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Antidiarrhoeal activity of loperamide: studies of its influence on ion transport across rabbit ileal mucosa in vitro

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SUMMARY Loperamide is a well-established antidiarrhoeal agent with effects on gastrointestinal motility. We have now shown that the drug influences ion transport. In isolated rabbit ileal mucosa loperamide caused a dose-related fall in potential difference and short-circuit current and reduced the serosa to mucosa flux of chloride. The electrical effects were inhibited by naloxone (10^{-6}M) suggesting that they were mediated by opiate receptors. Loperamide (10^{-6}M) inhibited secretion provoked by heat stable and heat labile *E. coli* toxins and by prostaglandin E_2. We conclude that loperamide is able to inhibit secretion mediated by cAMP or cGMP, and that this may be relevant to its antidiarrhoeal properties.

The antidiarrhoeal activity of opiates has long been thought to depend on their ability to influence intestinal motility. The recent demonstration, however, that morphine and certain enkephalins can influence ion transport across intestinal epithelium has prompted a re-examination of this hypothesis. Morphine enhances absorption of chloride across isolated rabbit ileal mucosa[1] and inhibits secretion provoked by a variety of secretagogues,[2] so that this antisecretory activity may be at least partly responsible for the antidiarrhoeal effect.

The commonly used antidiarrhoeal drug loperamide is an opiate derivative, which does not have central nervous system effects.[3] Like morphine it has been thought to exert its actions through effects on intestinal motility[4] but we demonstrate here that it also has antisecretory effects in isolated rabbit ileum.

Methods

Details of the methods used have been described previously[5] but, briefly, male New Zealand white rabbits (2 to 4 kg) were killed by air embolus and the distal ileum rapidly removed and bathed in oxygenated Ringer's bicarbonate buffer. Mucosa stripped of muscle layers was mounted in modified Ussing chambers[6] and bathed on each side by 10 ml isotonic buffer solution, pH 7·4, containing Na 146, K 4·2, Cl 125·8, HCO_3 26·6, H_2PO_4 0·2, HPO_4 1·2, Ca 1·2, Mg 1·2, and glucose 10 mmol/l. The buffer was stirred and oxygenated *via* a bubble lift mechanism by a 95%O_2/5%CO_2 mixture and maintained at 37°C.

ELECTRICAL MEASUREMENTS

The transmucosal potential difference and short-circuit current were measured as described previously[5] and the electrical resistance calculated.

RADIO-ISOTOPIC FLUXES

Sodium and chloride fluxes were measured using 0·5 μCi ^{22}Na and 2·5 μCi ^{36}Cl (Radiochemical Centre, Amersham) added to either mucosal or serosal reservoirs 20 minutes after mounting the tissue. After a further 20 minute equilibration period serial 1 ml samples were taken at 20 minute intervals, and replaced with 1 ml unlabelled buffer solution.

Eight pieces of mucosa from one rabbit were mounted in each experiment and these were paired provided that their resistances differed by less than 25%.[7] Unidirectional and net fluxes and residual ion fluxes were calculated as described previously.[5]

Pure loperamide hydrochloride powder was dissolved in Ringer's bicarbonate buffer with ethanol (4 ml/l) to improve solubility. The final ethanol concentration in the test and control chambers was 0·004%.

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In each experiment, after 20 minutes for equilibration, ion fluxes were determined over a 20 minute control period and then during three consecutive 20 minute flux periods after the addition of the drug or, to the controls, ethanol. In experiments with heat-labile *E. coli* toxin, which has a delayed action, the toxin was left in contact with the mucosa for 70 minutes before the three 20 minute flux periods began; loperamide was added 30 minutes before these flux periods. In experiments with prostaglandin E_2 or heat-stable *E. coli* toxin the secretagogue and loperamide were added simultaneously after the control flux period, immediately before the first of the three 20 minute flux periods. In each instance control fluxes were compared with the third flux period after loperamide.

Loperamide was kindly provided by Janssen Pharmaceuticals Ltd and the *E. coli* toxins by Dr B Drasar, London School of Hygiene and Tropical Medicine. Naloxone was obtained from Winthrop Laboratories and PGE_2 from Sigma Chemical Co.

