Basolateral K-Cl Cotransporter Regulates Colonic Potassium Absorption in Potassium Depletion*

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Active potassium absorption in the rat distal colon is electroneutral, Na⁺-independent, partially chloride-dependent, and energized by an apical membrane H,K-ATPase. Both dietary sodium and dietary potassium depletion substantially increase active potassium absorption. We have recently reported that sodium depletion up-regulates H,K-ATPase α-subunit mRNA and protein expression, whereas potassium depletion up-regulates H,K-ATPase β-subunit mRNA and protein expression. Because overall potassium absorption is non-conductive, K-Cl cotransport (KCC) at the basolateral membrane may also be involved in potassium absorption. Although KCC1 has not been cloned from the colon, we established, in Northern blot analysis with mRNA from the rat distal colon using rabbit kidney KCC1 cDNA as a probe, the presence of an expected size mRNA in the rat colon. This KCC1 mRNA is substantially increased by potassium depletion but only minimally by sodium depletion. KCC1-specific antibody identified a 155-kDa protein in rat colonic basolateral membrane. Potassium depletion but not sodium depletion resulted in an increase in KCC1 protein expression in basolateral membrane. The increase of colonic KCC1 mRNA abundance and KCC1 protein expression in potassium depletion of the rat colonic basolateral membrane suggests that K-Cl cotransporter: 1) is involved in transepithelial potassium absorption and 2) regulates the increase in potassium absorption induced by dietary potassium depletion. We conclude that active potassium absorption in the rat distal colon involves the coordinated regulation of both apical membrane H,K-ATPase and basolateral membrane KCC1 protein.

The K-Cl cotransporter (KCC),¹ a member of the cation-chloride cotransporter family, mediates the electroneutral, coupled transport of potassium and chloride (1, 2). The K-Cl cotransporter is important in the regulation of cell volume in non-epithelial cells and transepithelial potassium movement in epithelial cells (3–7). Four cDNAs encoding KCl cotransporter isoforms KCC1, KCC2, KCC3, and KCC4 have been cloned and characterized (8–12). Although KCC1 and KCC2 exhibit approximately 67% sequence homology, they are differentially expressed in rat tissues. Rat KCC1 is widely expressed in most tissues, whereas rat KCC2 is expressed only in brain. It has been proposed that KCC1 is a “housekeeping” isoform that regulates cell volume as well as transepithelial salt transport (8). The neuron-specific K-Cl cotransporter isoform KCC2 has recently been characterized as a K-Cl cotransporter and is a primary chloride extruder that promotes fast hyperpolarizing post-synaptic inhibition in the brain (13). K-Cl cotransport is also responsible for electroneutral K-Cl absorption in both proximal tubules and the cortical thick ascending limb of rabbit kidney (14).

Potassium transport is an important function of the mammalian large intestine, with evidence of both active potassium absorption and secretion in the distal colon. Active potassium absorption is electroneutral, Na⁺-dependent, chloride-dependent (in part), and is generally believed to be energized by an apical membrane H,K-ATPase (15–17). Because overall transepithelial potassium absorption is not conductive (18), the involvement of an electroneutral process (e.g. K-Cl cotransport) at the basolateral membrane has been proposed (19).

Both dietary sodium depletion (as a result of an increase in serum aldosterone) and dietary potassium depletion enhance active potassium absorption in the rat distal colon (16, 17). Apical membrane H,K-ATPase activity is increased in dietary sodium depletion but not in dietary potassium depletion (20). In addition to the increase in H,K-ATPase activity in sodium depletion, we have recently demonstrated that an up-regulation of colonic H,K-ATPase α subunit mRNA and protein expression may be responsible for the increase in active potassium absorption in dietary sodium depletion. Although an increase in either H,K-ATPase activity or its α subunit was not observed in potassium depletion, an up-regulation of H,K-ATPase β subunit expression at both mRNA and protein levels in dietary potassium depletion may be one of the factors responsible for the enhancement of potassium absorption in potassium depletion (21).

Because the increase in active potassium absorption by dietary potassium depletion that is observed in in vitro studies requires the presence of chloride (17), a role for basolateral K-Cl cotransport in transepithelial potassium movement is an interesting possibility (19). It is not known whether other factors also contribute to the enhanced potassium absorption induced by dietary potassium depletion in the rat distal colon. To date, there are no reports of K-Cl cotransport protein expression in the rat distal colon, although the rabbit kidney KCC1 cDNA identified a mRNA in the rat distal colon (8).

Because the up-regulation of active potassium absorption in dietary potassium depletion is chloride-dependent, suggesting that K-Cl cotransport may have a physiological role in colonic potassium absorption, the present study was designed to examine K-Cl cotransport protein expression in normal conditions and in dietary potassium depletion. These present experi-

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¹ The abbreviation used is: KCC, K-Cl cotransporter.

