Enhanced $K^+$ secretion in dextran sulfate-induced colitis reflects upregulation of large conductance apical $K^+$ channels (BK; Kcnma1)

Basalingappa M. Kanthesh,1 Geoffrey I. Sandle,2 and Vazhaikkurichi M. Rajendran1

1Department of Biochemistry and Molecular Biology, West Virginia University School of Medicine, Morgantown, West Virginia; and 2Leeds Institute of Biomedical and Clinical Sciences, St James’s University Hospital, Leeds, United Kingdom

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Kanthesh BM, Sandle GI, Rajendran VM. Enhanced $K^+$ secretion in dextran sulfate-induced colitis reflects upregulation of large conductance apical $K^+$ channels (BK; Kcnma1). Am J Physiol Cell Physiol 305: C972–C980, 2013.—Defective colonic Na$^+$ and Cl$^-$ absorption is a feature of active ulcerative colitis (UC), but little is known about changes in colonic $K^+$ transport. We therefore investigated colonic $K^+$ transport in a rat model of dextran sulfate-induced colitis. Colitis was induced in rat distal colon using 5% dextran sulfate sodium (DSS). Short-circuit current ($I_{sc}$, indicating electrogenic ion transport) and $^86$Rb ($K^+$ surrogate) fluxes were measured in colonic mucosa mounted in Ussing chambers under voltage-clamp conditions in the presence of mucosal orthovanadate (a P-type ATPase inhibitor). Serum aldosterone was measured by immunoassay. Control animals exhibited zero net $K^+$ flux. By contrast, DSS-treated animals exhibited active $K^+$ secretion, which was inhibited by 98, 76, and 22% by Ba$^{2+}$ (nonspecific $K^+$ channel blocker), iberiotoxin (IbTX; BK channel blocker), and TRAM-34 (IK channel blocker), respectively. Apical BK channel a-subunit mRNA abundance and protein expression, and serum aldosterone levels in DSS-treated animals, were enhanced 6-, 3-, and 6-fold respectively, compared with controls. Increasing intracellular Ca$^{2+}$ with carbachol (CCH), or intracellular cAMP with forskolin (FSK), stimulated both active $Cl^-$ secretion and active $K^+$ secretion in controls but had no or little effect in DSS-treated animals. In DSS-induced colitis, active $K^+$ secretion involves upregulation of apical BK channel expression, which may be aldosterone-dependent, whereas $Cl^-$ secretion is diminished. Since similar ion transport abnormalities occur in patients with UC, diarrhea in this disease may reflect increased colonic $K^+$ secretion (rather than increased $Cl^-$ secretion), as well as defective Na$^+$ and $Cl^-$ absorption.

In addition, some patients with severe UC develop hypokalemia, which may reflect increased fecal $K^+$ losses due, at least in part, to enhanced $K^+$ secretion across the inflamed colonic epithelium (28). However, the mechanism(s) responsible for increased colonic $K^+$ secretion in UC are unclear. Apical BK channel expression is increased in both acute and quiescent UC (28), but the precise role that enhanced BK channel expression plays in increased $K^+$ secretion is unknown, even though BMS (a BK channel opener) and DC-EBIO [an opener of intermediate conductance $K^+$ (IK) channels] induce BK and IK channel-mediated $K^+$ secretion respectively, in control rat distal colon (21, 29). In addition, in control rat distal colon, cAMP activates BK channel-mediated $K^+$ secretion, while aldosterone increases BK channel expression and induces BK channel-mediated $K^+$ secretion (29, 31). The aim of this study was to determine whether the expression of BK and/or IK channels was increased in DSS-induced colitis in rat distal colon and, if so, whether this resulted in enhanced colonic $K^+$ secretion. Our results indicate that in this experimental model of chronic colitis, increased colonic $K^+$ secretion reflects upregulation of apical BK channel expression, possibly as a result of increased serum aldosterone levels.

METHODS AND MATERIALS

Preparation of animals. Chronic active UC was induced in male Sprague-Dawley rats (Charles River, Raleigh, NC) weighing 126–150 g by feeding 5% DSS in water ad libitum for 5 alternate wk. Both control and DSS-treated rats were given standard rat chow. Other rats were fed a Na$^+$-free diet for 7 days to induce secondary hyperaldosteronism (31). Control and dietary Na$^+$-depleted (“aldo-treated”) rats were given water ad libitum. The experimental protocols were approved by the West Virginia University Institutional Animal Care and Use Committee.