All values are expressed as the mean ± 1 SEM. Statistical comparisons were performed using Student's *t* test for paired data. Values were held to be significant if $p<0.05$.

Results

ELECTRICAL RESPONSES TO LOPERAMIDE

After a 20 minute period of stabilisation the potential difference and short-circuit current of control tissues remained relatively constant, falling slowly by less than 1% in 10 minutes (n=20) while tissue resistance remained unchanged. Loperamide 10^{-7} and 10^{-6}M on the serosal side significantly reduced potential difference and short-circuit current ($p<0.01$) (Fig. 1) but left tissue resistance unchanged. The reduction at 10^{-8}M was not statistically significant (p 0·15). Loperamide 10^{-6}M had no effect when added to the mucosal side (Fig. 2). Pretreatment with naloxone 10^{-6}M, inhibited the fall in potential difference and short-circuit current induced by serosal loperamide 10^{-6}M ($p<0.02$) (Fig. 2), suggesting that loperamide exerted these electrical effects *via* opiate receptors.

RADIO-ISOTOPE FLUXES

Loperamide 10^{-6}M reduced the serosa to mucosa chloride flux but had no significant effect on net chloride movement (p 0·5). Short-circuit current was reduced but residual ion flux was unchanged (Table 1).

INFLUENCE OF LOPERAMIDE ON INTESTINAL SECRETION

Prostaglandin E_2 (PGE_2)

Serosally applied PGE_2 (10^{-5}M) increased potential difference, short-circuit current ($p<0.001$), and tissue resistance ($p<0.01$), the peak response occurring within 10 minutes. Simultaneous addition of loperamide 10^{-6}M did not influence the electrical response to PGE_2.

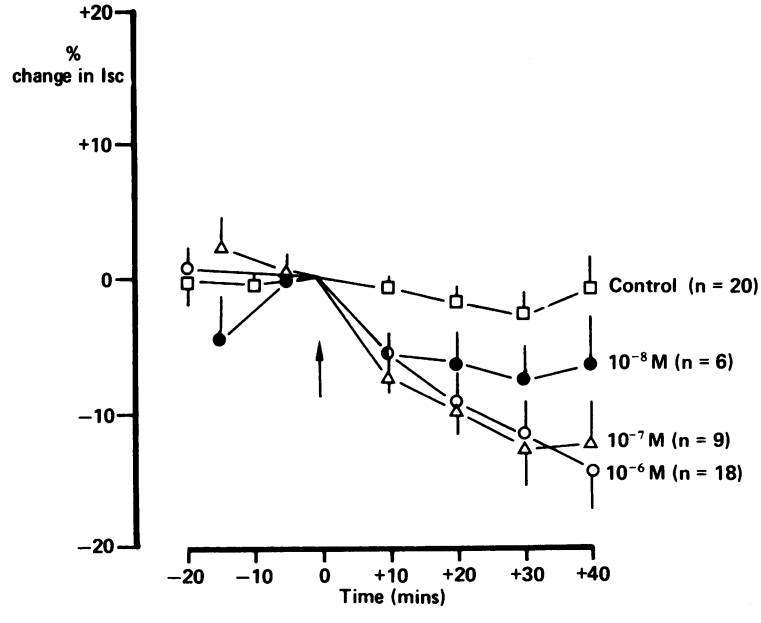

Fig. 1 *Effect on short-circuit current (Isc) of varying concentrations of loperamide on the serosal surface of rabbit ileal mucosa in vitro. Loperamide or control buffer was added as indicated by arrow.*

