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Involvement of inwardly rectifying K^+ channels in secretory responses of human ileal mucosa

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Abstract

In acute secretory diarrhoea the primary event driving fluid secretion is a transcellular, electrogenic, serosal to mucosal transport of chloride ions. Such transport requires the maintenance of an electrically negative cell membrane voltage, which is achieved through a basolateral outward leakage of potassium ions. The aim of this study was to investigate the nature of K^+ channel involvement in facilitating secretory processes in the human ileum. Muscle-stripped mucosal preparations of human ileal mucosa were set up in Ussing chambers for recording short-circuit current and transmucosal conductance. *Escherichia coli* heat-stable toxin and vasoactive intestinal peptide (VIP) produced concentration-dependent increases in short-circuit current. Responses to the heat-stable toxin were unaffected by basolateral application of 4-aminopyridine (5 mM), glibenclamide (10 μM) or a combination of charybdotoxin (0.3 μM) plus apamin (0.3 μM). However, basolateral barium (0.2–5 mM) caused a concentration-dependent inhibition. Responses to VIP were similarly affected by barium (0.05–1 mM). These results suggested that electrogenic chloride transport by human ileal mucosa required the presence of basolateral K^+ channels. The use of selective K^+-channel inhibitors and low concentrations of barium suggested that the channels involved might be of the inwardly rectifying type.

Introduction

Basolateral potassium channels play an important role in secretory processes of enterocytes that line the intestinal lumen. The mechanism responsible for the secretion of chloride relies on the potential difference across the apical membrane. As the efflux of accumulated chloride from the cell via apical chloride channels is an electrogenic process, it depolarizes the apical membrane, so reducing the driving force for continued chloride exit and attenuating the secretory process. The opening of potassium channels hyperpolarizes the basolateral membrane and since there is an electrical continuity between apical and basolateral membranes via the paracellular pathway, the potential difference across the apical membrane is restored so that chloride secretion is maintained (Field et al 1989; Dawson & Richards 1990).

Evidence for secretagogue activated basolateral potassium (K^+) channels in mammalian intestinal epithelial cells (enterocytes) came initially from work on cultured T_{84} cells (Dharmsathaphorn & Pandol 1986) and intact mucosal sheets of rat ileum (Hardcastle & Hardcastle 1986). Direct evidence of K^+ channels on basolateral membranes of enterocytes has been obtained from patch clamping T_{84} cells and isolated human colonic crypts (Devor & Frizzell 1993; Sandle et al 1994). Additionally, Epple et al (2001) have shown that loperamide inhibits a basolateral K^+ conductance in colon epithelial cells. It was this action rather than stimulation of opiate receptors that resulted in inhibition of secretory effects produced by forskolin, carbachol and the phorbol ester phenylmercuric acetate (PMA). Those investigations indicate that K^+-channel inhibition could reduce chloride secretion and thus provide new opportunities for treatment of secretory diarrhoeal disease, and although basolateral K^+ channels are involved in active absorption of nutrients (Brown & Sepúlveda 1985), it appears that sodium coupled glucose absorption, upon which oral rehydration therapy

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depends, is far less sensitive to barium than chloride secretion (Burleigh 1998). To date only limited information is available on basolateral K^+ channels in human small intestinal enterocytes (Brzuszczak et al 1996) and this region of the intestine is the site of action of two major bacterial enterotoxins: Cholera toxin, which acts partly through the release of vasoactive intestinal peptide (VIP; Cassuto et al (1981)) and *Escherichia coli* heat-stable enterotoxin. The aim of this invesigation was to determine whether inhibition of K^+ channels in human ileal enterocytes had anti-secretory potential and additionally to pharmacologically characterize, using selective K^+-channel inhibitors and low concentrations of barium, the nature of the K^+ channel involved.

