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# Berberine inhibition of electrogenic ion transport in rat colon

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1 The effects of the alkaloid berberine on basal and stimulated ion transport were investigated in voltage-clamped rat colonic epithelia.

2 Berberine (100-500 $\mu$M) reduced basal short circuit current (SCC) when applied basolaterally but not when applied apically.

3 SCC responses to mast cell activation by anti-rat IgE were significantly attenuated in the presence of berberine.

4 Berberine, applied to the basolateral bathing solution, also reduced SCC responses to the following agents which stimulate chloride secretion in rat colon: carbachol, forskolin, sodium nitroprusside, dibutyryl cyclic-AMP, heat-stable *E. coli* enterotoxin, 8-bromo-cyclic GMP and thapsigargin. Calcium mediated ion transport responses appear to be more sensitive to berberine inhibition than those which are cyclic GMP-mediated, which in turn are more sensitive than cyclic AMP-mediated responses.

5 Berberine added apically was without effect upon forskolin-stimulated ion transport. Cytochalasin D treatment of the lumenal surface of rat colon conferred apical-side sensitivity to berberine.

6 Berberine (at concentrations up to 500 $\mu$M) was without effect on generation of cyclic AMP by forskolin or on generation of cyclic GMP by sodium nitroprusside in isolated mucosal segments. Protein kinase A activity stimulated by dibutyryl cyclic AMP was unaffected by berberine (at concentrations up to 500 $\mu$M).

7 The precise mechanism of action of berberine remains to be elucidated. However, its site of action appears to be distal to second messenger production and may be at a level common to all stimuli of colonic chloride secretion.

**Keywords:** Berberine; rat colon; ion transport; cytochalasin D

## Introduction

Berberine, an alkaloid derived from a number of botanical sources including *Berberis aristata* and *Coptis chinensis*, has been used for over two thousand years in traditional Eastern medicine in the treatment of gastroenteritis and secretory diarrhoea (Tang & Eisenbrand, 1992) and is also effective in prevention and treatment of animal models of diarrhoea (Dutta *et al.*, 1972; Sabir *et al.*, 1977; Sack & Froelich, 1982). Berberine has a varied pharmacology including anti-microbial (Tang & Eisenbrand, 1992), anti-motility (Yamamoto *et al.*, 1993) and anti-secretory (Tai *et al.*, 1981; Guandalini *et al.*, 1987) activities, each of which may contribute to an anti-diarrhoeal effect. How each or all of these mechanisms may contribute to the thereapeutic usefulness of berberine in the treatment of diarrhoea is not yet firmly established.

Since berberine has a high binding affinity for mast cells (Berlin & Enerback, 1983), we began these studies to investigate whether berberine influenced mast cell-mediated chloride secretion in isolated, voltage-clamped rat colon (Taylor & Baird, 1993). It emerged that berberine exerts direct anti-secretory actions on epithelial cells. Since electrogenic chloride secretion in colonic epithelial cells may be stimulated by a number of separate but interacting intracellular mechanisms, the study was extended to examine the influence of berberine on ion transport responses to different secretagogues.

Intracellular mediators which have been implicated as major second messengers involved in regulation of ion transport across intestinal epithelia include adenosine 3':5'-cyclic monophosphate (cyclic AMP), guanosine 3':5'-cyclic monophosphate (cyclic GMP) and intracellular free calcium (Barrett & Dharmsathaphorn, 1991).

We used a pharmacological approach to investigate the effects of berberine on these transduction mechanisms using voltage-clamped rat colon. In order to stimulate cyclic AMP-mediated ion transport, we used dibutyryl cyclic AMP and forskolin which activates adenylyl cyclase (Bohme *et al.*, 1991). To activate cyclic GMP-dependent ion transport we used 8-bromo-cyclic GMP and heat stable enterotoxin (STa) which induces electrogenic chloride secretion by activating receptors for guanylin with consequent elevation in intracellular cyclic GMP (Forte *et al.*, 1993). To stimulate chloride secretion via elevation of intracellular free calcium we used thapsigargin (Brayden *et al.*, 1989), which inhibits calcium re-uptake into endoplasmic reticulum (Thastrup *et al.*, 1990).