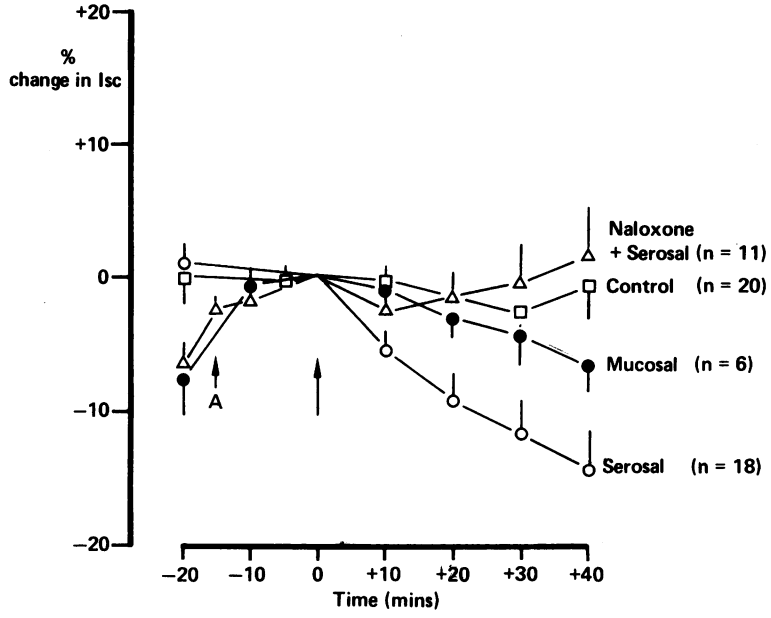

Fig. 2 *Influence of loperamide (10^{-6}M) on short-circuit current (Isc) when added to the mucosal or the serosal sides of rabbit ileal mucosa. The effect of naloxone added at point A on the response to serosal loperamide is shown. Loperamide or control buffer was added as indicated by arrow.*

PGE_2 decreased net sodium absorption by decreasing the mucosa to serosa sodium flux and induced net chloride secretion by increasing the serosa to mucosa chloride flux (Table 2). Short-circuit current and residual ion flux were both increased. Loperamide (10^{-6}M), added with PGE_2 (10^{-5}M), reduced the fall in net sodium absorption and prevented net chloride secretion and the rises in short-circuit current and residual ion flux induced by PGE_2 (Table 2).

Heat-labile E. coli toxin

The addition of 200 μl of a crude toxin-containing medium to the mucosal side of control tissues increased potential difference and short-circuit current within 10 minutes, increasing further over the next two hours. Loperamide (10^{-6}M) added 40 minutes after the toxin did not significantly influence this electrical response.

Unidirectional flux determinations 110 to 130 minutes after toxin addition showed a reduced net

Table 1 *Unidirectional and net Na and Cl fluxes over 20 minute periods immediately before and from 20 to 40 minutes after addition of (A) control solution or (B) loperamide*

	Na			*Cl*				
	ms	*sm*	*Net*	*ms*	*sm*	*Net*	*Isc*	J^RNet
n=5								
(A) Control solution								
Before	16·43	9·88	+6·55	9·24	8·03	+1·67	4·17	+2·32
	±1·51	±1·12	±0·89	±0·5	±0·57	±0·89	±0·26	±1·79
After	14·93	8·70	+6·51	8·70	7·99	+0·71	4·37	−1·30
	±1·29	±0·77	±1·48	±0·66	±0·41	±0·39	±0·36	±1·78
(B) After loperamide 10^{-6}M								
Before	16·57	10·50	+6·07	11·12	9·61	+1·52	5·33	+0·79
	±0·72	±0·46	±0·83	±0·65	±0·59	±0·30	±0·59	±0·99
After	14·88	10·24	+5·45	10·49	9·12**	+0·97	4·85*	+0·56
	±0·67	±0·90	±1·13	±0·72	±0·22	±0·72	±0·61	±2·03

Fluxes in μmol (μEq)/cm^2/h. + = net absorption. − = net secretion. ms = mucosa to serosa flux. sm = serosa to mucosa flux. Isc = short-circuit current. J^RNet = residual ion flux.
**p<0·02. *p<0·05. Significance of difference between flux periods before, and after addition of control or loperamide solution. Differences between fluxes not indicated by asterisks were not statistically significant – that is, p≥0·1.