Histology. Distal colons removed from anaesthetized control and DSS-treated rats were washed with ice-cold saline and fixed overnight in 10% neutral formalin. Sections (5 μm) cut from paraffin-embedded tissues were mounted on glass slides and stained with hematoxylin and eosin for histological evaluation. Coded slides were analyzed in blinded fashion by two investigators. The features considered were reactive epithelial changes, acute inflammatory changes within the lamina propria, acute cryptitis, crypt abscesses, crypt distortion, acute ulcer, healing ulcer, lymphoid aggregates, and transmural inflammation.

Cytokine assay. Proinflammatory cytokine levels in distal colonic epithelial cells from control and DSS-treated rats were quantified using a rat cytokine/chemokine panel (Milliplex MAP kit; Millipore). Mucosal sheers were freed of underlying muscle, manually homogenized using tight-fit Teflon-homogenizer and centrifuged (15 min at 2,000 g), and the resultant supernatants were used for cytokine assays. Filter plates dampened with assay buffer, were vacuum filtered before addition of either standard, control, or samples to appropriate wells. Premixed beads were added to each well, which were incubated...
overnight while shaking at 4°C. Following three washes, detection antibodies (25 μl) were added to each well and incubated for 2 h at room temperature. The wells were then treated with streptavidin-phycocerythrin (25 μl/well) and incubated for 30 min at room temperature. After three washes, beads resuspended in Milliplex sheath fluid (150 μl) were measured using the BioPlex Suspension System. Mucosal scrappings from four different control and DSS-treated rat distal colons were individually homogenized to prepare supernatants for cytokine assays. Cytokine levels were measured as picograms per milligrams of protein. Protein was measured as previously described (18).

**Aldosterone assays.** Serum aldosterone levels were measured using the aldosterone enzyme immunoassay kit, according to the manufacturer’s protocol (Enzo Life Sciences). Blood was collected directly from heart of six different anesthetized normal and UC rats and allowed to coagulate. Serum was collected by brief centrifugation. Triplicate assays were performed on all serum samples, and aldosterone levels were expressed as picomoles per milliliters of serum.

**Nitric oxide assays.** Distal colons obtained from four different control and DSS-treated rats were used for total nitric oxidized (NO) measurements. Each distal colon was divided into 10 segments, which were homogenized in Ringer solution and centrifuged (15 min at 2,000 g). The NO was measured in the resultant supernatants using a total NO detection kit, according to the manufacturer’s instruction (Enzo Life Sciences). In brief, NO that interacts covalently with target molecules (proteins) were measured as nitrate (NO3). Thus the total NO detection kit measured protein NO3 as an indicator of NO. This assay included conversion of NO3 to nitrite (NO2) by nitrate reductase, followed by the colorimetric detection of NO2 as a colored azo ionic species under study reflected its active (i.e., potential-independent) under open-circuit (mimicking in vivo) conditions, the net flux of the ionic species under study reflected its active (i.e., potential-independent) transport. The Ion and G were measured every 20 s using an automated multichannel voltage/current-clamp instrument (Physiological Instruments). Positive Ion represented either electrogenic Na+ absorption or electrogenic Cl− secretion, whereas negative Ion represented electrogenic K+ secretion.

For K+ flux studies, a trace of 86RbCl (1 μCi/chamber) was added to either the mucosal (m) or serosal (s) bath solutions. After a 45-min equilibration period, mucosa-to-serosa (m-s) and serosa-to-mucosa (s-m) K+ fluxes were measured under voltage-clamp conditions. Net fluxes were calculated from the difference between the m-s and s-m fluxes in tissue pairs that were matched based on differences in basal G values of <10%. Positive and negative values represented active absorption and active secretion, respectively. Epithelial sheets from control and DSS-treated rat distal colon exhibited basal G values of 5.5 ± 0.7 and 7.8 ± 0.8 mS, respectively, while the value in distal colon from aldo-treated rats was 29.3 ± 3.1 mS. Forskolin (FSK) significantly increased G in control distal colon from 5.5 ± 0.7 to 18.2 ± 1.6 mS but had no effect on G in either DSS-treated or aldo-treated distal colon (data not shown). Distal colonic epithelial sheets from control, DSS-treated, and aldo-treated animals exhibited stable values of G throughout the flux period (up to 60 min), indicating sustained tissue viability.