Materials and Methods

Setting up of mucosal preparations

Segments of human terminal ileum (taken within 20 cm of the ileocaecal junction) were obtained from thirty-five specimens of bowel resected at right hemicolectomy operations for carcinoma of caecum (n = 20) or carcinoma of ascending colon (n = 15). The procedure was approved by the Research Ethics Committee of the City and East London Health Authority. Eighteen patients were male and seventeen female, the average age was 69 years. Tissue was transported in Dulbecco's Modified Eagle's Medium plus Hams F-12 medium (1:1, v/v) with 10% foetal bovine serum added. Preparations were set up as described by Burleigh & Borman (1997). Briefly segments were opened longitudinally and pinned out, mucosa downwards, on a silastic mat in gassed (5% CO_2 in O_2) Krebs buffer, within 60 min of removal from the patient. Sheets of mucosa complete with submucosa were then prepared by removal of the outer muscle layers using sharp dissection, and mounted as a flat sheet in Ussing chambers (window area 0.64 cm^2) bathed either side by 10 mL of circulating, gassed Krebs buffer kept at 37 °C. Up to six individual preparations could be obtained from a specimen of resected bowel. To avoid activating electrogenic Na^+ absorption glucose was omitted from the Krebs buffer bathing the mucosal side of the intestine and replaced by an equimolar concentration of mannitol. The tissue was clamped at zero potential by a high impedance voltage clamp (DVC-1000, World-Precision Instruments) and transmural short-circuit current (Isc) was measured and continuously recorded. Using Ohm's law tissue conductance was calculated from the change in Isc when tissues were intermittently clamped at 2 mV for 20 s. Both current passing and voltage detecting electrodes utilized a system of silver/silver chloride half cells connected to large diameter (1-mL syringe barrels) agar bridges (4% agar in modified Krebs buffer, that is minus calcium and glucose). Krebs buffer contained (in mM): NaCl 118, $NaHCO_3$ 25, KCl 4.7, $MgCl_2$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5 and D-(+)-glucose or D-(+)–mannitol both at 11.5. $MgCl_2$ was substituted for $MgSO_4$ to avoid precipitation of barium.

Chemicals

4-Aminopyridine, apamin, barium chloride, charybdotoxin, dimethyl sulfoxide (DMSO), *Eschericia coli* heat-stable toxin (Sta), mannitol and vasoactive intestinal peptide (VIP) were purchased from Sigma. All other agents were of analytical grade. Toxins and VIP were dissolved in ultra high purity (UHP) water, and samples were frozen. Glibenclamide was dissolved in DMSO (final concentration 0.1%).

Statistical analysis

All data are expressed as arithmetic mean ± s.e.m., n = number of resected specimens of intestine. Statistical comparisons used Wilcoxon Matched-Pairs Signed Ranks test with $P < 0.05$ being taken to represent a significant difference.

Results

Short-circuit current response to *E. coli* heat-stable toxin and VIP

After 60-min equilibration, basal short-circuit current (Isc) was 46.0 ± 2.6 $\mu A\,cm^{-2}$ and tissue conductance was 15.5 ± 0.9 millisiemens cm^{-2} (n = 35). Mucosal (apical) application of heat-stable toxin (0.15–15 nM, n = 4) and serosal (basolateral) application of VIP (0.003–1.1 μM, n = 3) produced concentration-dependent increases in Isc of 2.5 ± 1.0 to 50.4 ± 8.1 and 29.2 ± 8.4 to 106.7 ± 11.0 $\mu A.cm^{-2}$, respectively (Figure 1).

Effect of K^+ channel inhibition on responses of mucosa to *E. coli* heat-stable toxin and VIP

Desensitization to *E. coli* heat-stable toxin did not occur. Pilot experiments with VIP showed a degree of desensitization which was minimized by adopting the following