Finally, we examined whether berberine influenced the generation of cyclic nucleotides or activation of the effector enzyme protein kinase A which regulates epithelial chloride channels (Finn *et al.*, 1992).

## Methods

### *Electrophysiological studies*

Non-fasted male Wistar rats (250-300 g) were killed by cervical dislocation. Segments of distal colon were opened and stripped of underlying smooth muscle by blunt dissection. Mucosal sheets of epithelium and attendant lamina propria were mounted in Ussing chambers (window area = 0.63 $cm^2$). Tissues, bathed on either side with Krebs Henseleit solution maintained at 37°C and oxygenated with 95%$O_2$/5% $CO_2$, were voltage clamped to zero potential difference with a DVC 1000 (World Precision Instruments, Stevenage, Herts). The composition of the Krebs Henseleit solution was (in mM) NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $KH_2PO_4$ 1.2, $NaHCO_3$ 25 and D-glucose 11.1. Short circuit current (SCC) was continuously monitored with a MacLab analogue to digital data

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acquisition system (A.D. Instruments, Hastings, Sussex). By convention, inward SCC is that which is accounted for by anion secretion, cation absorption or a combination of each. Drugs were added to either the apical (lumenal) side or the basolateral (serosal) side bathing solutions. Tissues were allowed to equilibrate until a steady state basal SCC was achieved. Voltage-clamp experiments using db cyclic AMP, 8-bromo-cyclic GMP and thapsigargin were carried out in the presence of tetrodotoxin (0.5 $\mu$M) in order to remove any neuronal contribution to stimulation of ion transport.

### *Nucleotide assay*

Segments of stripped epithelium (with attendant lamina propria) were maintained at 37°C in oxygenated Krebs-Henseleit solution. Tissues were stimulated over a 10 min period in the presence and, as controls, in the absence of berberine. Cytoplasm was extracted from tissues with 0.1 M HCl at room temperature for 90 min. Cell debris was discarded following centrifugation at 2000 *g* for 15 min at 5°C. Intracellular second messenger (cyclic AMP and cyclic GMP) levels in the remaining supernatant were assayed with radioimmunoassay kits (Amersham International plc, U.K.). Tissue protein content was determined by the method of Lowry *et al.* (1951). Assays were performed in duplicate.

### *Protein kinase assay*

As in the previous section, segments of stripped epithelium (with attendant lamina propria) were maintained at 37°C in oxygenated Krebs-Henseleit 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 (PKA) activity in distal colonic segments was estimated by a modified version of the method described by Giembycz & Diamond (1990). Tissues were homogenized for 15 s in 20 volumes of ice cold buffer: $KH_2PO_4$ 5 mM, $K_2HPO_4$ 5 mM, ethylenediaminetetraacetic acid 10 mM, dithiothreitol 10 mM, 3-isobutyl-1-methylxanthine 500 $\mu$M, NaCl 500 mM; pH 6.8. The resulting homogenate was separated by centrifugation at 12500 r.p.m. 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 radiolabelled ATP into the phosphate acceptor, kemptide. Assays were performed in triplicate.

### *Chemicals*

Berberine, carbachol, thapsigargin, heat stable *Escherichia coli* enterotoxin (STa), dibutyryl-cyclic AMP, 8-bromo-cyclic GMP, forskolin, sodium nitroprusside (SNP), tetrodotoxin, protein kinase inhibitor (type III; from porcine heart), cytochalasin D and kemptide were purchased from Sigma Chemical Co. Dorset. $^{32}P$ labelled ATP was from Amersham International plc, U.K. Anti-rat IgE immunoglobulins were obtained from Nordic Laboratories Ltd., Tilburg, the Netherlands.

### *Statistical analysis*

Paired preparations of mucosal segments from each rat were used throughout. Changes in ion transport ($\Delta$SCC) are given as peak values. Results are expressed as mean ± s.e. mean and statistical comparison was carried out with Student's two tailed paired *t* test or by analysis of variance where appropriate. $EC_{50}$ values were calculated as the concentration of agonist producing half maximal stimulation in individual experiments and the results are presented as the mean ± s.e.mean for groups of experiments.

