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Berberine inhibits ion transport in human colonic epithelia

Cormac T. Taylor [a], Des C. Winter [b,c], Maeve M. Skelly [a,d], Diarmuid P. O'Donoghue [d], Gerald C. O'Sullivan [b], Brian J. Harvey [c], Alan W. Baird [a,*]

[a] *Department of Pharmacology, University College Dublin, Belfield, Dublin 4, Ireland*
[b] *Department of Surgery, University College Cork, Cork, Ireland*
[c] *Department of Physiology, University College Cork, Cork, Ireland*
[d] *Division of Gastroenterology, St. Vincent's Hospital, Dublin 4, Ireland*

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Abstract

The effects of berberine on ion transport in both human colonic mucosal epithelia and an intestinal epithelial cell line (T84) were examined. Berberine (concentration range 0–500 μM) reduced both basal and stimulated ion transport responses in human colonic mucosae in a manner which was non-specific for Ca^{2+}- or cAMP-mediated signals. Similarly, in cultured intestinal epithelial monolayers, berberine inhibited Ca^{2+}- and cAMP-mediated responses indicating an inhibitory activity directly at the level of the epithelium rather than an indirect effect through other mucosal element(s). Berberine did not alter the rate of generation of cAMP by adenylyl cyclase or the activity of protein kinase A, the effector enzyme of the cAMP pathway. Berberine inhibited carbachol-stimulated $^{86}Rb^+$ efflux from T84 monolayers. Berberine also inhibited K^+ conductance in apically-permeabilised re-sected mucosae. These results indicate i) that berberine exerts an anti-secretory action directly upon epithelial cells and ii) the mechanism of action may be at the level of blockade of K^+ channels. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Berberine; Epithelium; Ion transport; K^+ channel

1. Introduction

In cases of secretory diarrhoea, fluid loss may occur due to transepithelial osmotic gradients established as a consequence of excessive transepithelial Cl^- secretion by intestinal epithelial cells (Donowitz et al., 1986; Field et al., 1989). Chloride ions enter epithelial cells across the basolateral membrane and exit across the apical membrane. This transcellular movement of negative chloride ions is followed by the paracellular movement of positive Na^+ ions and thus, transepithelial electrical equilibrium is sustained. In order to maintain an electrically favourable intracellular environment to sustain Cl^- secretion, K^+ is recycled at the basolateral membrane, an event which provides the electrochemical driving force for Cl^- secretion (Barrett, 1993; Reenstra, 1993).

Berberine is a plant alkaloid which has been used in traditional Eastern medicine for over 2 millennia in the treatment of gastroenteritis and secretory diarrhoea. In several small scale clinical trials, berberine has been effective in the treatment of secretory diarrhoea (Lahiri and Dutta, 1967; Chauhan et al., 1970; Sharda, 1970; Sharma et al., 1970; Tang and Eisenbrand, 1992). Although its therapeutic benefit has been attributed in part to anti-microbial (Iwasa et al., 1998) and anti-motility (Yamamoto et al., 1993) actions, berberine has been shown to prevent epithelial electrolyte secretion in vitro in rabbit (Guandalini et al., 1987) and rat (Tai et al., 1981; Taylor and Baird, 1995) intestine. However the mechanism of anti-secretory action of berberine remains to be elucidated.

In this investigation, we examined the actions of berberine in a human model of intestinal ion transport. Using electrophysiological techniques, berberine was found to be an inhibitor of ion transport in human colon in vitro. This inhibitory effect may be an important component in berberine's overall anti-diarrhoeal activity. A reductionist approach was then adopted to investigate the effects of berberine in cultured monolayers of human colonic epithelial cells. Using T84 epithelial monolayers, berberine was found to have an anti-secretory action directly at the level of the intestinal epithelium. Biochemical techniques were

* Corresponding author. Tel.: +353-1-706-1557; Fax: +353-1-269-2749; E-mail: abaird@macollamh.ucd.ie

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then employed to determine whether the action of berberine was at a level distal to second messenger (cAMP) generation or activation of the effector enzyme protein kinase A. Finally, using an isotope efflux assay and nystatin-permeabilised human colonic mucosae, evidence was obtained to suggest that berberine may act through inhibition of basolaterally located K^+ channels, the functioning of which is essential for Cl^- secretion regardless of stimulus.

