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Effects of Antibiotics on Epithelial Ion Transport in the Rabbit Distal Colon In-vitro

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Abstract

One side-effect of the therapeutic use of antimicrobial agents is respiratory paralysis as a result of inhibition of skeletal neuromuscular transmission; cholinergic neuro-effector motor transmission in the gastrointestinal tract is inhibited by the same classes of antimicrobial agent. Study of the effects of several classes of antibiotic compound on intestinal motility has suggested that antibiotic-induced alterations of intestinal motility may be related to the onset of diarrhoea or the development of antibiotic-associated colitis. These compounds may, however, also initiate or exacerbate diarrhoea by altering control of epithelial function, a possibility that has not previously been rigorously investigated. This series of experiments investigated the effect of six antibiotics on rabbit distal colonic epithelial ion transport.

Of all the antibiotics studied, only ampicillin was without effect. Clindamycin, erythromycin, gentamicin and lincomycin, each reduced the response of the epithelium to electrical field stimulation. In addition, the lincosamides clindamycin and lincomycin reduced basal short circuit current and the epithelial response to acetylcholine. Vancomycin had no effect on the response to electrical field stimulation or acetylcholine but enhanced the secretory action of prostaglandin E_2.

These data suggest that, in addition to their ability to alter intestinal motility, a number of potential antibiotic interactions with the epithelium and its innervation may contribute to the pathogenesis of antibiotic-associated diarrhoea and colitis.

One of the more dramatic and potentially life-threatening side-effects of the therapeutic use of antimicrobial agents is respiratory paralysis caused by an inhibition of skeletal neuromuscular transmission. This effect at cholinergic motor synapses was initially described by Molitor et al (1946) and has since been extensively reviewed (Pittinger & Adamson 1972; Singh et al 1980). In general, cholinergic neuro-effector motor transmission in the gastrointestinal tract is inhibited by the same classes of antimicrobial agent that have effects at the skeletal neuromuscular junction (Lees & Percy 1981a, b; Percy & Christensen 1985).

Therapy with a variety of antibiotic compounds has been linked to adverse gastrointestinal side-effects ranging from abdominal discomfort to antibiotic-associated diarrhoea and antibiotic-associated colitis (Donta 1977; Mogg et al 1979). These effects appear to be related to the colonic overgrowth of the bacterium *Clostridium difficile* and its subsequent toxin production (George et al 1978). In this context it is curious to note that even antibiotics considered of value in the treatment of antibiotic-associated colitis, such as vancomycin and metronidazole, have been implicated in its pathogenesis (Mogg et al 1979; Saginur et al 1980; Daly & Chowdary 1983; Johnson et al 1989; Oliva et al 1989).

It has previously been suggested from both in-vitro (Lees & Percy 1981a; Percy & Christensen 1985) and in-vivo (Itoh et al 1984; Heyman et al 1988; Pilot & Quin 1988; Caron et al 1991) evidence that antibiotic-induced alterations in the functioning of intestinal smooth muscle and its intrinsic innervation might contribute to pathogenesis of antibiotic-associated diarrhoea and colitis. Antibiotic-induced depression of intestinal motility, particularly the muscularis mucosae, may facilitate antibiotic-resistant bacterial overgrowth leading to colitis (Lees & Percy 1981a; Percy & Christensen 1985). Additional effects may include a reduction in transit time resulting in reduced water absorption, thus precipitating diarrhoea. A second and equally important component of the gut that has not been rigorously investigated as a target for the non-antibacterial actions of this group of compounds is the mucosa. Epithelial ion transport across the mucosa is under continuous independent neurohumoral modulation (Cooke 1986). Since many antibiotics are known to alter neurotransmission in other systems, this raised the possibility that the intestinal mucosa might be similarly affected. It was surprising to find, therefore, that although the effects of certain antibiotics on respiratory and bladder epithelia have been described (Wills 1981; Cloutier et al 1990; Tamaoki et al 1992), their actions on intestinal absorption and secretion have not yet been studied in a rigorous and systematic manner.