## Results

### *Effects of berberine upon basal and stimulated SCC*

Resting values for SCC were $51.1 \pm 4.1$ $\mu$A cm$^{-2}$ ($n=35$) which, after 30 min equilibration, were stable over several hours. Berberine applied to the basolateral side of voltage-clamped mucosae caused a significant drop in basal SCC of $-19.0 \pm 4.3$ $\mu$A cm$^{-2}$ for 100 $\mu$M berberine ($n=35$; $P<0.01$) and $-27.3 \pm 4.8$ $\mu$A cm$^{-2}$ for 500 $\mu$M berberine ($n=29$; $P<0.01$). This effect was not observed when berberine (500 $\mu$M) was applied apically ($\Delta SCC = 1.4 \pm 0.8$ $\mu$A cm$^{-2}$; $n=6$). Since berberine caused a fall in SCC which was stable over time, responses to secretagogues in the presence of berberine are expressed throughout as $\Delta$SCC from the new, steady baseline.

Anti-rat IgE antiserum (1:200 dilution) stimulated an immediate, transient inward SCC of $27.7 \pm 8.8$ $\mu$A cm$^{-2}$ ($n=10$) in rat colonic mucosae. Responses to anti-IgE were significantly reduced in the presence of 100 $\mu$M berberine by $90 \pm 3\%$ ($P<0.05$; $n=5$) and were abolished in the presence of 500 $\mu$M berberine (Figure 1).

In separate experiments, stimulation of SCC by carbachol (1–300 $\mu$M added basolaterally) produced a concentration-dependent increase in SCC ($EC_{50} = 5.9 \pm 0.9$ $\mu$M; maximum response $= 163.2 \pm 21.3$ $\mu$A cm$^{-2}$). Carbachol stimulation of ion transport in rat colon was inhibited by berberine (100 $\mu$M, $P<0.01$ and 500 $\mu$M, $P<0.001$) added basolaterally (Figure 2a). Apical addition of berberine (500 $\mu$M) did not alter SCC responses to carbachol which were superimposable with controls ($n=6$). Reversibility of the anti-secretory action of berberine was also investigated. Using a single concentration of carbachol (100 $\mu$M), SCC responses were $184.8 \pm 45.1$ $\mu$A cm$^{-2}$. Following washout with three changes of Krebs Henseleit solution, responses to a second challenge with carbachol (100 $\mu$M) in the presence of berberine (500 $\mu$M) were significantly reduced to $5.6 \pm 3.3$ $\mu$A cm$^{-2}$ ($P<0.05$; $n=5$). A third challenge with carbachol (100 $\mu$M) following a second washout produced a change in SCC of $89.0 \pm 18.3$ $\mu$A cm$^{-2}$ showing that the effects of berberine were not irreversible.

SNP stimulated an inward SCC which was abolished in the presence of 100 $\mu$M bumetanide ($P<0.01$; $n=6$). Berberine also abolished SCC responses to SNP (Figure 2b).

### *Effects of berberine upon the actions of directly acting secretagogues*

We used a number of agents to stimulate colonic chloride secretion by a direct action on epithelial cells. Dibutyryl cyclic

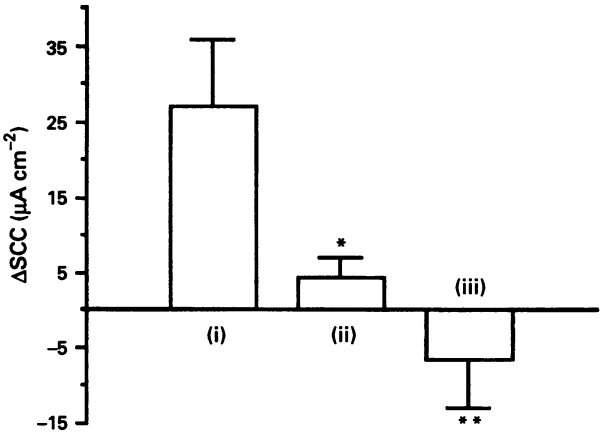


**Figure 1** Antibodies raised against rat IgE (1:200 dilution of antisera) stimulated a transient inward SCC in voltage-clamped rat colon (i). This response was significantly attenuated by 100 $\mu$M berberine (ii; $*P<0.05$) and abolished by 500 $\mu$M berberine (iii; $**P<0.01$). $n=5$ throughout.