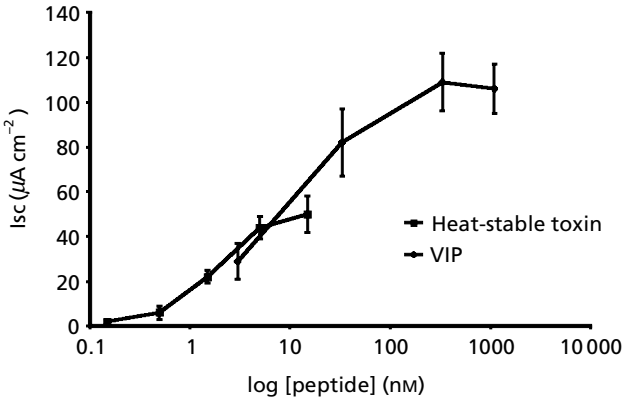

Figure 1 Effect of *Escherichia coli* heat-stable toxin and vasoactive intestinal peptide (VIP) on short-circuit current (Isc) of human ileal mucosal preparations. Each point represents mean ± s.e.m. from n = 4 (*E. coli* heat-stable toxin) or n = 3 (VIP) resected specimens of ileum.

procedures: using sub-maximal effective concentrations; repeated washing (i.e. four replacements of Ussing reservoir fluid) of preparations immediately after response had plateaued; and allowing sufficient time between first and second additions of VIP (110–120 min). All K^+-channel inhibitors were added to the serosal domain. Following equilibration, a single concentration of heat-stable toxin (5 nM) or VIP (10 nM) was given to the mucosal or serosal domain, respectively. After washout of the secretagogue and return of short-circuit current to basal levels, antagonist or vehicle were added for 30 min before reapplication of heat-stable toxin or VIP. At high concentrations barium (>1 mM) did increase basal Isc as did 4-aminopyridine (5 mM). Neither had an effect on tissue conductance. Responses to *E. coli* heat-stable toxin were not significantly reduced in the presence of H_2O (100 μL, n = 21, $P > 0.05$) or DMSO (10 μL, n = 9, $P > 0.05$, Figure 2). Responses to VIP were also unaffected by H_2O (100 μL, n = 13, $P > 0.05$, Figure 3). Barium (0.2–5 mM) exerted a concentration-dependent inhibition of short-circuit current responses to heat-stable toxin (n = 6–12, $P < 0.05$, Figure 2). Barium (0.05–1 mM) also inhibited responses to VIP in a concentration-dependent fashion (n = 6–8, $P < 0.05$, Figure 3). Glibenclamide (10 μM, n = 9), 4-aminopyridine (5 mM, n = 6) and a combination of charybdotoxin plus apamin (each 0.3 μM, n = 9) were all without effect on responses to *E. coli* heat-stable toxin ($P > 0.05$, Figure 2).

Discussion

Short-circuit current responses of human ileum to *E. coli* heat-stable toxin were quantitatively similar to those previously obtained from human jejunum (Kuhn et al 1994). Those authors concluded that the toxin increased electrogenic chloride secretion across human isolated jejunal mucosa by stimulation of cGMP production. VIP also produced chloride secretion by human jejunum when the peptide was infused into healthy volunteers. The secretion was active as it occurred against electrical and chemical gradients (Krejs et al 1980). Chloride secretion across the apical membrane of enterocytes is an electrogenic process. It requires an outward conductance of K^+ ions across the basolateral membrane to maintain the electrical driving force for Cl^- ion exit (Dawson & Richards 1990). The short-circuit current technique detects changes in electrogenic transport of ions and has been used to indicate the functional consequences of K^+-channel inhibition