2. Materials and methods

2.1. Chemicals

Berberine, carbachol, dibutyryl-cyclic AMP, nystatin, forskolin, protein kinase inhibitor (type III) and kemptide were purchased from Sigma Chemical, UK Ionophore A23187 was from Research Biochemicals International (Natick, MA, USA), ^{32}P labelled ATP and $^{86}Rb^+$ were obtained from Amersham International, UK, and T84 cells were from American Type Culture Collection (Rockville, MD, USA). Tissue culture media, supplements and trypsin were all obtained from Gibco, UK Stock solutions were prepared in the appropriate bathing solution. Control experiments were treated with the same volume of vehicle to which each test tissue was exposed.

2.2. Tissue preparation

Human tissue was obtained from patients undergoing colonic resection for adenocarcinoma as described previously (Stack et al., 1995). Segments of tissue were removed from an area marginal to the lesion and were shown to be histologically normal. Tissues were transported to the laboratory in Krebs–Hensleit solution where smooth muscle was removed by blunt dissection leaving a mucosal sheet consisting of an epithelial layer with attendant lamina propria. The composition of Krebs–Hensleit bathing solution was (in mM): NaCl (113), KCl (4.7), KH_2PO_4 (1.2), $MgSO_4 \cdot 7H_2O$ (1.2), $CaCl_2 \cdot 2H_2O$ (1.9), $NaHCO_3$ (25) and glucose (12.1). Ethical approval was obtained from the Ethics Committees of each of the Hospitals which participated in the studies.

2.3. Cell culture

T84 cells were grown to confluence on 75 cm^2 tissue culture flasks in a 1:1 mixture of Dulbecco modified Eagle's medium and Ham's F-12 medium, supplemented with foetal calf serum (10%), L-glutamine (1%), penicillin/streptomycin (1%) and non-essential amino acids (1%). Confluent monolayers were subcultured every 7–10 days using trypsin/EDTA (1 ×).

For ion transport studies, 10^6 T84 cells were plated on Snapwell® porous cell culture inserts (polycarbonate membrane, tissue culture treated, 12 mm diameter, 0.4 μm pore size from Costar (Cambridge, MA, USA)). Nutrient medium was provided to both apical and basolateral domains of the monolayers. After 10–14 days culture, confluent monolayers were mounted in specially modified Ussing chambers for ion transport measurement.

2.4. Short-circuit current measurement

Stripped human mucosae were mounted in Ussing chambers with a window area of 0.63 cm^2. T84 monolayers were mounted in specially modified chambers with an exposed area of 1.13 cm^2 (Snapwell®, Costar). Preparations were bathed on either side with 10 ml of Krebs–Hensleit solution recirculated in reservoirs maintained at 37°C. The solution was gassed with 95% O_2–5% CO_2 to maintain pH at 7.4. When mounted, each preparation was connected to a voltage clamp (DVC-1000; World Precision Instruments, Stevenage, Herts, UK) with current and voltage electrodes placed on either side. Preparations were clamped to zero potential difference and were allowed to equilibrate for 30–60 min. Short circuit current (SCC; $\mu A/cm^2$) was continuously monitored using a MacLab® digital to analogue data acquisition system (AD Instruments, Hastings, UK). In all cases, paired preparations of tissue were used.

Prior to permeabilisation with nystatin, the apical bath solution was changed to a nystatin bathing solution (NBS) that is designed to mimic the intracellular ionic milieu (high K^+, low permeant anion and low ionised Ca^{2+}). The NBS, equilibrated with air and with the pH corrected with KOH, contained (in mM): K gluconate (123), NaCl (17), $MgCl_2$ (3.0), KH_2PO_4 (1.2), K_2HPO_4 (2.9), glucose (11.0), *N*-[2-Hydroxyethyl]piperazine-*N*-[2-ethane-sulfonic acid] (HEPES) (6.0). The concentrations of $CaCl_2$ and EGTA for the desired free Ca^{2+} were determined with a software package (Chang et al., 1988). The apical membrane was treated with the ionophore nystatin (100 μg ml^{-1}) and the current allowed to reach a steady state. Where indicated, the K^+ gradient was altered by varying the apical bath K^+ concentration by replacement with sodium. Experiments were carried out at pCa 7 in order to approximate the 'normal' environment and to allow for agonist-induced increases in intracellular Ca^{2+} concentration. The basolateral side was bathed in a low Cl^-, bicarbonate-buffered solution (all but 23 mM Cl^- replaced by gluconate) such that there was an equal concentration of Cl^- on both sides of the epithelium. The K^+-dependent SCC following permeabilization of the apical membrane of human colonic mucosal epithelium is termed I_K.