AMP stimulated a concentration-dependent increase in SCC which was inhibited by 100 $\mu$M berberine and abolished by 500 $\mu$M berberine (Figure 3a). Forskolin stimulated an inward SCC which was concentration-dependent ($EC_{50}=5.8\pm1.6$ $\mu$M) and reached a peak value of $86.8\pm21.2$ $\mu$A cm$^{-2}$ ($n=5$). A single concentration of forskolin (10 $\mu$M; $n=12$) provoked a change in SCC of $63.1\pm8.5$ $\mu$A cm$^{-2}$ which was significantly attenuated by 100 $\mu$M and 500 $\mu$M berberine by $53\pm11\%$ ($P<0.05$) and $72\pm12\%$ ($P<0.01$) respectively.

Basolateral addition of STa to mucosal sheets caused a slow in onset and sustained increase in SCC which did not reach a maximum value within the concentration-range used. This action of STa was virtually abolished in the presence of 100 $\mu$M berberine (Figure 3b). 8-Bromo-cyclic GMP, a lipid-soluble and stable analogue of cyclic GMP also stimulated inward SCC in a concentration-dependent fashion (1–300 $\mu$M). Responses to 8-bromo-cyclic GMP were inhibited by 100 $\mu$M ($P<0.05$; $n=6$) and abolished by 500 $\mu$M ($P<0.01$; $n=6$) berberine (Figure 3c).

Thapsigargin evoked a concentration-dependent increase in SCC when applied to the basolateral bathing solution. Berberine at a concentration of 100 $\mu$M abolished SCC responses to increasing concentrations of thapsigargin (0.01–30 $\mu$M; $P<0.01$; Figure 3d).

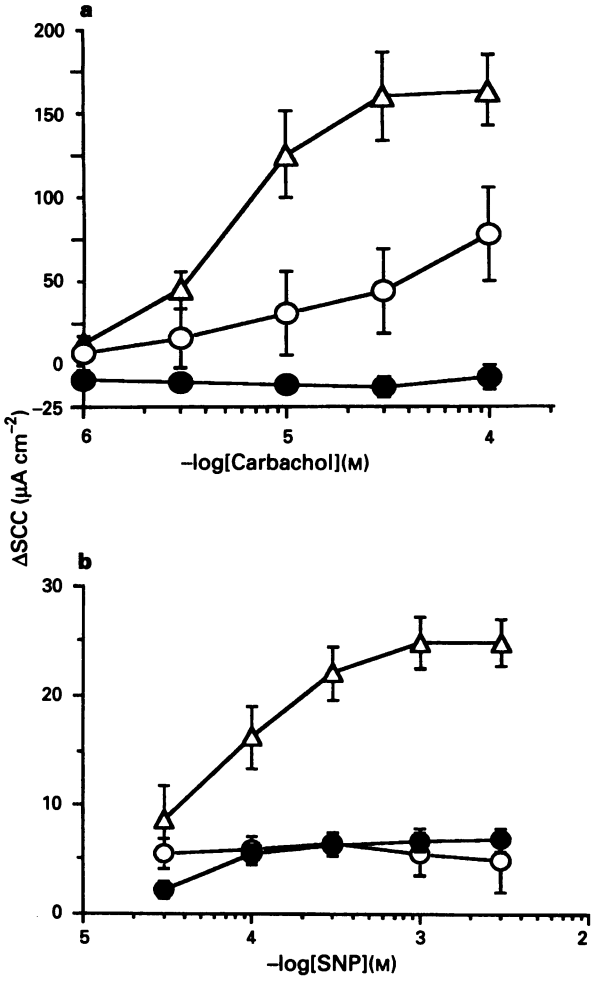


**Figure 2** Effects of berberine on ion transport responses in voltage-clamped rat colon. (a) Carbachol (1–100 $\mu$M) stimulated inward SCC (△) which was significantly inhibited by 100 $\mu$M berberine (○; $P<0.01$) and abolished by 500 $\mu$M beberine (●; $P<0.001$). (b) Sodium nitroprusside (SNP; 30 $\mu$M–3 mM) stimulated inward SCC in a concentration-dependent manner (△). Responses to SNP were abolished in the presence of bumetanide (100 $\mu$M; ●; $P<0.01$), and berberine (100 $\mu$M; ○; $P<0.01$). $n=6$ throughout.