Early in-vitro studies suggested that neomycin could stimulate the uptake of D-glucose by rabbit small intestinal brush-border membranes because of the positively charged nature of the antibiotic molecule (Lemaire et al 1982). In contrast, candicidin, a polyene antibiotic, reduced glucose transport by rat small intestinal epithelium because of increased Na^+ movement across the apical membrane producing a decrease in its electrochemical gradient (Roschina et al 1980). Apical application of duramycin, a peptide antibiotic, has been shown to enhance Na^+ absorption by cultured human colonic epithelial cells (Roberts et al 1991). Clindamycin is known to depress water absorption in the rat jejunum and proximal colon while inducing net fluid secretion in the ileum (Giannella et al 1981). The mechanism by which this effect is achieved has not

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been elucidated. Most recently it has been demonstrated in-vitro that the presence of azlocillin and tobramycin in the bathing medium enhances the responses of the rat rectal colonic mucosa to vasoactive intestinal polypeptide, without affecting basal short circuit current (Schulzke et al 1995). Again, the basis for this phenomenon is unclear.

Thus, on the basis of these previous observations the aim of this study was to investigate in-vitro the effects of several antibiotics on colonic mucosal function. Colonic epithelial ion transport is normally regulated by cholinergic and non-cholinergic nerves (Cooke 1986) and humoral agents such as prostaglandin E_2 (PGE_2) (Goldhill et al 1993). Thus the present study investigated the effects of a variety of antibiotics on rabbit colonic epithelial responses to exogenous acetylcholine, PGE_2 and electrical field stimulation (EFS).

Methods and Materials

Male New Zealand White rabbits (3–4 kg) were killed by pentobarbitone overdose (60 mg kg^{-1}). The abdomen was opened along the linea alba and the distal 5 cm of colon, immediately proximal to the pelvic brim, was removed. Tissues were opened along their mesenteric border and rinsed in Krebs solution to remove residual faecal material. The preparation was then pinned, mucosal surface down, in a Petri dish in oxygenated Krebs solution. The muscularis propria was removed by sharp dissection and the remaining tissue mounted between two halves of an Ussing chamber. Both halves of the chamber were circulated with oxygenated Krebs solution. Potential difference was monitored using one pair of agar salt bridges connected to a voltage-current clamp (DVC 1000, WPI, Sarasota, FL, USA). A pair of current-passing electrodes was connected to this apparatus and short circuit current (SCC) automatically determined. Compensation for fluid resistance and potential difference arising from electrode asymmetry was made before mounting tissues. At 1-min intervals the current required to alter the clamping potential from 0–5 mV was determined and tissue resistance calculated using Ohm's law.

Effects of antibiotics on epithelial responses to acetylcholine and PGE_2

Once the SCC had reached a stable value tissues were pretreated for 25 min with distilled water (vehicle control) or with one of the antibiotics ampicillin (0·1 mg mL^{-1}), clindamycin (0·1 mg mL^{-1}), erythromycin (0·1 mg mL^{-1}), gentamicin (0·1 mg mL^{-1}), lincomycin (1 mg mL^{-1}), or vancomycin (1 mg mL^{-1}). In one series of experiments the antibiotics were added to the serosal solution, in a second series to the mucosal solution. This paradigm mimics the effects of both systemic administration and oral ingestion. Antibiotic concentrations used were based on those previously found to be effective on a variety of intestinal nerve muscle preparations (Lees & Percy 1981a, b; Percy & Christensen 1985) and are expressed as equivalent potency. Acetylcholine (10^{-9}–10^{-3} M) or PGE_2 ($2{\cdot}8 \times 10^{-9}$–$2{\cdot}8 \times 10^{-6}$ M) were then added to the serosal solution for 5 or 10 min, respectively, in a non-cumulative fashion. The serosal solution (with or without antibiotic) was replaced after each concentration of either acetylcholine or PGE_2, whereas the mucosal solution (with or without antibiotic) was replaced between concentration–response curves.