2.5. Cyclic nucleotide assay

Segments of stripped epithelium (with attendant lamina propria) were maintained at 37°C in oxygenated Krebs–Hensleit solution. Tissues were stimulated over a 10-min

period in the presence or in the absence of berberine and the appropriate stimulus. Cytoplasm was extracted from tissues using 0.1 M hydrochloric acid at room temperature for 90 min. Cell debris was discarded following centrifugation at $2000 \times g$ for 15 min at 5°C. Cyclic AMP levels in the remaining supernatant were assayed using a commercially available radioimmunoassay kit (Amersham International). Assays were performed in duplicate.

2.6. Protein kinase assay

As in Section 2.5, segments of stripped epithelium (with attendant lamina propria) were maintained at 37°C in oxygenated Krebs–Hensleit solution. Tissues were stimulated over a 10-min period in the presence and, as controls, in the absence of berberine. Cyclic AMP-dependent protein kinase A activity in distal colonic segments was estimated using a modified version of the method described by Giembycz and Diamond (1990). Tissues were homogenised for 15 sec in 20 vol. of ice cold buffer (5 mM KH_2PO_4, 10 mM ethylenediaminetetraacetic acid, 10 mM dithiothreitol, 500 μM 3-isobutyl-1-methylxanthine, 500 mM NaCl; pH 6.8). The resulting homogenate was separated by centrifugation at 12,500 rpm for 20 min. The pellet was used for protein determination and the supernatant was used to determine enzyme activity which was estimated by measuring incorporation of ^{32}P from radiolabeled ATP into the phosphate acceptor kemptide. Assays were performed in triplicate.

2.7. Radioisotope efflux studies

Basal and stimulated $^{86}Rb^+$ efflux was measured from sub-confluent monolayers of T84 cells which were subcultured onto 6-well plates at a density of 2.5×10^6 cells/well and allowed to grow to 60–80% confluence before use. Rb^+ is transported in the same manner as K^+ and consequently represents an effective tracer for K^+ movement (Venglarik et al., 1990). T84 cells grown on 6-well plates were incubated for one hour in 'efflux buffer' containing (in mM): NaCl (135), KCl (3), $MgCl_2$ (1.5), $CaCl_2 \cdot 2H_2O$ (1.9), HEPES (20) and glucose (10) with $^{86}Rb^+$ (1 μCi ml^{-1}).

After the loading period, cells were washed three times in fresh efflux buffer. Aliquots were sampled at 1-min intervals and replaced with an equal volume of fresh buffer. Radioactivity was estimated by liquid scintillation counting using standard techniques. Efflux curves were constructed by plotting the fraction of counts remaining in the cell monolayer against time. Rate constants were determined by fitting efflux curves to exponential functions.

2.8. Data analysis

Paired preparations of mucosal tissues were used throughout. T84 cells were used within five passage numbers and individual experiments were designed so that each test procedure had a corresponding control from the same batch. Changes in ion transport (ΔSCC) are given as peak values. Results are expressed as mean ± S.E.M. for n experiments and statistical comparison was carried out using a two tailed paired Student's t-test or by repeated measures analysis of variance where appropriate.

3. Results

3.1. Berberine inhibits SCC responses to Ca^{2+} and cAMP agonists in intact human colonic mucosae

Basal SCC levels in voltage clamped human colonic mucosae were 48.4 ± 9.0 μA/cm^2 ($n = 22$). The basolateral application of 500 μM berberine caused virtual abolition of basal SCC. Basolateral carbachol (1–500 μM) caused a concentration-dependent increase in SCC (max $\Delta SCC = 63.3 \pm 15.1$ μA/cm^2; $n = 10$; $EC_{50} = 93.0 \pm 24.5$ μM) which was virtually abolished by 500 μM berberine (max $\Delta SCC = 2.7 \pm 2.9$ μA/cm^2; $P < 0.05$; $n = 5$; Fig. 1).

Basolaterally applied forskolin (0.1–50 μM) caused a concentration-dependent increase in SCC (max $\Delta SCC = 55.7 \pm 14.9$ μA/cm^2; $n = 9$; $EC_{50} = 21.7 \pm 2.4$ μM) which was inhibited by 500 μM berberine (max $\Delta SCC = 11.6 \pm 3.2$ μA/cm^2; $P < 0.05$; $n = 5$; Fig. 2). These data reveal that berberine inhibits ion transport responses in human resected colonic tissue in a manner which is not selective for cAMP or for Ca^{2+} signalling pathways. Berberine (500 μM) applied to the apical bathing solution was without effect upon basal or stimulated SCC.