### *Effects of cytochalasin D upon apical-side sensitivity to berberine*

SCC responses to a single concentration (30 $\mu$M) of forskolin ($72.8\pm9.2$ $\mu$A cm$^{-2}$; $n=17$) were not significantly altered in the presence of berberine (500 $\mu$M) applied to the apical-side bathing solution ($\Delta$SCC $=83.0\pm15.4$ $\mu$A cm$^{-2}$; $n=5$). Following cytochalasin D treatment (1 $\mu$g ml$^{-1}$; apical side for 1 h), apically applied berberine significantly reduced SCC responses to forskolin ($\Delta$SCC $=48.3\pm11.7$ $\mu$A cm$^{-2}$; $P<0.05$; $n=5$). In matched, control experiments cytochalasin D alone (1 $\mu$g ml$^{-1}$; apical side for 1 h) did not alter ion transport responses to 30 $\mu$M forskolin ($\Delta$SCC $=64.9\pm9.4$ $\mu$A cm$^{-2}$; $n=5$).

### *Effects of berberine upon cyclic nucleotide generation and protein kinase A activity*

Basal levels of cyclic AMP ($51.6\pm18.6$ fmol $\mu$g$^{-1}$ protein) were significantly elevated by forskolin (10 $\mu$M; $907\pm164$ fmol $\mu$g$^{-1}$ protein; $P<0.001$). This effect was not altered when the experiment was carried out in the presence of berberine ($978\pm20$ fmol $\mu$g$^{-1}$ protein; $n=5$ throughout) in a concentration (100 $\mu$M) which significantly inhibited ion transport responses to forskolin (Figure 4a).

Basal levels of cyclic GMP ($143\pm25$ fmol mg$^{-1}$ protein) were significantly elevated in tissues stimulated with SNP (1 mM; $496\pm76$ fmol mg$^{-1}$ protein; $P<0.05$). Berberine (500 $\mu$M) did not alter cyclic GMP elevation in response to SNP ($469\pm72$ fmol mg$^{-1}$ protein; $n=6$ throughout; Figure 4b).

Basal PKA activity ($9.7\pm2.2$ ng ATP $\mu$g$^{-1}$ protein) was elevated by dibutyryl cyclic AMP (500 $\mu$M; $15.4\pm2.8$ ng ATP $\mu$g$^{-1}$ protein; $P<0.01$). The PKA inhibitor (1 $\mu$g ml$^{-1}$) reduced basal and stimulated activity ($1.9\pm0.5$ ng ATP $\mu$g$^{-1}$ protein; $P<0.005$). PKA activity stimulated by dibutyryl cyclic AMP was not altered in the presence of berberine at concentrations of 100 $\mu$M ($18.9\pm4.1$ ng ATP $\mu$g$^{-1}$ protein) or 500 $\mu$M ($17.2\pm3.6$ ng ATP $\mu$g$^{-1}$ protein; $n=7$ throughout; Figure 4c).