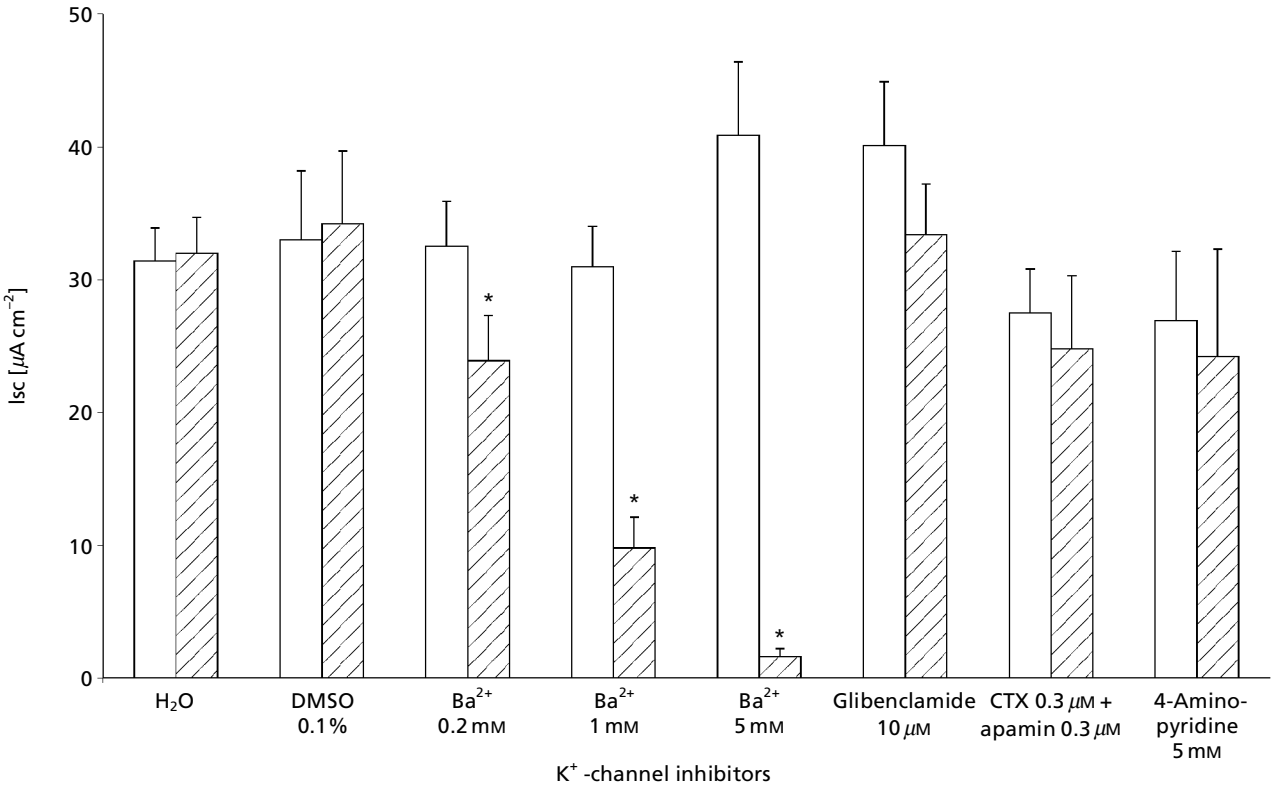

Figure 2 Effect of K^+-channel inhibitors on short-circuit current (Isc) responses of human ileal mucosa to *Escherichia coli* heat stable toxin (5 nM mucosally). Columns show paired responses of mucosa to heat-stable toxin before (open) and after (hatched) exposure to control vehicles (H_2O, or DMSO for glibenclamide) or K^+-channel inhibitors (CTX, charybdotoxin). Bars represent means ± s.e.m. from n ≥ 6 resected specimens of ileum. *$P < 0.05$ between second paired response obtained in presence of barium compared with first paired response obtained in absence of the blocker.

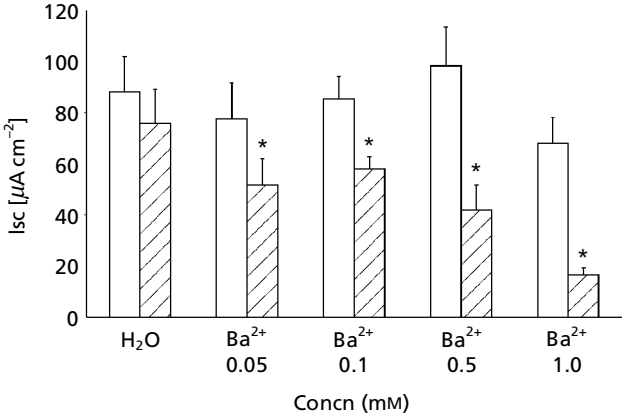

Figure 3 Effect of barium on short-circuit current (Isc) responses of human ileal mucosa to vasoactive intestinal peptide (VIP, 10 nM serosally). Columns show paired responses of mucosa to VIP before (open) and after (hatched) exposure to H_2O or Ba^{2+}. Bars represent means ± s.e.m. from $n \geq 6$ resected specimens of ileum. $^*P < 0.05$ between second paired response obtained in presence of barium compared with first paired response obtained in the absence of the blocker.