Effects of antibiotics on epithelial responses to stimulation of secretomotor neurons

Electrical field stimulation (EFS) of submucosal nerves was performed as previously described (Biagi et al 1990). Square wave electrical pulses from a stimulator (Grass S88, Quincy, MA, USA) were passed through a stimulus isolation unit (Grass SIU5) to a pair of aluminium foil electrodes parallel to the plane of the tissue, juxtaposed between the chamber halves and the serosal surface. Stimulus pulses with a duration of 0·5 ms, an amplitude of 40 V and a frequency of 10 Hz were applied for 2-min periods. These conditions were chosen to ensure that all secretomotor neurons were activated and, therefore, that inter-neuronal transmission was not a factor in the overall response. It has previously been shown that epithelial responses under these stimulus conditions are tetrodotoxin-sensitive and result from neuronal activation (Biagi et al 1990).

To determine the effects of individual antibiotics on electrically evoked epithelial responses two trains of pulses were applied sequentially. Following the first train, SCC was allowed to return to basal levels and the antibiotic under study was applied to either the serosal or mucosal solution. EFS was repeated after a further 25-min equilibration period. In preliminary experiments it was shown that responses to repeated trains of EFS separated by a minimum of 10 min were unchanged, and that distilled water vehicle had no measurable effect on the epithelial response to EFS.

Statistical analysis

Changes in SCC elicited by drug application or EFS were expressed as $\mu A\ cm^{-2}$. When concentration–response curves were compared, multivariate analysis of variance was performed. The effects of antibiotics on basal parameters and the response to EFS were assessed statistically using a Student's paired *t*-test. In each case a P value of less than 0·05 was considered to demonstrate a significant difference.

Drugs and solutions

All experimental protocols were performed using a Krebs solution of composition (mM): NaCl, 118·5; KCl, 4·75; $CaCl_2$, 2·54; NaH_2PO_4, 1·19; $MgSO_4$, 1·19; $NaHCO_3$, 25·0; glucose, 11·0. Acetylcholine chloride was dissolved in Krebs solution adjusted to pH 4·0 with 0·1 M HCl. Fresh solutions were prepared each day. PGE_2 (Advanced Magnetics, Cambridge, MA, USA) was dissolved in a modified Krebs solution of composition (mM): NaCl, 143; KCl, 4·75; $CaCl_2$, 2·54. Dilutions were prepared daily. The PGE_2 stock solution was stored at −20°C when not in use. All drugs were added to the circulating Krebs solution in a volume of 1% of the total volume. With the exception of PGE_2 all chemicals were obtained from Sigma, (St Louis, MO, USA).

The experiments were performed in accordance with the principles described in the Guide for the Care and Use of Laboratory Animals, Publication No. DHHS (NIH) 86–23.

Results

Effects of antibiotics on basal SCC

Serosal but not mucosal addition of either clindamycin or lincomycin reduced basal SCC by $31{\cdot}6 \pm 9{\cdot}5\%$ ($n = 9$; $P < 0{\cdot}05$) and $39{\cdot}3 \pm 8{\cdot}5\%$ ($n = 7$; $P < 0{\cdot}01$), respectively. In both cases tissue resistance was unaltered. The addition of