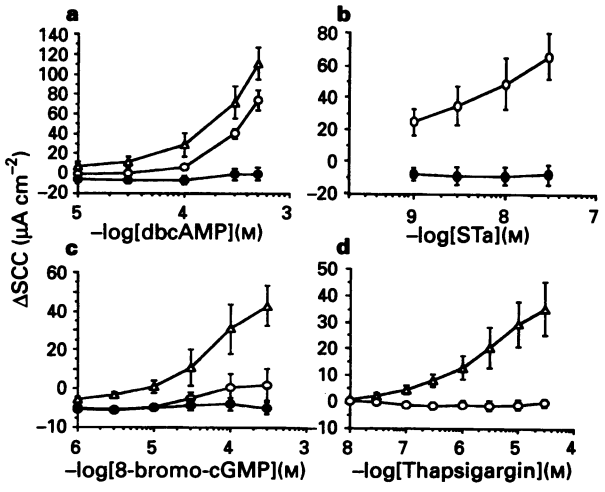


**Figure 3** Effects of berberine on directly acting secretagogues in voltage-clamped rat colon. (a) Dibutyryl cyclic AMP (dbcAMP; 10–500 $\mu$M) stimulated inward SCC (△) which was inhibited by 100 $\mu$M berberine (○) and virtually abolished by 500 $\mu$M berberine (●; $P<0.01$). (b) Heat stable enterotoxin (STa; 1–30 nM; ○) stimulated inward SCC in voltage-clamped rat colon. In the presence of 100 $\mu$M berberine, SCC responses to STa were abolished (●; $P<0.05$). (c) 8-Bromo-cyclic GMP (1–300 $\mu$M; △) stimulated SCC in voltage-clamped rat colon which was reduced in the presence of 100 $\mu$M berberine (○; $P<0.05$) and was abolished in the presence of 500 $\mu$M berberine (●; $P<0.01$). (d) Thapsigargin (0.01–30 $\mu$M) stimulated inward SCC (△) which was abolished in the presence of 100 $\mu$M beberine (○; $P<0.01$). $n=6$ throughout.

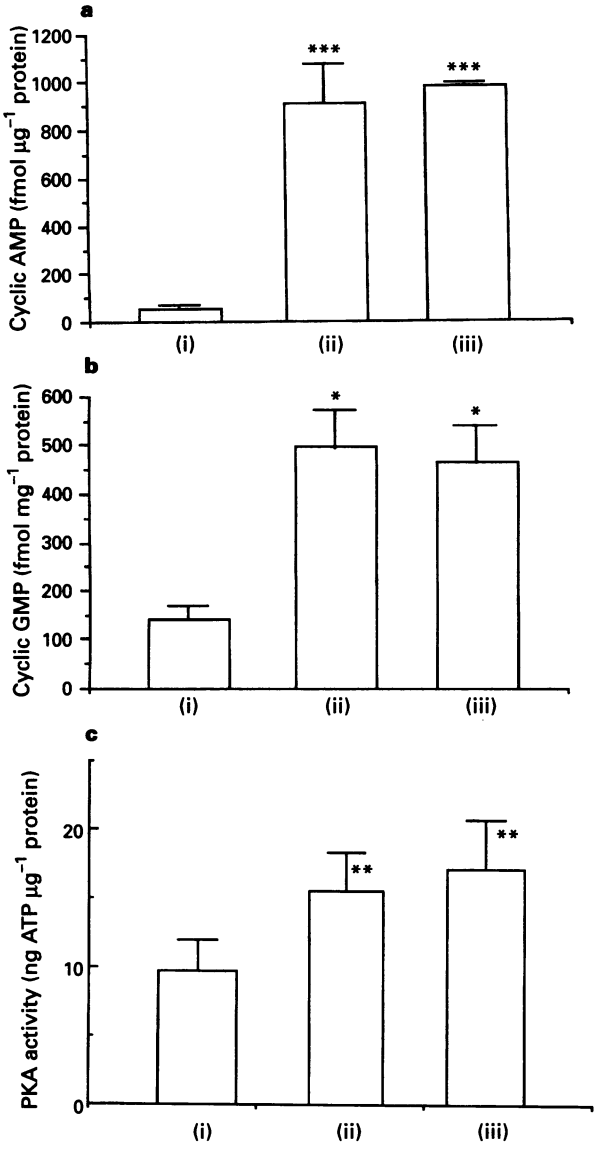


**Figure 4** Effects of berberine on cyclic nucleotide generation and protein kinase A activity in rat colon. (a) Basal levels of cyclic AMP (i) in rat colonic mucosae were significantly elevated by 10 $\mu$M forskolin (ii; $P<0.001$), This response to forskolin was not altered in the presence of 100 $\mu$M berberine (iii; $P<0.001$ when compared with basal levels). $n=5$ throughout. (b) Basal levels of cyclic GMP (i) in rat colonic mucosae were significantly elevated by sodium nitroprusside (ii; 1 mM; $P<0.05$). Cyclic GMP responses to sodium nitroprusside were not altered in the presence of 500 $\mu$M berberine (iii; $P<0.05$ when compared with basal levels). $n=6$ throughout. (c) Protein kinase A activity in rat colonic mucosae (i), determined by ATP incorporation into kemptide, was elevated in response to 500 $\mu$M dibutyryl cyclic AMP (ii; $P<0.01$ when compared with basal activity), were unaffected in the presence of 500 $\mu$M berberine (iii; $P<0.01$ when compared with basal levels). $n=7$ throughout.