K+ fluxes were measured over 15-min periods. At the end of each period, 0.5-ml samples were withdrawn from the bath opposite to the isotope-containing side. Following sample removal, 0.5 ml of regular Ringer were added to maintain bath volume. Basal fluxes were measured immediately following an equilibrium period. Following the basal flux period, either carbachol (CCH; 100 μM), a cholinergic agonist that transiently increases cellular free Ca2+, or FSK (10 μM), an adenylyl cyclase inhibitor that increases cellular cAMP levels, was added to the serosal bath. In additional experiments, the effects of mucosal Ba2+ (a nonspecific K+ channel blocker), TRAM-34 (a specific IK channel blocker; 10 μM), and iberiotoxin (IbTX; a BK channel blocker; 100 nM) were examined on the changes in Ion, G, and K+ fluxes induced by CCH and FSK. The K+ fluxes are presented as microequivalents per hour per centimeter squared, while Ion are presented as nanoamperes per centimeter squared. G values are presented as mSiemens (mS).

**Western blots.** Western blot analyses were performed on epithelial cell homogenates of distal colon using anti-Kcnm4-abc (4), BK α-subunit (MaxiKo; Santa Cruz Biotechnology, Hercules, CA), and actin (Santa Cruz Biotechnology) antibodies, as described previously (31). Epithelial cells suspended (1.20) in ice-cold lysis buffer [50 mM Tris, pH 8.0, 0.5% SDS, 1 mM PMSF, 4 mM EDTA, and 1 tablet of protease inhibitor (Roche Applied Science, Indianapolis, IN)] were homogenized with a tight fit Teflon homogenizer. The homogenate was centrifuged (15 min at 2,000 g), and 16-μl aliquots of supernatant were mixed with equal volumes of Laemmli buffer and heated at 95°C for 5 min. Heat-treated aliquots were immediately placed in liquid nitrogen and stored at −80°C. Frozen samples were heated at 40°C for 1–2 min, and 10-μl samples (20 μg protein) resolved on 14% polyacrylamide gels were transferred onto nitrocellulose membranes. Blots were incubated with primary antibodies [Kcnm1α (1:400); anti-Kcnm4-abc (1:3,000); and β-actin (1:2,500)] and then with horseradish peroxidase-conjugated goat anti-rabbit IgG, and immune complexes were detected using enhanced chemiluminescence (GE Healthcare, Buckinghamshire, UK). The stripped blots were processed with anti-actin antibody and horseradish peroxidase-conjugated donkey anti-mouse IgG, and immune complexes were detected using enhanced chemiluminescence (GE Healthcare, Buckinghamshire, UK). The stripped blots were processed with anti-actin antibody and horseradish peroxidase-conjugated donkey anti-mouse IgG, and immune complexes were detected using enhanced chemiluminescence (GE Healthcare, Buckinghamshire, UK). The stripped blots were processed with anti-actin antibody and horseradish peroxidase-conjugated donkey anti-mouse IgG, and immune complexes were detected using enhanced chemiluminescence (GE Healthcare, Buckinghamshire, UK). The stripped blots were processed with anti-actin antibody and horseradish peroxidase-conjugated donkey anti-mouse IgG, and immune complexes were detected using enhanced chemiluminescence (GE Healthcare, Buckinghamshire, UK). The stripped blots were processed with anti-actin antibody and horseradish peroxidase-conjugated donkey anti-mouse IgG, and immune complexes were detected using enhanced chemiluminescence (GE Healthcare, Buckinghamshire, UK).
IbTX (100 μM in Ringer solution; Sigma-Aldrich), and charybdoxin (20 μM in Ringer solution; Sigma-Aldrich), and amiloride (20 mM in methanol, Sigma-Aldrich), TRAM-34 (50 mM in DMSO; Tocris Bioscience, Ellisville, MO), and dotoxin (20 μM in Ringer solution; Sigma-Aldrich). All other molecular grade chemicals used were purchased from Sigma-Aldrich.