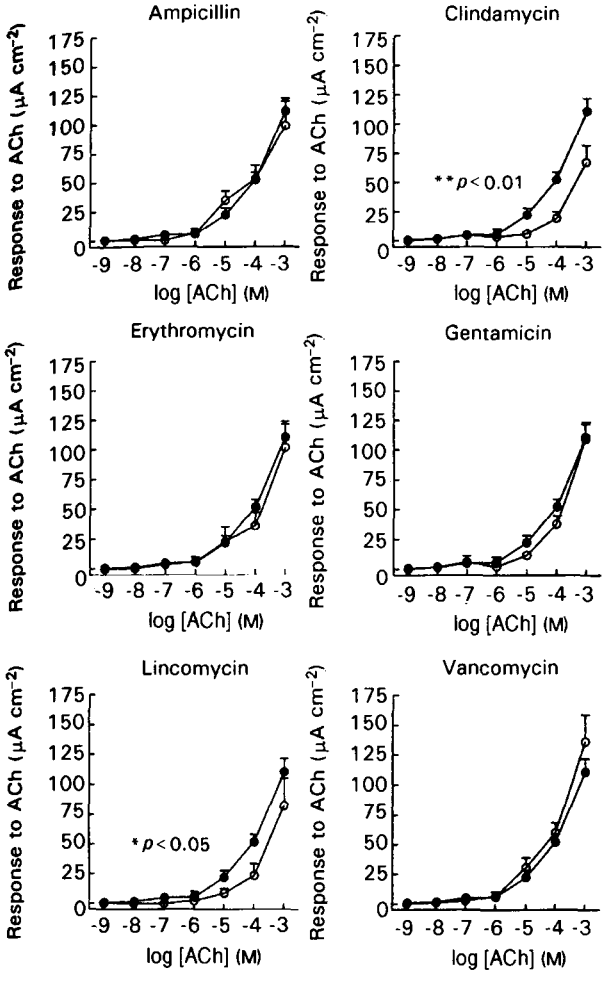

FIG. 1. Effects of ampicillin (0·1 mg mL^{-1}), clindamycin (0·1 mg mL^{-1}), erythromycin (0·1 mg mL^{-1}), gentamicin (0·1 mg mL^{-1}), lincomycin (1 mg mL^{-1}) and vancomycin (1 mg mL^{-1}) on rabbit distal colonic epithelial responses to acetylcholine (ACh). Antibiotics were added to the serosal solution. Antibiotic ○, control ●. Data are expressed as peak changes in short circuit current over baseline (μA cm^{-2}).

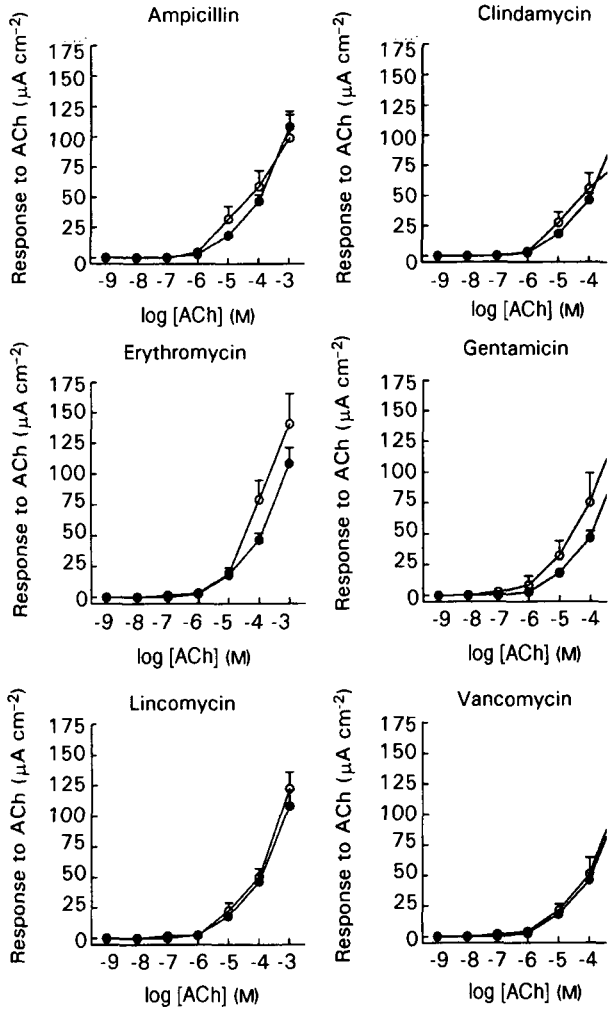

FIG. 2. Effects of ampicillin (0·1 mg mL^{-1}), clindamycin (0·1 mg mL^{-1}), erythromycin (0·1 mg mL^{-1}), gentamicin (0·1 mg mL^{-1}), lincomycin (1 mg mL^{-1}) and vancomycin (1 mg mL^{-1}) on rabbit distal colonic epithelial responses to acetylcholine (ACh). Antibiotics were added to the mucosal solution. Antibiotic ○, control ●. Data are expressed as peak changes in short circuit current over baseline (μA cm^{-2}).

drug vehicle had no measurable effect on any electrical parameter. None of the other antibiotics studied significantly altered basal SCC.