3.2. Berberine inhibits ion transport responses in T84 monolayers

Basal SCC levels in T84 monolayers were 3.4 ± 2.6 μA/cm^2 ($n = 26$). Berberine did not significantly alter

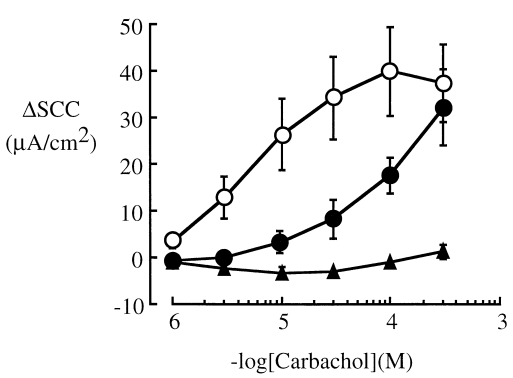

Fig. 1. Carbachol (1–500 μM) stimulated a concentration-dependent inward short circuit current response in human colonic mucosa (open circles; $n = 10$) which was reduced by berberine at a concentration of 100 μM (closed circles; $n = 5$) and virtually abolished by a higher concentration (500 μM; triangles; $n = 5$) of berberine.

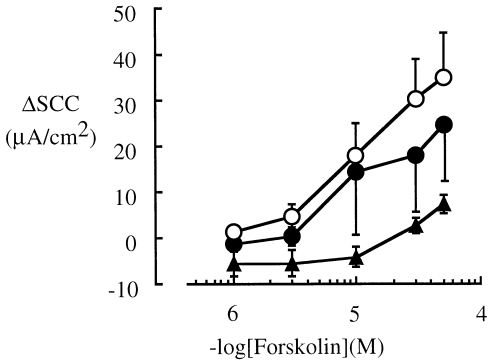

Fig. 2. Forskolin (0.1–50 μM) stimulated a concentration-dependent inward short circuit current response in human colonic mucosa (open circles; $n = 9$) which was significantly reduced by berberine at a concentration of 500 μM (closed circles; $P < 0.05$; $n = 5$) but not significantly altered in the presence of a lower concentration of berberine (100 μM; triangles; $n = 5$).

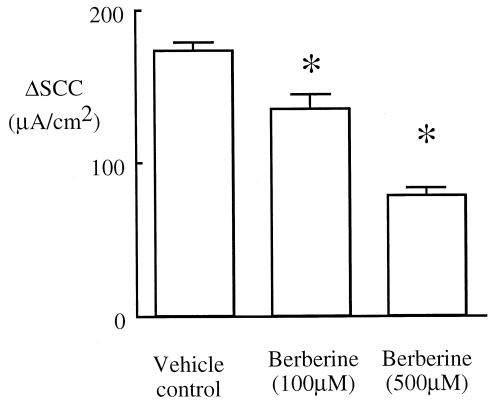

Fig. 4. Carbachol-stimulated short circuit current in T84 monolayers was significantly inhibited following 15-min pre-treatment with 100 and 500 μM berberine (* $P < 0.05$; $n = 8$ in each case).

basal SCC in T84 monolayers ($\Delta SCC = -0.1 \pm 2.1$ μA cm^{-2}; $P > 0.05$). Application of forskolin (10 μM) to the basolateral side of the monolayers stimulated a SCC response of 38.3 ± 11.1 μA/cm^2 which was inhibited by berberine (100 and 500 μM) by 28 and 59%, respectively; Fig. 3). Berberine was inhibitory only when present on the basolateral side of the monolayer and not when delivered to the apical domain (data not shown).

The Ca^{2+} agonist carbachol (100 μM) was applied to the basolateral side of voltage clamped T84 monolayers and caused an inward SCC of 172.8 ± 4.0 μA/cm^2 ($n = 8$) This response was significantly inhibited in the presence of basolateral berberine (100 and 500 μM by 22 and 45%, respectively; Fig. 4).

3.3. Effect of berberine on cyclic nucleotide generation and protein kinase A activity

Basal levels of cAMP in human colonic mucosal segments (50.8 ± 35.4 fmol μg^{-1} protein) were significantly elevated following treatment with forskolin (10 μM; 227.9 ± 91.5 fmol μg^{-1} protein; $P < 0.05$; $n = 5$). This increase in cAMP synthesis was not inhibited by 100 μM (268.4 ± 61.4 fmol μg^{-1} protein) or 500 μM (221.4 ± 79.5 fmol μg^{-1} protein) berberine.