Statistics. Results represent means ± SE of six tissue pairs from six rats. Statistical analyses were performed using unpaired or paired Students’ t-test or Bonferroni’s one-way ANOVA post hoc test using OriginPro 8.0 (OriginLab, Northampton, MA). P < 0.05 was considered to be statistically significant.

Stock solutions. Stock solutions were DSS (5% in water; MP Biomedical), amiloride (20 mM in methanol, Sigma-Aldrich), TRAM-34 (50 mM in DMSO; Tocris Bioscience, Ellisville, MO), IbTX (100 μM in Ringer solution; Sigma-Aldrich), and charybdoxin (20 μM in Ringer solution; Tocris Bioscience, Ellisville, MO). All other molecular grade chemicals used were purchased from Sigma-Aldrich.

RESULTS

Colonic histology and proinflammatory cytokines. Control distal colon and the inflammatory response in DSS-treated distal colon were evaluated histologically and by assay of proinflammatory cytokines (Fig. 1 and Table 1). Control distal colon exhibited straight crypts and an intact surface epithelium, without any inflammatory cells in the lamina propria (Fig. 1A). By contrast, in DSS-treated distal colon, there was loss and distortion of the crypts (Fig. 1B), crypt abscess (Fig. 1C and D), and an excess of inflammatory cells within the lamina propria (Fig. 1D), these being the typical histological features of human UC (9). Levels of the proinflammatory cytokines TNF-α, IL-1β, IL-6, and INF-γ were significantly increased in epithelial cells from DSS-treated distal colon (Table 1). These findings are similar to those previously reported in the mouse model of chronic UC induced by DSS treatment (1) and establish our experimental model of rat colitis as one suitable for ion transport studies in this disease.

Luminal stool and water contents. Bloody and often profuse diarrhea is the commonest symptom in patients with active UC (13, 27). To establish whether the luminal water content was increased in DSS-treated rat colon, the luminal stool and water contents in distal colon were measured. The wet weight of the luminal contents from DSS-treated distal colon was 2.2-fold greater than that from control distal colon (control vs. DSS-treated colon: 0.88 ± 0.10 vs. 1.95 ± 0.18 g/colon; P < 0.001). By contrast, the dry weights of the luminal contents were identical in DSS-treated and control distal colon (Fig. 2B). The greater wet weight in DSS-treated distal colon reflected a 3.3-fold increase in the weight of luminal water in DSS-treated colon (1.47 ± 0.17 g/colon) compared with that in controls (0.45 ± 0.07 g/colon; P < 0.001; Fig. 2C). The increased luminal water content in DSS-treated rat distal colon is perhaps not surprising given that the animals develop diarrhea, and most likely reflects enhanced water secretion and/or inhibition of water absorption.

Table 1. Proinflammatory cytokine levels in control and DSS-treated rat distal colon

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Control, pg/mg protein</th>
<th>DSS treated, pg/mg protein</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>10.5 ± 0.01</td>
<td>17.7 ± 0.6</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>IL-1β</td>
<td>48.2 ± 2.3</td>
<td>106.8 ± 0.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-6 (GRO/KC)</td>
<td>76.2 ± 1.4</td>
<td>178.9 ± 0.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>INF-γ</td>
<td>51.8 ± 2.3</td>
<td>175.8 ± 2.2</td>
<td>&lt;0.001</td>
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DSS, dextran sulfate sodium.
BK channel expression. It has been suggested that enhanced apical BK channel expression in human UC may underlie increased colonic K⁺ secretion, resulting in excessive fecal K⁺ losses in some patients with severe disease (28). We therefore evaluated apical BK channel expression in DSS-treated distal colon by Western blot analyses. In keeping with previous studies in human UC (28), BKα-subunit protein expression was enhanced approximately threefold in plasma membranes from DSS-treated distal colon (Fig. 3, A and C). Since IK channels are also present in rat distal colon (4), Western blot analyses were performed using Kcnn4a-b antibody, which detects apical (37 kDa) and basolateral (40 kDa) IK channel proteins (4). As shown in Fig. 3, B and D, neither apical nor basolateral IK channel expression was altered in DSS-treated distal colon. In additional studies, acute colitis induced by just 1 wk of 5% DSS treatment had no effect on either BKα-subunit or IK protein expression in the distal colon (data not shown).