Effects of antibiotics on epithelial responses to acetylcholine

Acetylcholine caused a concentration-dependent increase in SCC (Figs 1, 2). Serosal but not mucosal application of clindamycin or lincomycin significantly reduced this effect (Fig. 1). None of the other antibiotics studied had a significant effect on acetylcholine-induced responses (Figs 1, 2).

Effects of antibiotics on epithelial responses to PGE_2

PGE_2 caused a concentration-dependent increase in SCC (Figs 3, 4). Serosal but not mucosal application of vancomycin significantly increased this effect (Fig. 3). None of the other antibiotics tested significantly altered the responses of this tissue to PGE_2 (Figs 3, 4).

Effects of antibiotics on epithelial responses to EFS

In the absence of any antibiotic, EFS increased SCC (115·6 ± 9·0 μA cm^{-2}; n = 31). Serosal but not mucosal addition of clindamycin, erythromycin, gentamicin and lincomycin each reduced the response to EFS (Table 1). Neither ampicillin nor vancomycin measurably altered the epithelial response to EFS when applied either serosally or mucosally.

Discussion

The effects of several classes of antibiotic compound on the innervation and motor responses of the guinea-pig ileum, rabbit colon and opossum colonic muscularis mucosae have previously been characterized (Lees & Percy 1981a,b; Percy & Christensen 1985). This study demonstrates that, in general, these same pharmacological agents also affect rabbit colonic epithelial ion transport.

The rabbit colonic epithelium is innervated by cholinergic secretomotor fibres which, in part, contribute to the secretory response to EFS (Biagi et al 1990). In this study the colonic epithelial response to EFS and to exogenous acetylcholine was attenuated by the serosal application of clindamycin and

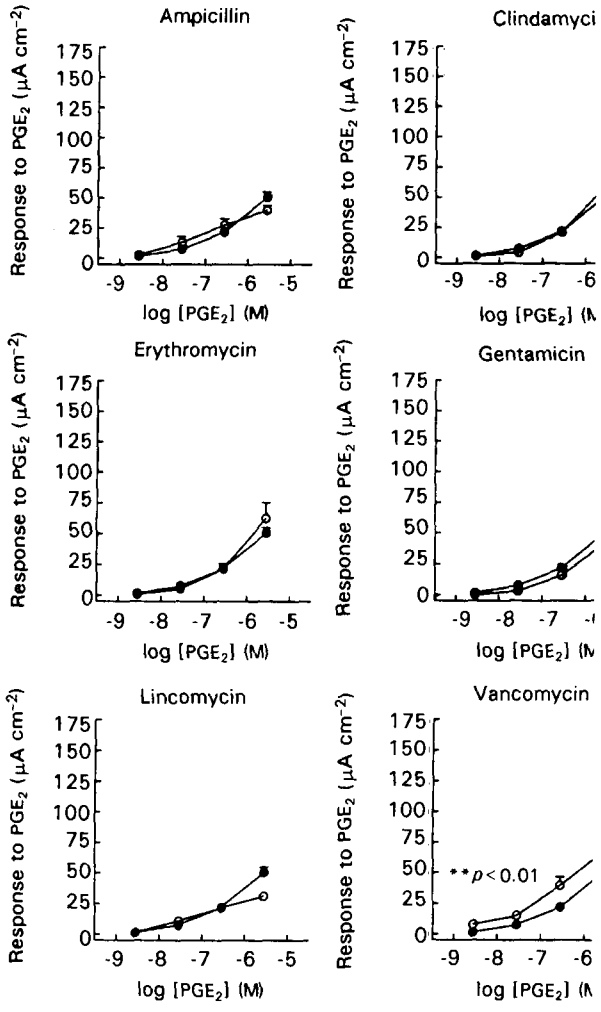

FIG. 3. Effects of ampicillin (0·1 mg mL^{-1}), clindamycin (0·1 mg mL^{-1}), erythromycin (0·1 mg mL^{-1}), gentamicin (0·1 mg mL^{-1}), lincomycin (1 mg mL^{-1}) and vancomycin (1 mg mL^{-1}) on rabbit distal colonic epithelial responses to prostaglandin E_2 (PGE_2). Antibiotics were added to the serosal solution. Antibiotic ○, control ●. Data are expressed as peak changes in short circuit current over baseline ($\mu A\ cm^{-2}$).