In human colonic mucosal segments, basal protein kinase A activity (3.4 ± 0.7 ng ATP μg^{-1} protein) was elevated in tissues stimulated with dibutyryl cAMP (500 μM; 6.2 ± 1.4 ng ATP μg^{-1} protein; $P < 0.05$; $n = 6$). Protein kinase A activation was not significantly altered by 100 μM (4.8 ± 0.8 ng ATP μg^{-1} protein) or 500 μM (5.6 ± 0.2 ng ATP μg^{-1} protein) berberine. As a control, the specific protein kinase A inhibitor (Type III, isolated from porcine hearts; Sigma) significantly reduced both basal and stimulated protein kinase A activity (0.5 ± 0.1 ng ATP μg^{-1} protein; $P < 0.05$; $n = 6$).

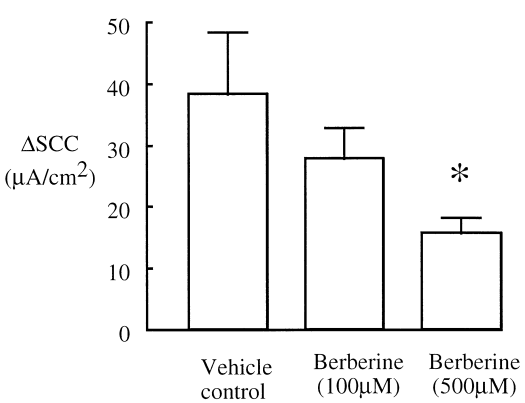

Fig. 3. Forskolin (10 μM) stimulated an inward short circuit current response in T84 monolayers grown on semi-permeable supports ($n = 26$). In tissues pre-treated with berberine for 15-min responses to forskolin were attenuated by 100 μM berberine ($n = 5$) and significantly inhibited by 500 μM berberine (* $P < 0.05$; $n = 8$).

3.4. Effect of berberine on stimulated $^{86}Rb^+$ efflux from T84 monolayers

In sub-confluent T84 monolayers grown on plastic tissue culture 6-well plates, basal apparent rate constants (K_a) for $^{86}Rb^+$ efflux were constant over 5 min. Stimulation of the cells with the agonist carbachol resulted in a significant increase in the rate of $^{86}Rb^+$ efflux. Both 100 and 500 μM berberine inhibited carbachol activated $^{86}Rb^+$ efflux (Data summarised in Table 1). Berberine alone had no effect on rate constants when compared with vehicle-treated controls.

In similar experiments, the ionophore A23187 (1 μM) produced a more rapid and vigorous $^{86}Rb^+$ efflux ($K_a = 1.131 \pm 0.116$, during the first minute of challenge) than was obtained with carbachol. $^{86}Rb^+$ efflux in the presence of A23187 was not altered by 100 μM berberine but was significantly reduced in the presence of 500 μM berberine ($K_a = 1.003 \pm 0.121$; $n = 6$; $P < 0.05$). Such data implicate basolaterally located K^+ channels as potential targets for the anti-secretory activity of berberine.

Table 1
Effect of berberine on carbachol-stimulated $^{86}Rb^+$ efflux from T84 cells

Treatment	K_a
Vehicle	0.098 ± 0.021
Carbachol (100 μM)	0.147 ± 0.012[a]
Carbachol + 100 μM berberine	0.093 ± 0.008
Carbachol + 500 μM berberine	0.080 ± 0.010

$^{86}Rb^+$ release from T84 cells, apparent rate constant (K_a) was determined 2 min after carbachol challenge. Values are means ± S.E. for six experiments. Carbachol significantly increased K_a, a response which was reduced in the presence of berberine.
[a] $P < 0.005$ when control responses to carbachol are compared to each of the other values.

3.5. Berberine inhibits K^+ currents in nystatin-permeabilised colonic mucosae

We returned to the native tissue to investigate the putative inhibition of secretion through blockade of basolateral K^+ channels by using nystatin-permeabilised human colonic mucosae in order to elucidate the sensitivity of I_K to berberine. Under these conditions (low Cl^- solutions, mucosa to serosa K^+ gradient, apical permeabilisation with nystatin) a linear relationship between SCC and K^+ gradient is observed. I_K represents transport through basolateral K^+ channels because under these conditions SCC is inhibited by barium, tolbutamide and tetrapentylammonium (data not shown). A decrease in I_K was observed in response to serosally applied berberine at concentrations of 100 and 500 μM (Fig. 5). No inhibition of I_K occurred when the mucosal side was exposed to berberine (results not shown). We went on to examine the effect of berberine on carbachol-activated increases in I_K. Carbachol (100 μM) induced a rapid onset activation of