We next determined whether the increased BKα-subunit protein expression in DSS-treated distal colon occurred at a transcriptional or posttranscriptional level by measuring BKα-subunit mRNA abundance in colonic epithelial cells. RT-PCR analyses indicated that BKα-subunit mRNA abundance was enhanced approximately sixfold in DSS-treated distal colon (Fig. 4A), which suggests that increased BK channel expression in DSS-treated distal colon reflects regulation at the level of transcription. By contrast, in the case of IK channels, the abundances of both apical and basolateral IK channel-
specific mRNAs were significantly increased in DSS-treated distal colon (Fig. 4, B and C), despite there being no changes in IK channel protein expression (Fig. 3, B and D).

**Ion transport studies.** Ion flux studies were performed to identify whether Ca$$^{2+}$$- or cAMP-induced Cl$$^-$$ secretion, which drive fluid secretion and/or K$$^+$$ secretion, is altered in DSS-treated distal colon. We avoided epithelial sodium channel (ENaC)-related $I_{sc}$ and interference by apical H-K-ATPase-mediated active K$$^+$$ absorption by measuring $I_{sc}$ and K$$^+$$ fluxes in the mucosal presence of 0.1 mM amiloride (to inhibit ENaC) and 1 mM VO$_4$ (to inhibit apical H-K-ATPase; Ref. 6). Control distal colon exhibited a positive $I_{sc}$, reflecting a mucosa-negative transepithelial membrane potential under basal condition (Fig. 5A). However, in DSS-treated distal colon, there was a negative $I_{sc}$, reflecting a mucosa-positive transepithelial membrane potential, and possibly K$$^+$$ secretion (Fig. 5B). This observation differs from other studies, which showed a marked decrease in the mucosa-negative transepithelial membrane potential (but not a mucosa-positive transepithelial membrane potential) in a DSS-induced model of acute colitis in mouse colon (24). This disparity may reflect a species difference, a technical difference (stripped mucosa vs. whole intestine), or the different chronicities of the colitis.

**Electrogenic Cl$$^-$$ secretion.** In control distal colon, serosal CCH (100 μM) that elicits a transient increase in intracellular free Ca$$^{2+}$$ enhanced the $I_{sc}$, reflecting stimulation of electrogenic Cl$$^-$$ secretion (Fig. 5A). In DSS-treated distal colon, CCH had no effect on $I_{sc}$ (Fig. 5B), and a similar failure of CCH to stimulate Cl$$^-$$ secretion has been reported in the mouse model of DSS-induced colitis (14, 24). The absence of CCH-induced Cl$$^-$$ secretion suggests that either the Ca$$^{2+}$$-dependent Cl$$^-$$ channel (CaCC) and/or the Ca$$^{2+}$$-dependent K$$^+$$ channels that provide the driving force for electrogenic Cl$$^-$$ secretion, or the protein kinase C (PKC) signaling pathway, may be impaired in DSS-treated distal colon.

cAMP-dependent Cl$$^-$$ secretion was also examined in control and DSS-treated distal colon. In control distal colon, serosal FSK stimulated electrogenic Cl$$^-$$ secretion, as previously described (29), and subsequent addition of CCH further enhanced the Cl$$^-$$ secretory response (Fig. 6A). In DSS-treated distal colon (Fig. 6B), CCH did not enhance Cl$$^-$$ secretion, possibly due to impaired Cl$$^-$$ secretion or absence of electrogenic Cl$$^-$$ secretion. This observation was confirmed by measuring the mRNA of Cl$$^-$$ channel genes (Fig. 6C).

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**Fig. 4.** Large conductance K$$^+$$ (BK$$^\alpha$$) and intermediate conductance K$$^+$$ (IK; Kcnn4b and Kcnn4c) channel-specific mRNA abundance in control and DSS-treated rat distal colon. BK$$^\alpha$$ (A), Kcnn4b (B), and Kcnn4c (C)-specific mRNA from control (open bars) and DSS-treated (closed bars) rats quantified by RT-PCR. Data represent means ± SE values from 3 different animals in both groups. *$P < 0.001$, compared with control; **$P < 0.001$, compared with control.