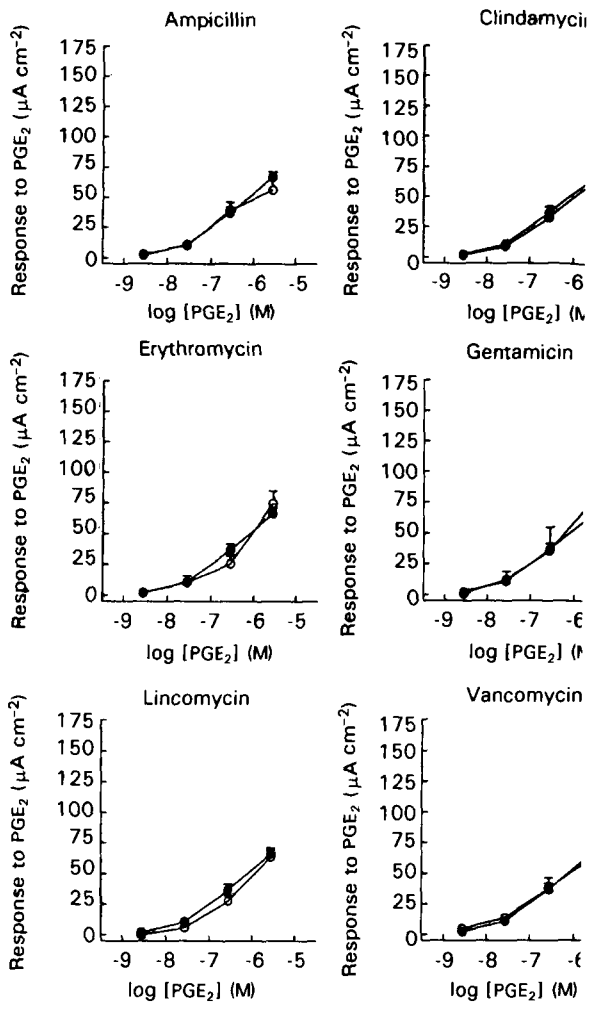

FIG. 4. Effects of ampicillin (0·1 mg mL^{-1}), clindamycin (0·1 mg mL^{-1}), erythromycin (0·1 mg mL^{-1}), gentamicin (0·1 mg mL^{-1}), lincomycin (1 mg mL^{-1}) and vancomycin (1 mg mL^{-1}) on rabbit distal colonic epithelial responses to prostaglandin E_2 (PGE_2). Antibiotics were added to the mucosal solution. Antibiotic ○, control ●. Data are expressed as peak changes in short circuit current over baseline ($\mu A\ cm^{-2}$).

lincomycin. This suggests that these lincosamides depress neuro-epithelial cholinergic neurotransmission via a post-junctional action. Similar actions of clindamycin have been reported in both the longitudinal muscle of the guinea-pig ileum (Lees & Percy 1981a, b) and opossum colonic muscularis mucosae (Percy & Christensen 1985). In contrast to rabbit colonic epithelium, lincomycin does not, however, alter the response of the opossum colonic muscularis mucosae to acetylcholine. It is not possible from these data to determine whether these drugs have additional pre-junctional actions on cholinergic neuroepithelial transmission, because such an action would be masked by its post-junctional effects. Multiple sites of action of this antibiotic cannot, however, be excluded because it has local anaesthetic-like actions (Wright & Collier 1976) and reduces neurotransmitter release (Singh et al 1978) at the skeletal neuromuscular junction.

A further epithelial effect of the lincosamide class of antibiotic was to reduce basal SCC. Cholinergic fibres do not contribute to basal epithelial activity in the rabbit distal colon (Goldhill et al 1993), which provides additional evidence that both clindamycin and lincomycin have effects other than those on cholinergic neurons. Further studies are required to determine the mechanism(s) underlying this observation and to assess whether it represents alterations in either or both sodium absorption and chloride secretion. It would, however, appear that electrogenic sodium absorption is unaffected by clindamycin or lincomycin as the response to the sodium channel blocker, amiloride, was not changed by either of these antibiotics (unpublished observations).