(Dharmsathaphorn & Pandol 1986). High concentrations of barium (above 1 mM) have been reported to block K^+ currents through the following K^+ channels: K_M, K_A, K_{IR}, K_V, BK_{CA}, IK_{CA} SK_{CA}, K_{ATP}, K_{ACH} and $K_{5\text{-}HT}$ (Cook & Quast 1990). The choice of a non-selective K^+-channel blocker was necessary to establish whether a K^+ channel of any type played a role in secretory responses of human ileal enterocytes to *E. coli* heat-stable toxin and VIP. The inhibitory actions of barium prompted the use of more selective K^+-channel inhibitors. The lack of effect of 4-aminopyridine, a combination of charybdotoxin plus apamin (shown to be more effective than giving the inhibitors separately, Zygmunt & Hogestätt 1996) or of glibenclamide indicated that the K^+ channels facilitating Cl^- ion secretion were not voltage activated (Cook & Quast 1990), calcium activated (Cook & Quast 1990) or ATP-sensitive (Ashford 1990).

Re-evaluation of the actions of barium showed that significant inhibition of *E. coli* heat-stable toxin or VIP-induced ion secretion could be obtained by relatively low concentrations of barium i.e. less than 1 mM. In low concentrations barium is considered to be the most effective and selective extracellular inhibitor of inwardly rectifying K^+ channels of arteriolar smooth muscle. For arteriolar smooth muscle half block of K_V channels requires millimolar concentrations, for K_{Ca} channels there is little block even at 10 mM. K_{ATP} channels are more sensitive to barium, half block occurring at 100 μM (Nelson & Quayle 1995), however the fact that glibenclamide was without effect in human ileal enterocytes demonstrated that these channels were not required for the secretagogues action. Apart from arteriolar smooth muscle barium blocks K_{IR} channels in human lung cancer cells (5 μM–5 mM (Sakai et al 2002)) and bovine endothelial cells (500 μM (Pasyk et al 1992)).

The inward rectifying potassium channel is activated by hyperpolarization and passes an inward current at membrane potentials negative to E_K. However, under more normal physiological conditions i.e. at membrane potentials less negative than the equilibrium potential for K^+, they can pass outward K^+ current and may serve to stabilize the membrane potential (Bolton & Beech 1992). Such outward currents would also hyperpolarize the cell thus providing the driving force for chloride ion secretion through the apical membrane. It appears that the basolateral K^+ channels on human ileal enterocytes, which play a role in chloride ion secretion, are less sensitive to barium than K_{IR} channels found on arteriolar smooth muscle cells. Inhibition of K_{IR} channels by barium is greater at more negative membrane potentials (Nelson & Quayle 1995) and in human ileal enterocytes, where chloride ion secretion is depolarizing the cell, there is reduced sensitivity to barium. However, whether this would explain a tenfold difference in sensitivity is debatable. A more probable explanation might lie in the nature of the channel itself. Like other K_{IR} channels the G protein gated K^+ channel (K_g) is effectively blocked by extracellular barium, but at concentrations approximately ten times those required to block the classical K_{IR} channels (Yamada et al 1998).

In conclusion this investigation, utilizing intact ileal mucosa from the human small intestine, has demonstrated that K^+-channel inhibition reduced short-circuit current responses of human ileal mucosa to *E. coli* heat-stable toxin and VIP. Selective pharmacological inhibition of K_V, K_{Ca} and K_{ATP} channels did not reduce responses to the heat-stable toxin, while sub-millimolar concentrations of barium inhibited both *E. coli* heat-stable toxin and VIP. It is likely that an inwardly rectifying potassium channel played an important role in maintaining electrogenic chloride secretion produced by *E. coli* heat-stable toxin and VIP in the human ileum. In the absence of any safe, effective anti-secretory therapy this investigation demonstrated that selective inhibition of K^+ channels may represent a potential target for development of anti-secretory drugs.

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