**Fig. 5.** Effect of carbachol (CCH) on $I_{sc}$ and net K$$^+$$ fluxes in control and DSS-treated rat distal colon. Time course of short-circuit current ($I_{sc}$; A and B) and net K$$^+$$ fluxes (C and D) before and after serosal addition of CCH (50 μM) under voltage-clamp conditions in control and DSS-treated rat distal colon. Net K$$^+$$ fluxes were measured in the absence (open bars) and presence (closed bars) of CCH. *$P < 0.001$, compared with basal value in controls; **$P < 0.001$ compared with basal value in controls.
distal colon, cAMP-induced Cl− secretion was reduced by 80%, while the addition of CCH had no further effect (Fig. 6B).

Electrogenic K+ secretion. In parallel experiments, net K+ fluxes were measured in control and DSS-treated distal colon (Fig. 5, C and D). In controls, net K+ flux was zero under basal condition (in presence of mucosal VO4), while CCH stimulated active K+ secretion (basal 0.02 ± 0.04 μeq h−1 cm−2 vs. CCH −0.12 ± 0.05 μeq h−1 cm−2; P < 0.001; Fig. 5C). By contrast, in DSS-treated colon, there was significant active K+ secretion under basal conditions (−0.42 ± 0.06 μeq h−1 cm−2) compared with controls (0.02 ± 0. μeq h−1 cm−2; P < 0.001), although CCH did not elicit any additional K+ secretory response. As shown in Fig. 6C, FSK induced active K+ secretion in control distal colon, but subsequent addition of CCH did not alter FSK-induced K+ secretion (Fig. 6C), despite enhancing FSK-enhanced Cl− secretion (Fig. 6A). In DSS-treated distal colon, active K+ secretion was present under basal conditions, but neither cAMP nor CCH had any additional effect (Fig. 6D). These observations indicate that up-regulation of BK channel expression in DSS-treated distal colon (Fig. 3, A and C) is associated with a substantial amount of active K+ secretion, which is insensitive to FSK and CCH.

A variety of K+ channel blockers were used to identify the different types of K+ channel that might be involved in the active K+ secretory process in DSS-treated distal colon. Active K+ secretion was inhibited completely by Ba2+ (a nonspecific K+ channel blocker), inhibited 25% by TRAM-34 (an IK channel blocker), and inhibited 80% by IbTX (a BK channel blocker; Fig. 7), which suggests that active K+ secretion in DSS-treated distal colon is mediated primarily via apical BK channels.

Roles of aldosterone and NO. Aldosterone enhances apical BK channel expression and stimulates BK channel-mediated K+ secretion, while NO nitrosylates and activates BK channels expressed (15, 26, 31, 32). Based on previous studies, we envisaged that in DSS-treated rats impaired colonic Na+ absorption might result in raised serum aldosterone levels and that mucosal inflammation might increase NO concentrations in colonic epithelial cells (36, 38). We found that serum aldosterone and epithelial cell NO concentrations were enhanced by 5.8- and 2.7-fold, respectively, in DSS-treated rats compared with controls (Table 2). Increased serum aldosterone levels in DSS-treated rats were similar to those in rats fed a high-K+ diet but markedly lower than those in rats fed a Na+-free diet. These observations suggest that in DSS-treated rats aldosterone may have a role in the upregulation of BK

| Table 2. Serum aldosterone and epithelial cell nitric oxide concentrations in control and DSS-treated rat distal colon |
|-------------------------------------------------|-----------------|-----------------|
|aldosterone, pg/ml serum                        | Control         | DSS Treated     | P    |
|Total NO, pmol/mg protein                       | 239.8 ± 26.9    | 651.5 ± 85.2    | <0.001 |
|                                                | 236.2 ± 27.8    | 1,386.6 ± 208.2 | <0.001 |

NO, nitric oxide.
channel expression and aldosterone and/or NO may be involved in the active $K^+$ secretory process.