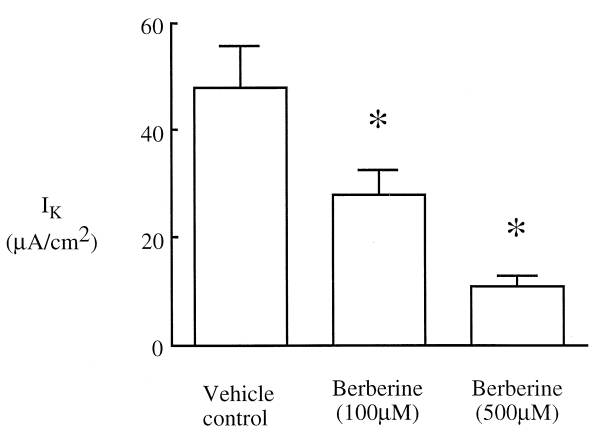

Fig. 5. Berberine (100 and 500 μM) inhibited (*: $P < 0.05$) K^+-dependent current (I_K) across the basolateral membrane of human colonic mucosae following permeabilisation of the apical membrane with nystatin under an imposed K^+ gradient of 120 mM. The drop in current was immediate in onset and complete within 10 min of berberine treatment. These data suggest that berberine blocks basolateral K^+ channels under basal conditions in human colonic mucosae. $N = 6$ throughout.

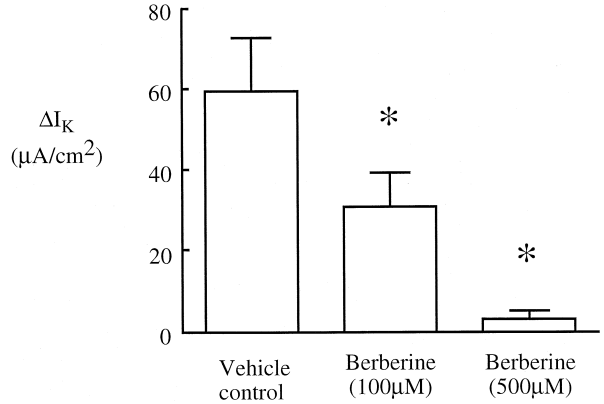

Fig. 6. Carbachol (100 μM) induced activation of basolateral K^+ channels in permeabilised human colonic mucosae (ΔI_K). Berberine pre-treatment for 15 min, at concentrations of 100 and 500 μM, had a significant (*: $P < 0.05$) inhibitory influence on the stimulatory effect of carbachol on basolateral K^+ channels. $N = 6$ throughout.

basolateral K^+ channels in nystatin permeabilised human colonic mucosae with a peak increase in I_K (ΔI_K) of 59.8 ± 13.3 μA/cm^2. Berberine reduced I_K responses with inhibition occurring at 100 μM ($\Delta I_K = 31.2 \pm 8.7$ μA/cm^2) and 500 μM ($\Delta I_K = 3.1 \pm 1.9$ μA/cm^2; $n = 6$; Fig. 6).

4. Discussion

Intact human colonic mucosae and the tumour-derived epithelial cell line T84 secrete chloride ions in response to both cAMP- and Ca^{2+}-regulated secretory pathways which orchestrate the activity of specific ion transport mechanisms (MacVinish et al., 1993; Barrett, 1993). Using (i) forskolin, which stimulates Cl^- secretion in intestinal epithelial cells by elevating intracellular cAMP and activation of protein kinase A (Riordan, 1993; Barrett, 1993) and (ii) carbachol, which causes epithelial Cl^- secretion by elevating intracellular Ca^{2+} (Dharmsathaphorn and Pandol, 1986), we stimulated SCC in voltage clamped human colonic mucosae in vitro. Berberine significantly inhibited both basal and stimulated transepithelial Cl^- secretion in isolated human colonic mucosae when present in the basolateral domain. Berberine reduction of basal SCC in voltage clamped human colon was immediate in inset and complete within 5–10 min. In a limited number of experiments berberine also rapidly reduced the sustained SCC response to forskolin. These results confirm that berberine will attenuate secretory processes which are already stimulated, consistent with its capacity to reduce diarrhoea associated with ongoing disease.