## Discussion

Secretory diarrhoea occurs when the intestine, normally a predominantly absorptive organ becomes a net secretory organ resulting in the establishment of an osmotic gradient across the intestinal epithelium which favours the movement of water in a net blood-to-lumen direction (Donowitz *et al.*, 1986). Colonic chloride secretion may occur as a consequence of neuronal, immunological, blood-borne or toxic stimuli (Field *et al.*, 1989; Barrett & Dharmsathaphorn, 1991). Since berberine has high affinity for some (Berlin & Enerback, 1983) but not all (Arizono *et al.*, 1987) mast cells, we began this investigation to study whether berberine inhibits mast cell-mediated ion transport in rat isolated colon. Antibodies raised against rat-IgE activate mast cells and/or eosinophils in the lamina propria of rat colonic mucosa which indirectly stimulate epithelial chloride secretion (Baird *et al.*, 1985; O'Malley *et al.*, 1993). Our results showed that berberine effectively inhibited mast cell-dependent stimulation of chloride secretion, consistent with our hypothesis that the alkaloid prevents mast cell activation. However, the anti-secretory action of berberine was not specific for immune-driven ion transport. Berberine also inhibited ion transport responses to carbachol which stimulates chloride secretion by activating epithelial muscarinic receptors (Dickenson *et al.*, 1992) although a significant contribution of the effects of carbachol upon ion transport in voltage-clamped rat colon is tetrodotoxin-sensitive (O'Malley *et al.*, 1995). Ion transport responses to SNP which stimulates neuronally mediated chloride secretion (Tamai & Gaginella, 1993) were also significantly attenuated in the presence of berberine. Since the anti-secretory action of berberine on carbachol-induced ion transport was reversible, it is unlikely that berberine exerts a permanent toxic effect on the preparation.

We examined the effects of berberine on direct stimulation of electrogenic ion transport. The cellular basis of epithelial chloride secretion is complex (Dawson, 1993). Intracellular second messengers which regulate chloride secretion in the gut include cyclic AMP, cyclic GMP and intracellular calcium (Barrett & Dharmsathaphorn, 1991) and synergism between these secretagogues has been reported (McRoberts *et al.*, 1985; Cartwright *et al.*, 1985; Cliff & Frizzell, 1990; Lindeman & Chase, 1992; Calderaro *et al.*, 1993).

Our results show that berberine significantly inhibits ion transport responses to forskolin in rat voltage-clamped colon. Forskolin is a diterpene which stimulates chloride secretion in rat colon by activation of adenylate cyclase (Bohme *et al.*, 1991). We also found that berberine inhibits SCC responses to dibutyryl cyclic AMP, a lipid-soluble and stable analogue of cyclic AMP which mimics the action of endogenous cyclic AMP to activate transepithelial chloride secretion (Kockerling & Fromm, 1993). Thus we have two lines of evidence to indicate that berberine interferes with chloride secretion which occurs as a consequence of activation of the cyclic AMP-dependent second messenger system.

SCC responses to STa, which stimulates colonic chloride secretion via a cyclic GMP-dependent second messenger system by activating guanylin receptors (Forte *et al.*, 1993; Cuthbert *et al.*, 1994), were inhibited by berberine. Since it has been proposed that STa may also stimulate intestinal electrolyte transport via a calcium-dependent pathway (Greenberg *et al.*, 1992), we examined the effects of berberine on ion transport responses to 8-bromo-cyclic GMP. This cyclic GMP analogue, which directly activates protein kinase G to evoke chloride secretion in rat colonic epithelium (Nobles *et al.*, 1991; Lin *et al.*, 1995), stimulated SCC which was also inhibited by berberine.

Calcium-dependent chloride secretion which is stimulated by thapsigargin (Brayden *et al.*, 1989) was abolished by berberine at concentrations of 100 $\mu$M in experiments where we used cumulative concentration-response curves to the agonist.

