyclonal antibodies that are directed against each of these three proteins demonstrate transporter-specific fluorescence on apical membranes of these nephron segments (9, 14). Expression of these transporters in Xenopus laevis oocytes gives rise to the expected ion transport function [Na-K-2Cl cotransporter (2) or Na-Cl cotransporter (2) or K⁺ channel (8)]. Moreover the biophysical, electrophysiological and regulatory characteristics of the cloned channel correspond closely to that of the native low conductance, inwardly rectifying (and ATP- and pH-regulated) K⁺ channels found in the apical membranes of TAL (8, 20).

The cation-chloride transporters are glycoproteins with molecular weights of ~150 and 165 kDa for the Na-Cl and Na-K-2Cl cotransporters, respectively (Fig. 1B). These cotransporter proteins have similar overall topologies with a large hydrophobic central region of possibly 12 membrane-crossing helices flanked by large hydrophilic regions that appear to face the interior of the cell. N-linked sugar residues are present on the large extracellular loop between the 7th and 8th membrane-spanning segment: two for the Na-Cl cotransporter and up to three for the Na-K-2Cl cotransporter. The specific ion and diuretic binding regions on these cotransporters have not been identified.

The ROMK inwardly rectifying K⁺ channel has a novel topology that consists of only two potential membrane-spanning helices flanked by cytosolic amino and carboxy terminals (Fig. 1C). It has been suggested that the functional ROMK channel may be composed of four identical subunits that form a central pore, but...
this has not been verified. Several alternatively spliced isoforms of ROMK have been identified in the rat (1, 8, 20) and human (16) kidney. In the rat, three ROMK isoforms with distinct amino terminals have been identified that are differentially expressed along the nephron from medullary TAL to outer medullary collecting duct (1, 11).

Lessons from mutations in the human Na-K-2Cl cotransporter (Slc12a1; chromosome 16) and the apical K⁺ recycling channel (ROMK; KCNJ1; chromosome 11) in the TAL and the Na-Cl cotransporter (Slc12a3; chromosome 16q13) in the DCT have been cloned. B: proposed topology of the bumetanide-sensitive Na-K-2Cl and thiazide-sensitive Na-Cl cotransporters. C: topology model of the ROMK inward-rectifying K⁺ channel.

Fig. 1. Salt transporters contributing to the separation of NaCl from water in the loop of Henle (TAL) and the distal convoluted tubule (DCT). A: cell models of ion transport in the DCT and the TAL of Henle. The Na-K-2Cl cotransporter (BSC1; gene Slc12a1; chromosome 16) and the apical K⁺ recycling channel (ROMK; KCNJ1; chromosome 11) in the TAL and the Na-Cl cotransporter (TSC1; Slc12a3; chromosome 16q13) in the DCT have been cloned. B: proposed topology of the bumetanide-sensitive Na-K-2Cl and thiazide-sensitive Na-Cl cotransporters. C: topology model of the ROMK inward-rectifying K⁺ channel.
encode the apical Na-K-2Cl cotransporter in TAL and the Na-CI cotransporter in DCT, respectively. The finding that mutations in KCNJ 1 (ROMK) also lead to an identical clinical syndrome provides strong evidence that ROMK encodes the apical K⁺ channel in the TAL and that normal function of this K⁺ channel is vital to maintenance of NaCl reabsorption by the TAL. The deficits in concentrating and dilution capacity that these individuals exhibit is readily explained by their loss of function of Na-CI, Na-K-2Cl, or K⁺ channels, which are vital to the separation of NaCl and water in the nephron.

Summary. The cloning of the apical transport proteins involved in NaCl absorption in the TAL and DCT has ushered in a new era that promises to provide molecular details of the urinary concentrating and diluting processes. We now have at hand tools to examine the regulation of these proteins and genes under a variety of circumstances that can affect the concentrating and diluting capacity.

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