Apical sensitivity to berberine in rat intact colon was not observed but, like the action of loperamide, can be pharmacologically conferred using cytochalasin D which increases permeability of the intestinal epithelium similar to the increase observed in inflammatory disease (Baird et

al., 1997). While ion transport responses to both cAMP and Ca^{2+} channel agonists were inhibited, responses to Ca^{2+}-dependent pathways (carbachol) in human colon appear to be more sensitive to inhibition by berberine than responses to cyclic AMP-dependent pathways (forskolin). This finding is in keeping with the differing sensitivity of cyclic nucleotide- vs. Ca^{2+}-induced secretion to berberine that we reported in rat colon (Taylor and Baird, 1995). cAMP-mediated secretion was less sensitive to berberine inhibition than Ca^{2+}-dependent secretion and this may indicate that cAMP signals rely on serosal K^+ conductance through channels which are differentially sensitive to berberine. It is possible that cAMP activates voltage-sensitive K^+ channels in the serosal membrane due to the cellular depolarisation that accompanies apical Cl^- efflux (Cartwright et al., 1985; Mandel et al., 1986; Bajnath et al., 1991; Lohrmann et al., 1995; Schultheiß and Diener, 1997). These channels may display relative resistance (compared with K_{Ca} channels) to blockade by berberine, thereby explaining the differential inhibition of secretory events.

The intact human colonic mucosa is complex in architecture containing a wide variety of cell types including immunocytes, neurones, fibroblasts and other cell types which can influence epithelial ion transport (Perdue and McKay, 1994). Accordingly, a reductionist approach was used to study the effects of berberine on cultured intestinal epithelial monolayers, thus allowing investigation of the impact of berberine on an isolated epithelium in the absence of any other cell type. The T84 cell line has been widely used as a model in the study of electrogenic Cl^- secretion (Dharmsathaphorn and Madara, 1990). As we found with native, intact tissue, carbachol and forskolin stimulated a short circuit current response in voltage clamped T84 monolayers. The effect of each of these agonists was significantly inhibited by berberine thus confirming that the alkaloid is having its effects at least in part through a direct action on epithelial cells. As with intact tissue, berberine was ineffective when added to the apical bathing solution.

We examined the effects of berberine on various elements of the cAMP second messenger pathway using intact tissue. Forskolin activates adenylyl cyclase, the enzyme responsible for the synthesis of cyclic AMP (Mandel et al., 1986). Cyclic AMP activates protein kinase A (Cohn, 1987) which directly phosphorylates apical Cl^- channels (Cohn et al., 1992; Berger et al., 1993). We first examined the effects of berberine on adenylyl cyclase activity in intact human colonic segments using cAMP production as a measure of enzyme activity. Forskolin stimulated a dramatic increase in cellular cAMP, an event which was not inhibited by berberine. In separate experiments, protein kinase A activity in segments of intact human colon was determined using a modified version of the assay described by Giembycz and Diamond (1990) based on the measurement of protein kinase A-mediated phosphate transfer from radio-labelled ATP to the phosphate acceptor kemptide. Neither basal nor cAMP-stimulated protein kinase A activity in isolated human mucosal segments was effected by the presence of berberine indicating that protein kinase A inhibition is not a site of inhibitory action of berberine.

We then returned to the T84 human epithelial cell line to explore a mechanistic basis for the anti-secretory activity of berberine. In order for sustained Cl^- secretion from epithelial cells to occur, regardless of stimulus, there must be compensatory cation movement out of the cell (Reenstra, 1993; Sandle et al., 1994). Carbachol induced increases in the rate of $^{86}Rb^+$ efflux from T84 cells were significantly inhibited by berberine. Since rubidium efflux reflects K^+ movement (Venglarik et al., 1990) these results indicate that the alkaloid may be have its anti-secretory actions through inhibition of basolateral K^+ recycling. There was no difference in effects of berberine upon forskolin stimulated $^{86}Rb^+$ efflux between each of the concentrations used. In contrast, A23187 stimulated a much more vigorous efflux which was not inhibited by 100 μM berberine. The differences in apparent sensitivity may be accounted for by relatively different magnitude of challenge as indicated by the different level of the response to each of the stimuli. It should also be noted that $^{86}Rb^+$ efflux is not the most accurate method of measuring K^+ channel activity. For example Rb^+ can block K^+ channels (Latorre and Miller, 1983) and some K^+ channels are impermeable to Rb^+ (Foster et al., 1989). For these reasons we turned again to the intact tissue model.