It is of note that colonic epithelial ion transport is not altered by the mucosal addition of lincosamides. This is similar to previous reports describing pro-secretory effects of clindamycin on fluid transport only at high concentrations (500 $\mu g\ mL^{-1}$) (Giannella et al 1981). Those authors did, however, report that mucosal administration of clindamycin caused fluid secretion in the small intestine and speculated that this may contribute to antibiotic-associated diarrhoea. It has previously been suggested that antibiotic-induced inhibition of

Table 1. Effects of antibiotics on epithelial responses to electric field stimulation. Tissues were stimulated by two consecutive trains of pulses. The first was a control pulse whereas the second was applied 25 min after treatment with an antibiotic administered either serosally or mucosally. The difference in the response to these two trains of stimulation (μA cm^{-2}) are given.

	Ampicillin (0·1 mg mL^{-1})	Clindamycin (0·1 mg mL^{-1})	Erythromycin (0·1 mg mL^{-1})	Gentamicin (0·1 mg mL^{-1})	Lincomycin (1 mg mL^{-1})	Vancomycin (1 mg mL^{-1})
Serosal (n = 5)	7·96 ± 2·65	− 44·24 ± 15·04*	− 59·29 ± 7·07**	− 19·46 ± 7·07*	− 15·92 ± 2·65**	8·84 ± 6·19
Mucosal (n = 5)	7·16 ± 9·55	12·3 ± 10·20	1·06 ± 7·16	8·84 ± 12·56	− 3·53 ± 2·83	0·97 ± 10·79

Responses to two control trains of stimuli applied 25 min apart did not differ significantly from one another. Data were analysed using a paired Student's t-test. Data differing significantly from control are indicated as *$P < 0·05$; **$P < 0·01$.

muscularis propria or muscularis mucosae movement may increase bacterial adherence and proliferation (Lees & Percy 1981a; Percy & Christensen 1985). This in turn has the potential to precipitate the onset of antibiotic-associated colitis, a second side-effect of the lincosamides. Depression of colonic epithelial secretion could amplify this effect by further facilitating bacterial adherence. Serum concentrations of clindamycin achieved following intravenous or intramuscular administration (Sande & Mandell 1990) approach those used in this study, and the effects of clindamycin described herein are, therefore, clinically relevant. Development of clindamycin analogues without these effects would, furthermore, represent a major step forward in antimicrobial therapy, because although clindamycin is a highly favourable antibiotic, it is used with caution because of the incidence of antibiotic-associated colitis (up to 10%) and antibiotic-associated diarrhoea (up to 20%) (Sande & Mandell 1990).

It has previously been shown that erythromycin alters opossum colonic muscularis mucosae function, depressing spontaneous contractile activity and the tissue's responses to acetylcholine, while causing little change in the muscle's response to electrical stimulation of its intrinsic innervation. In contrast, erythromycin attenuated the SCC response of the rabbit distal colonic epithelium to EFS without affecting basal SCC or its responses to acetylcholine. Thus, the data from this study provide no support for a post-junctional inhibitory effect of erythromycin on this system. In this respect the actions of erythromycin on the epithelium differ from its effects on the opossum colonic muscularis mucosae. It has, however, previously been suggested that, in addition to its post-junctional effects, erythromycin has a pre-junctional inhibitory effect on the release of substance P and of acetylcholine from nerves in the myenteric plexus of the guinea-pig ileum (Minocha & Galligan 1991). The data from the present study are consistent with erythromycin exhibiting similar pre-junctional effects on the rabbit colonic submucosal plexus. The inhibitory effect that erythromycin has on the epithelial response to EFS could result in a reduction of fluid secretion and, as described for the lincosamides, facilitate bacterial adherence to the gut wall and thus contribute to the development of colitis.