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MATERIALS AND METHODS

Male Harlan Sprague-Dawley rats weighing ~200–250 g were purchased from Charles River Laboratories (Wilmington, MA). The animals were divided into three groups. The control group was fed normal rat food that contained 4.4 g of sodium/kg and 9.5 g of potassium/kg. The sodium-depleted group was given a sodium-free diet for 1 week. The potassium-depleted group was given a potassium-free diet (0.6 mg of potassium/kg) for 3 weeks. All rats were allowed free access to water. On the last day of the experimental diet periods, the animals were killed, and their distal and proximal colons were immediately removed and washed with diethyl pyrocarbonate-treated sterilized saline. Colonocytes were prepared as described previously (20).

RNA Preparation—Total RNA was isolated from colonocytes using Trizol reagent (Life Technologies, Inc.) as recommended by the manufacturer. Poly(A)+ mRNA was prepared from total RNA using Oligotex reagent (Qiagen) according to the manufacturer’s recommendations.

Northern Blot Analysis—Northern blot analyses were performed using poly(A)+ mRNA as described previously (20) except that a 32P-labeled 1.1-kilobase BamHI and EcoRI fragment of rabbit KCC1 cDNA was used as a probe (1 × 106 cpm/ml). Hybridization was performed at 42 °C in a Hybaid oven for 18 h. Blots were washed for 15 min in 1× SSC (0.15 M NaCl and 0.015 M sodium citrate, pH 7.0) and 0.1% SDS at 65 °C, exposed to x-ray film, and developed.

Isolation of Apical and Basolateral Membranes—Apical and basolateral membranes were isolated by methods previously described in detail (22, 23). Purity of apical membranes was assessed by H,K-ATPase (10–12-fold enrichment compared with homogenate) (15, 24), whereas that of basolateral membranes was assessed by Na,K-ATPase (12–15-fold enrichment) (24). H,K-ATPase activity was not detected in basolateral membranes, and Na,K-ATPase activity was only minimally present in apical membranes (15, 24).

Western Blot Analysis—Western blot analyses were performed as described previously (20, 21) except for the use of rabbit KCC1 polyclonal antibody. A cDNA fragment corresponding to the 42-kDa C terminus of rabbit KCC1 protein was bacterially expressed as a GST-KCC1 fusion protein. The affinity purified GST-KCC1 fusion protein was minimally increased compared with that of controls (p < 0.05).

The abundance of mRNA prepared from rat proximal colons of control, sodium-depleted, and potassium-depleted rats using KCC1 cDNA as a probe was assessed by Northern blot analysis and is shown in Fig. 2A; a densitometric analysis is presented in Fig. 2B. mRNA expression of KCC1 in the proximal colon of potassium-depleted rats was not altered compared with that of controls. KCC1 mRNA expression in the proximal colon was also not affected by dietary sodium depletion compared with that of controls.

Expression of KCC1 Protein—Western blot analysis was performed with basolateral membranes using an antibody raised against the entire C terminus of rabbit KCC1. The KCC1 antibody identified a protein in the rat distal colon that was identical in size to that previously seen in the rabbit colon. This protein was therefore designated as KCC1 protein. The Western blot analysis of KCC1 protein expression in basolateral membranes of the distal colons of normal, dietary sodium-depleted, and dietary potassium-depleted rats is presented in Fig. 3A, and a densitometric analysis is shown in Fig. 3B. KCC1 protein expression was increased in basolateral membranes of dietary potassium-depleted rats compared with those of controls (cf lanes 7–9 with lanes 1–3 of Fig. 3A) (p < 0.002). In contrast, KCC1 protein expression was reduced (p < 0.01) in basolateral membranes of sodium-depleted rats compared with those of controls (cf lanes 4–6 with lanes 1–3 of Fig. 3A).

Fig. 4 presents the Western blot analyses of KCC1 protein in the apical membranes of the rat distal colon in control, sodium-depleted, and potassium-depleted conditions. Although KCC1
significant differences were present. *, p < 0.01 compared with control; **, p < 0.002 compared with dietary sodium-depleted distal colons, a faint band was seen in apical membranes of normal or dietary sodium-depleted distal colons, suggesting that this band probably represents contamination by basolateral membranes.