To further explore aldosterone-enhanced BK channel-mediated $K^+$ secretion, the effects of FSK on $I_{sc}$ and $K^+$ transport were studies in aldosterone-treated distal colon. As shown in Fig. 8A, before the addition of FSK, the negative $I_{sc}$ ($-50.8 \pm 5.6 \mu A/cm^2$) was consistent with $K^+$ secretion and the addition of FSK reversed the $I_{sc}$ to a positive value ($148 \pm 11.8 \mu A/cm^2$), reflecting stimulation of electrogenic Cl$^-$ secretion. Although net $K^+$ secretion was present under basal conditions in aldosterone-treated distal colon, as previously described (31), FSK did not further enhance $K^+$ secretion (Fig. 8B), a situation similar to that seen in DSS-treated distal colon (Fig. 5D). Taken together, these observations suggest that aldosterone may have a regulatory role in BK channel-mediated $K^+$ secretion in DSS-treated distal colon.

Given that previous studies have indicated that NO activates BK channels (26) and mucosal NO levels were significantly raised in DSS-treated distal colon (Table 2), we examined the effect of NO donor (DPTA-NONOate; a slow NO donor) on $I_{sc}$ and $K^+$ fluxes in control distal colon. The addition of DPTA-NONOate initially stimulated $I_{sc}$, which then declined to less than the basal value (Fig. 9A). The initial rise in $I_{sc}$ suggests that NO activated Cl$^-$ secretion, as reported previously (33), although in that study the effect of NO on $I_{sc}$ was followed for only 5 min. The decrease in $I_{sc}$ we observed at later time points after the addition of DPTA-NONOate is consistent with NO-stimulated active $K^+$ secretion (Fig. 9B). We also studied the effect of cAMP on NO-stimulated $I_{sc}$ and $K^+$ secretion, using 8-bromoadenosine $3',5'$-cyclic monophosphate (8BrcAMP) instead of FSK, since NO inhibits adenylate cyclase. 8BrcAMP in the continued presence of DPTA-NONOate did not affect $I_{sc}$ but further enhanced net $K^+$ secretion. The observation that 8BrcAMP further enhanced $K^+$ secretion already activated by NO suggests that NO and cAMP stimulate active $K^+$ secretion by nitrosylation and phosphorylation, respectively.

**DISCUSSION**

In the present study, administration of 5% DSS to rats for 5 alternate wk resulted in chronic active inflammation and an increase in the luminal water content in the distal colon, which demonstrated similar clinical and histological features to those seen in human UC. Recent studies have revealed enhanced colonic BK channel expression in human UC (28), which could conceivably result in increased colonic $K^+$ secretion (3, 12) and excessive fecal $K^+$ losses in this disease. We found that DSS-treated rat distal colon exhibited a significant level of active $K^+$ secretion, which was associated with a marked enhancement of BK channel expression. This conclusion is based on the following observations: 1) under basal conditions, a serosa-positive transepithelial potential difference was present in control distal colon, whereas a serosa-negative potential difference was present in DSS-treated distal colon, consistent with the induction of active $K^+$ secretion in this experimental model of colitis (Figs. 5 and 6); 2) mucosal IbTX (a BK channel blocker) inhibited active $K^+$ secretion in DSS-treated distal colon (Fig. 7); and 3) BK-specific mRNA abundance and protein expression in DSS-treated distal colon were increased six- and threefold, respectively, compared with the levels in control distal colon. Furthermore, BK channel activity stimu-
lates the transcription factor NF-κB, which upregulates proinflamma-
tory cytokine production (23). It has been suggested that
immune dysregulation has a pathogenic role in UC (30,
37), and in keeping with this notion, antibiotics inhibit the
colonic inflammatory response induced by DSS, which is
similar to that in patients with UC (22, 25).