These data indicate that berberine acts as as a non-selective inhibitor of ion transport in rat colon, although calcium-mediated ion transport responses appear to be more sensitive to berberine inhibition than those which are cyclic GMP-mediated, which in turn are more sensitive than cyclic AMP-mediated responses. This profile of sensitivity is similar to that of loperamide which exerts a greater anti-secretory effect against SCC responses mediated by cyclic AMP than SCC responses mediated by cyclic GMP in rabbit ileal mucosa (Hughes *et al.*, 1982). In rat colon (Diener *et al.*, 1988), loperamide blocked cyclic AMP-mediated secretion at ten times higher concentrations than were required to block secretion mediated by calcium.

Another feature of the action of berberine which is shared with that of loperamide is that of sidedness. Beberine, like loperamide (Hughes *et al.*, 1982), reduced basal and stimulated SCC when applied basolaterally but not when applied apically.

Our findings with berberine are consistent with other reports using rat voltage-clamped ileum. Tai *et al.* (1981) showed greater sensitivity to basolateral than apical application of berberine. Also using rat small intestine, Guandalini *et al.* (1987) demonstrated that the anti-secretory effect of berberine was limited only to basolateral-side application. Since berberine is effective in the treatment of experimentally-induced diarrhoea when given orally (Dutta *et al.*, 1972), we investigated whether sensitivity to apically-applied berberine could be conferred. Treatment of rat colonic epithelia with cytochalasin D, which enhances paracellular permeability by opening intercellular tight junctions (Madara *et al.*, 1986), resulted in apical-side sensitivity of the tissue to berberine. Following cytochalasin D treatment, apically-applied berberine significantly reduced ion transport responses to forskolin. In control preparations, cytochalasin D alone did not alter the capacity of tissues to respond to forskolin.

We studied the effects of berberine on cyclic nucleotide generation in mucosal segments using forskolin to stimulate cyclic AMP and SNP to stimulate cyclic GMP. Berberine, at concentrations which significantly attenuated SCC responses to each of the agonists, was without effect upon stimulated elevation of either cyclic AMP or cyclic GMP. Thus, inhibition of ion transport responses by berberine seems unlikely to be mediated by interference with cyclic nucleotide formation.

Epithelial chloride secretion may be regulated by protein kinase A-dependent phosphorylation of channel-associated proteins (Tabcharani *et al.*, 1991; Anderson *et al.*, 1992). In our study we used an indirect measure of cyclic AMP-dependent protein kinase A activity based on the phosphorylation of the acceptor molecule, kemptide (Giembycz & Diamond, 1990). Protein kinase A activity in colonic mucosa was significantly stimulated by dibutyryl-cyclic AMP in concentrations which, in separate experiments, evoked electrogenic chloride secretion across voltage-clamped tissues. Basal and stimulated protein kinase A levels were significantly inhibited by a purified cyclic AMP-dependent protein kinase inhibitor (Type III from porcine heart; Walsh *et al.*, 1971) but were unaffected by berberine.

In summary, berberine is a non-selective inhibitor of transepithelial ion transport across rat distal colonic mucosae, an effect which may be important in the anti-diarrhoeal action of the drug. Although the precise mechanism of action of berberine remains to be elucidated, its site of action appears to be distal to second messenger production and may be at a level common to all stimuli of colonic chloride secretion. Although it shares some pharmacological properties with loperamide, berberine does not appear to act through opioid receptors (Guandalini *et al.*, 1987). An interesting possibility arises from the recent observation that berberine inhibits potassium currents in non-epithelial cell types (Huang, 1992; Sun & Li, 1993). Chloride secretion across intestinal epithelia is dependent upon activation of basolateral potassium channels which may be cyclic AMP and/or calcium-activated (Mandel *et al.*, 1986; Matthews *et al.*, 1993; Reenstra, 1993; Lohrmann *et al.*, 1995). Potassium channel opening leads to hyperpolarization of epithelial cells and maintenance of a favourable gradient for chloride exit at the apical membrane (Sandle *et al.*, 1994). Thus berberine may display an anti-secretory effect by inhibition of basolateral potassium channel opening which would result in attenuation of the chloride secretory response regardless of stimulus.

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