Further evidence to support our hypothesis was provided by our work with nystatin-permeabilised human colonic mucosae. Nystatin molecules form cation permeable pores in the apical cell membranes, thereby removing the apical membrane resistance barrier and allowing free cation transport (Akaike and Harata, 1994; Lewis et al., 1997). The contribution of apical K^+ conductance to the effects of secretagogues or berberine has not been examined. However, since SCC and I_K closely parallel each other in response to stimulation and inhibition it is likely that the K^+ channels principally involved are located at the basolateral membrane. The I_K in human colon described here is unaffected by apical amiloride or basolateral bumetanide demonstrating that neither electrogenic sodium absorption nor Cl^- secretion occur following nystatin addition, i.e., both absorptive and secretory (crypt) cells are permeabilized (results not shown). Carbachol increased basolateral K^+ conductance in permeabilised human colonic mucosae. This is in keeping with results in rat colon (Schultheiß and Diener, 1997) and the human colonic epithelial cell line T84 (Tabcharani et al., 1994). Such an activation of K^+ channels by carbachol would hyperpolarize the membrane of secretory cells and provide the driving force for Cl^- efflux into the lumen. Our data clearly show inhibition of basal I_K and carbachol-stimulated I_K in permeabilised human colon.

K^+ channels are widely distributed in many mammalian cell types and their contribution to complex reflexes is often difficult to study. For example, K^+ channel activation was found to decrease SCC in ileal rabbit mucosa (Homaidan and Broutman, 1994) and to exert anti-diarrhoeal activity in a mouse model (Poggioli et al., 1995). These findings seem to be contradictory to the hypothesis that K^+ channel blockade is anti-secretory. However, apparent contradictions may be explained in the first case by different regulatory effects of K^+ channels in small and large intestine and, in the second, by activation of K^+ channels on other elements (e.g., smooth muscle or nerves) which influence intestinal transit time in vivo. The contribution of apical K^+ conductance to the effects of secretagogues or berberine has not been examined in this study. However, since SCC and I_K closely parallel each other in response to stimulation and inhibition is likely that the K^+ channels principally involved are located at the basolateral membrane.

At least two types of basolateral K^+ channels exist in human colonic mucosal epithelium (Iliev and Marino, 1993; Lomax et al., 1996) and these conductances are pharmacologically distinct. For example, the Ca^{2+}-activated basolateral K^+ channel is inhibited by charybdotoxin whereas the cAMP-dependent K^+ conductance is sensitive to barium salts (Mandel et al., 1986; Baro et al., 1994). Berberine, like clotrimazole (Rufo et al., 1996) and levamisole (Mun et al., 1998), inhibits both cAMP- and Ca^{2+}-dependent epithelial ion transport processes and therefore each of these drugs would reduce Cl^- secretion regardless of its stimulus pathway, perhaps increasing the range of therapeutic applications of these drugs. Despite an obligate dependence of Cl^- secretion on basolateral K^+ channels for maintaining the charge difference which drives Cl^- secretion, cAMP fails to directly increase basolateral K^+ conductances in colonic cell lines (Bajnath et al., 1991; Reenstra, 1993) and rat colon (Schultheiß and Diener, 1997). Consequently, it is not immediately clear why berberine inhibits both Ca^{2+}-mediated responses to carbachol as well as cAMP-mediated responses to forskolin in human intact mucosae. Activation of a Ca^{2+}-dependent K^+ channel by cAMP in rabbit colonic epithelial cells has been reported (Loo and Kaunitz, 1989). Further experiments are required to fully identify the molecular target(s) of berberine.

5. Conclusion

Evidence provided in this study shows that berberine inhibits ion transport in human colonic epithelia. Data suggest inhibition of a basolateral K^+ conductance on epithelial cells as the mechanism of action of this anti-diarrhoeal drug. Berberine has already been reported as a K^+ channel blocking agent in neurones (Wu and Gin, 1997), coronary arteries (Huang, 1992), guinea pig ventricular myocytes (Wang and Zheng, 1997), guinea pig gallbladder (Wehner et al., 1990), human myeloma cells (Wu et al., 1998) and a model of ventricular fibrillation (Sun and Li, 1993). Berberine may be added to a growing list of protonated amine-containing compounds including chromanol derivatives (Suessbrich et al., 1996), clotrimazole (Rufo et al., 1996), levamisole (Mun et al., 1998) and ammonia itself (Prasad et al., 1995), each of which inhibits a basolateral membrane K^+ conductance which maintains the electrical gradient of epithelial cells favouring apical Cl^- efflux (Tabcharani et al., 1994). Such basolateral K^+ channels have been identified as novel and potentially important targets for anti-secretory drugs on the treatment of secretory diarrhoea (Lohrmann et al., 1995). It is interesting that a drug which has been widely used for millenia is a member of a ‘new’ pharmacological family.

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