Although the precise mechanism of BK channel upregula-
tion in DSS-induced colitis is unclear, we found in DSS-treated
animals that serum aldosterone and mucosal NO concentra-
tions were increased 6.9- and 2.7-fold, respectively, compared
with controls (Table 2). It is possible that hyperaldosteronism
occurred secondary to excessive fecal Na+ losses in DSS-
treated distal colon, since NHE3-mediated Na+ absorption is
inhibited in this experimental model of colitis (Nanda Kumar
NS, Binder HJ, Rajendran VM; unpublished observations),
and NHE3-mediated electroneutral Na+ absorption and ENaC-
mediated electrogenic Na+ absorption are both markedly
impaired or absent in patients with active UC (2, 34). Hyperal-
dosteronism has been shown to enhance BK channel expres-
sion and induces BK channel-mediated active K+ secretion in
mammalian colon (31, 32), and it is possible that the increases
in BK channel expression and net K+ secretion we observed in
DSS-treated distal colon reflected, at least in part, the increase
in serum aldosterone concentration. Hyperaldosteronism sec-
ondary to feeding either a high-K+ diet or a Na+-free diet
increase BK channel expression and induce net K+ secretion in
rat distal colon (7, 20). However, it should be noted that dietary
K+ enrichment results in a modest increase in serum aldoste-
rone concentration, which is associated with net K+ secretion
alone, whereas dietary Na+ depletion results in a much higher
serum aldosterone concentration, which is associated with
stimulation of ENaC-mediated electrogenic Na+ absorption, in
addition to net K+ secretion (7, 11, 20, 31). Interestingly,
serum aldosterone concentrations in the DSS-treated animals
were similar to those reported in rats fed a high-K+ diet (20),
and both cases exhibit an increase in K+ secretion without any
change in ENaC-mediated Na+ absorption.

The total NO concentration in cells from DSS-treated distal
colon was substantially higher than in control distal colon
(Table 2), raising the possibility that NO may have a role in K+
secretion in this model of colitis. In addition to our observation
that the NO donor NONOate stimulated K+ secretion in
control distal colon (Fig. 9), NO has been shown to activate BK
channels directly by nitrosylation, and indirectly by cGMP-
dependent phosphorylation (8, 15, 19). NO also increases the
number of active BK channels in colonic myocyte membranes
(16). However, it should be noted that net K+ secretion in
distal colon from ald-o-treated animals (Fig. 8B) was 12-fold
greater than net K+ secretion stimulated by NO in distal colon
from control animals (Fig. 9B), which suggests that aldosterone
and NO stimulate colonic K+ secretion by different mecha-
nisms.

BK channels consist of a pore-forming α-subunit and a
regulatory β-subunit. Two characteristically distinct splice
variants of the BKα-subunit are present in mammalian colon,
a 58 amino acid peptide that is present in the COOH terminus
of the STREX variant being absent from the ZERO variant.
cAMP inhibits the STREX variant but activates the ZERO
variant. RT-PCR analyses and IbTX-inhibition of cAMP-stimu-
lated K+ secretion have confirmed that ZERO transcripts
encode apical BK channels in rat and human colon (unpub-
lished observations) (5), and the BK α-subunit protein has been
localized to the apical membrane of colonic epithelial cells
using immunofluorescence (28, 32, 40). The addition of cAMP
had no further stimulatory effect on aldosterone-induced K+
secretion (Fig. 8B), possibly because the BK channels were
already maximally activated. However, cAMP elicited a sig-
ificant increase in NO-induced K+ secretion (Fig. 9B), which
most likely reflected the additive effect of phosphorylation and
nitrosylation on BK channel activity. This leads us to speculate
that in both human UC and DSS-induced colitis a combination
of raised intracellular concentrations of NO and cAMP (sec-
ondary to raised mucosal prostaglandin E2 concentrations)
may result in sufficient BK channel activity to sustain K+ secretion
across the inflamed colonic mucosa.

In summary, we have demonstrated that a significant level of
active K+ secretion occurs in DSS-induced colitis and this is
mediated by upregulated apical BK channels. Furthermore,
increases in serum aldosterone and/or mucosal NO concentra-
tions may enhance BK channel expression/activity as part of
the K+ secretory process. Based on these findings, we specu-
late that similar changes in human UC may account for the
excessive fecal K+ losses and hypokalemia that sometimes
occur in patients with this disease.

GRANTS

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

Author contributions: B.M.K. and V.M.R. performed experiments; B.M.K.,
G.I.S., and V.M.R. analyzed data; G.I.S. and V.M.R. interpreted results of
experiments; G.I.S. and V.M.R. edited and revised manuscript; V.M.R. prepared
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K⁺ SECRETION IN DSS-INDUCED COLITIS