Table 2 *Unidirectional and net Na and Cl fluxes over 20 minute periods immediately before and from 40 to 60 minutes after addition of (A) PGE_2 or (B) PGE_2 + loperamide*

	Na			*Cl*				
	ms	*sm*	*Net*	*ms*	*sm*	*Net*	*Isc*	J^RNet
n=6								
(A)								
Control	18·34	11·19	+7·14	11·6	9·72	+1·89	5·02	−0·23
	±1·64	±0·93	±0·97	±0·48	±0·67	±0·7	±0·76	±1·07
PGE_2	14·04*	11·64	+2·39**	10·44	11·55*	−1·20***	6·73***	+3·15*
	±0·94	±0·75	±1·07	±0·34	±1·28	±0·99	±0·87	±0·88
n=9								
(B)								
Control	17·88	10·73	+7·16	10·54	8·24	+2·29	5·16	+0·30
	±1·10	±0·93	±0·88	±0·51	±0·26	±0·48	±1·72	±1·01
PGE_2 + loperamide 10^{-6}M	15·69	10·22	+5·47***†	10·72	9·64***	+1·08	6·08	+1·69
	±0·97	±0·60	±0·82	±0·84	±0·54	±0·92	±0·64	±1·07

Fluxes in μmol (μEq)/cm²/h. + = net absorption. − = net secretion. ms = mucosa to serosa flux. sm = serosa to mucosa flux. Isc = short-circuit current. J^RNet = residual ion flux.
***p<0·01. **p<0·02. *p<0·05. Significance of difference from its paired control. †p<0·025 – significance of difference from response to PGE_2 alone.

sodium absorption, due to a decrease in mucosa to serosa flux, and a net chloride secretion due to a reduction in the mucosa to serosa chloride flux. Loperamide (10^{-6}M) added 40 minutes after the toxin – that is, 30 minutes before flux determinations – reduced these flux responses (Table 3).

Heat-stable E. coli toxin

Addition of 200 μl of a crude toxin-containing medium to the mucosal side caused an immediate increase in potential difference and short-circuit current but left tissue resistance unchanged. Similar electrical effects were obtained using a similar medium without toxin. Loperamide (10^{-6}M) added simultaneously did not modify the electrical response to the toxin.

The control medium had no effect on sodium or chloride fluxes but the toxin solution decreased net sodium absorption by reducing the mucosa to serosa and increasing the serosa to mucosa sodium fluxes, although neither of the unidirectional flux changes were individually significant. The toxin almost abolished net chloride absorption because of an increase in serosa to mucosa chloride flux. Simultaneous addition of loperamide (10^{-6}M) to the serosal side prevented the changes in chloride transport induced by the toxin on the mucosal side but sodium absorption was still reduced (Table 4).

Table 3 *Unidirectional and net Na and Cl fluxes over 20 minute periods immediately before and from 70 to 90 minutes after addition of (A) E. coli heat-labile toxin or (B) E. coli heat-labile toxin + loperamide*

	Na			*Cl*				
	ms	*sm*	*Net*	*ms*	*sm*	*Net*	*Isc*	J^RNet
n=6								
(A)								
Control	13·54	8·39	+5·14	10·43	8·45	+1·98	3·55	+0·39
	±0·98	±0·59	±0·76	±0·70	±0·40	±0·90	±0·22	±0·67
Heat-labile toxin	11·01*	9·06	+1·96***	8·66*	10·16	−1·49*	4·78**	+1·33
	±1·37	±0·70	±0·99	±0·82	±0·70	±0·72	±0·47	±0·82
n=6								
(B)								
Control	13·61	9·39	+4·25	11·66	9·92	+1·74	4·76	+2·25
	±1·81	±0·85	±1·04	±1·04	±0·68	±1·06	±0·82	±1·01
Heat-labile toxin + loperamide	13·10	10·37	+3·46	10·61	10·93	−0·32	6·13	+2·74
	±1·33	±1·02	±1·43	±1·27	±1·70	±0·83	±1·21	±0·94

Fluxes in μmol (μEq)/cm²/h. + = net absorption. − = net secretion. ms = mucosa to serosa flux. sm = serosa to mucosa flux. Isc = short-circuit current. J^RNet = residual ion flux.
***p<0·01. **p<0·02. *p<0·05.