It is important to note that the incidence of antibiotic-associated colitis is approximately 5-fold less after treatment with erythromycin than after that with clindamycin (Bartlett 1981). It is possible that this relates to the observation that clindamycin inhibits both basal and nerve-stimulated epithelial transport, whereas erythromycin only affects the latter. Like clindamycin, serum concentrations of erythromycin achieved after intravenous administration (Sande & Mandell 1990) approach those used in this study, illustrating that, as with clindamycin, the epithelial effects of erythromycin described here are of clinical relevance.

When added to the serosal solution, gentamicin attenuated the epithelial response to EFS without reducing its responses to acetylcholine. This effect is similar to that of erythromycin and, likewise, may reflect pre-junctional depression of cholinergic and/or non-cholinergic neuroepithelial transmission. This conclusion is supported not only by evidence from the skeletal neuromuscular junction (Timmerman et al 1959; Brazil & Prado-Franceshi 1969; Dretchen et al 1972), but also by its actions on the myenteric plexus of the guinea-pig ileum (Lees & Percy 1981a, b) and the opossum colonic muscularis mucosae (Percy & Christensen 1985). Although qualitatively similar, in the present study the effects of gentamicin were found to be very much smaller than those of erythromycin. It is interesting to note, therefore, that whereas antibiotic-associated colitis is occasionally seen after erythromycin treatment, gentamicin is not generally considered to have significant gastrointestinal side-effects (Bartlett 1981).

Prostaglandins are known to cause fluid secretion and are thought to contribute to the diarrhoea observed in many diseases of the intestinal tract (Rask–Madsen 1987). Antibiotic-associated colitis often occurs after administration of lincosamides, and results from the overgrowth of antibiotic-resistant *Clostridium difficile*. The increased colonic PGE_2 levels measured during *C. difficile* colitis may contribute to the diarrhoea associated with this disease (Lauritsen et al 1988).

Vancomycin is often used in the treatment of *C. difficile* colitis. As we have shown that vancomycin augments the epithelial response to PGE_2, a paradoxical situation may arise in clinical practice whereby the secretory response to increased PGE_2 levels is enhanced during antimicrobial therapy. In other words the anti-diarrhoeal effects of vancomycin may be self-limiting. Despite this observation, vancomycin remains the antibiotic of choice in the treatment of antibiotic-associated colitis and it is possible that part of its efficacy results from an increase in fluid secretion resulting in a decreased ability of *C. difficile* to adhere to the colonic epithelium. The mechanism by which vancomycin enhances the epithelial effects of PGE_2 is unclear from this study and has not been previously reported. This effect is, however, likely to be clinically relevant because very high levels of vancomycin are present in stool following oral administration of the drug for the treatment of *C. difficile* colitis (Sande & Mandell 1990).

In summary, the data from this study demonstrate two types of interaction between antibiotics and colonic epithelial ion transport. Clindamycin, lincomycin, erythromycin and gentamicin all have effects consistent with reduced secretion. Each of these antibiotics also depresses the motor function of the

muscularis mucosae and we speculate that, in certain clinical situations, these effects in tandem may facilitate overgrowth of colitis-inducing bacteria that are resistant to antimicrobial treatment.

Clindamycin affects colonic ion transport and muscle function by a variety of mechanisms; in contrast, gentamicin acts through only one pathway, namely depression of secretomotor neuron function. It is interesting to note, therefore, that this complexity is mirrored by the relative risk of developing colitis after treatment with these antibiotics. Whereas there may be a correlation between risk of colitis and the non-antimicrobial actions of certain antibiotics, other factors are clearly associated with the etiology of antibiotic-associated colitis. This is exemplified by ampicillin, which is often implicated in antibiotic-associated colitis (Bartlett 1981), but which does not appear to alter ion transport and has little or no effect on small and large intestinal motility or movement of the muscularis mucosae (Lees & Percy 1981a; Percy & Christensen 1985). In contrast, the epithelial effects of vancomycin are consistent with a pro-secretory action. Whereas this may limit the ability of vancomycin to reduce diarrhoea, it may hasten the expulsion of *C. difficile* effectively attenuating its damaging effects on the colonic mucosa.

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