**DISCUSSION**

The mammalian distal colon serves as an important regulatory system for the maintenance of overall potassium balance with the presence of both potassium absorptive and secretory processes (16–18). Studies under voltage clamp conditions have characterized active potassium transport processes. In normal animals active potassium absorption is present and is electroneutral, Na\(^+\) independent, and, in part, chloride-dependent. Active potassium absorption involves both apical and basolateral membrane transport processes and is generally believed to be energized by an apical membrane H,K-ATPase (15). It is likely that there are at least two different H,K-ATPase isoforms, one that is ouabain-sensitive and one that is ouabain-insensitive (26–29). The ouabain-insensitive H,K-ATPase is present exclusively in surface cells and is the H,K-ATPase that has been cloned and expressed (26–32).

Potassium transport in the rat distal colon is altered by changes in dietary potassium balance (17). An increase in dietary potassium stimulates potassium secretion but does not alter active potassium absorption, whereas dietary potassium depletion enhances active potassium absorption but does not alter active potassium absorption, whereas dietary potassium depletion does not affect active potassium absorption (17). Active potassium absorption is also substantially increased by aldosterone in experiments with dietary sodium depletion or with aldosterone administered via subcutaneous infusion (33). Only after aldosterone-induced potassium secretion is inhibited is the increase in active potassium absorption demonstrated or unmasked (16). The cellular mechanisms by which dietary potassium depletion and aldosterone increase active potassium absorption differ (20, 21). Aldosterone increases both apical membrane H,K-ATPase activity and H,K-ATPase \(\beta\) subunit (HK\(\beta\)) mRNA and protein but does not alter H,K-ATPase \(\alpha\) subunit (HK\(\alpha\)) mRNA and protein (20, 21). In contrast, dietary potassium depletion does not affect H,K-ATPase activity or HK\(\alpha\) mRNA and protein expression but increases HK\(\beta\) mRNA and protein expression (20, 21). It is not known how the changes in the \(\beta\) subunit of H,K-ATPase induced by dietary potassium depletion are linked to the increase in active potassium secretion or whether another mechanism(s) is responsible for the increase in potassium absorption by dietary potassium depletion.

The stimulation of active potassium absorption both by dict-
tary potassium depletion and aldosterone is dependent on chloride. In the absence of chloride neither potassium depletion nor aldosterone enhances active potassium absorption (16, 17). This observation and the non-conductive nature of active potassium absorption initially suggested a possible role for basolateral K-Cl cotransport. The present results provide compelling supportive evidence that the chloride-dependent potassium absorption induced by dietary potassium depletion may be mediated by K-Cl cotransport at the basolateral membrane. Figs. 1 and 3 demonstrate that dietary potassium depletion increases KCC1 mRNA and protein expression at the basolateral membrane. The increase in potassium absorption that is induced by aldosterone does not appear to involve basolateral membrane K-Cl cotransporter, because KCC1 mRNA was increased modestly, but KCC1 protein expression was reduced by dietary sodium depletion (Figs. 1 and 3).

Furthermore, KCC1 mRNA is not increased by dietary potassium depletion initially suggested a possible role for basolateral K-Cl cotransport. The present results provide compelling supportive evidence that the chloride-dependent potassium absorption in the proximal colon, where active potassium absorption induced by dietary potassium depletion was reduced by dietary sodium depletion (Figs. 1 and 3).

Although dietary potassium absorption also in -sodium chloride-dependent active potassium absorption (16, 17). This observation and the non-conductive nature of active potassium absorption initially suggested a possible role for basolateral K-Cl cotransport. The present results provide compelling supportive evidence that the chloride-dependent potassium absorption induced by dietary potassium depletion may be mediated by K-Cl cotransport at the basolateral membrane. Figs. 1 and 3 demonstrate that dietary potassium depletion increases KCC1 mRNA and protein expression at the basolateral membrane. The increase in potassium absorption that is induced by aldosterone does not appear to involve basolateral membrane K-Cl cotransporter, because KCC1 mRNA was increased modestly, but KCC1 protein expression was reduced by dietary sodium depletion (Figs. 1 and 3).

There is limited additional evidence of the regulation of K-Cl cotransport by potassium. K-Cl cotransport is enhanced by K-ethylmaleimide in sheep erythrocytes when exposed to low [K+]. (25). Although dietary potassium absorption also increases renal potassium absorption, a specific role for K-Cl cotransport in the enhancement of renal tubular potassium absorption by potassium depletion has not been established. In contrast, preliminary studies revealed that KCC1 protein expression in the rat renal cortex was substantially enhanced in the presence of dietary potassium depletion (16).

These present results establish that the increase in active potassium absorption induced by dietary potassium depletion is associated with an increase in KCC1 protein expression in the basolateral membranes of the distal colon. Therefore, our previous experiments (20, 21) and this present study permit the conclusion that active potassium absorption in the rat distal colon is regulated by both apical and basolateral transport proteins: H,K-ATPase at the former and KCC1 at the latter.

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