The toxin caused an increase in short-circuit current but not residual ion flux, whereas toxin and loperamide together caused an increase in both short-circuit current and residual ion flux (Table 4).

Discussion

Loperamide is a butyramide derivative and an opiate agonist[8] which improves the diarrhoea due to many causes.[9–14] It has profound effects on intestinal motility causing a dose-related inhibition of longitudinal and circular muscle activity during pressure-induced peristaltic reflexes in guinea-pig isolated ileum.[15] It slows the progression of ingested charcoal through the gastrointestinal tract of mice.[3] The antidiarrhoeal effect of loperamide was attributed to these actions on motility.[4] The observations that prostaglandin and bisacodyl-induced secretion in rats was inhibited by loperamide,[10 17] however, and that atropine, although moderately effective in reducing peristalsis, is not an effective antidiarrhoeal agent[16] suggest that loperamide may also have effects on intestinal absorption or secretion. Sandhu *et al*[18] showed that pretreating rats with loperamide inhibited secretion induced *in vivo* by cholera toxin and prostaglandin E_2 supporting this possibility. Our data *in vitro* clearly indicate that loperamide inhibits the secretion provoked by three different types of secretagogue in an isolated preparation of ileal mucosa where effects on motility can be entirely obviated. These results are in keeping with recent reports from this laboratory[1 2] and elsewhere[19 20] that opiates affect intestinal absorption and inhibit secretion. Morphine stimulated chloride absorption under basal conditions[1] and inhibited the secretion due to cholera toxin, prostaglandin E_2, or acetylcholine.[2]

In the present study, loperamide did not influence net transport in unstimulated tissue, although it reduced serosa to mucosa chloride fluxes. Loperamide, however, slightly reduced the potential difference and short-circuit current in these tissues, and presumably the ion transport effects, which must have accompanied this electrical response, were small and difficult to detect by our technique. There is little doubt, however, that loperamide inhibits the actively secreting mucosa. Prostaglandin E_2 and heat labile *E. coli* toxin stimulate secretion by activating cAMP production,[21 22] while heat stable *E. coli* toxin is believed to exert its effect by stimulating cGMP production.[23] Thus the antisecretory effect of loperamide cannot be explained simply by inhibition of one of these nucleotides alone. The increased cAMP content of intestinal mucosa after cholera toxin stimulation was not inhibited by loperamide in one study,[18] suggesting that the antisecretory effect was exerted at a site distal to the activation of cAMP; this may be a site common to both cAMP and cGMP.

Naloxone inhibited the electrical effects of

Table 4 *Unidirectional and net Na and Cl fluxes over 20 minute periods immediately before and from 60 to 80 minutes after addition of (A) toxin-free control medium, (B) E. coli heat-stable toxin and (C) toxin + loperamide*

	Na			*Cl*				
	ms	*sm*	*Net*	*ms*	*sm*	*Net*	*Isc*	J^RNet
n=5								
(A)								
Control	16·95	11·30	+5·66	12·46	10·58	+1·88	4·27	+0·49
	±1·77	±1·06	±0·82	±0·88	±1·14	±0·93	±0·75	±0·34
Control medium	17·25	10·30	+7·55	13·17	10·83	+2·97	5·59	−1·24
	±2·19	±1·27	(±1·27)	±1·72	±1·51	(±0·15)	±0·75	(±0·01)
n=8								
(B)								
Control	15·89	9·90	+7·24	11·99	10·09	+2·70	4·88	−0·46
	±2·26	±0·97	±1·23	±1·13	±0·52	±0·72	±0·69	±0·87
Heat-stable toxin	14·13	10·52	+4·86*	11·59	11·48*	+0·53**	6·23**	+0·72
	±2·14	±0·83	±1·54	±1·03	±0·91	±0·80	±1·00	±1·27
n=5								
(B)								
Control	16·55	9·43	+7·73	12·32	9·35	+2·98	4·28	+0·96
	±0·64	±0·73	±1·14	±0·93	±0·52	±0·84	±0·26	±1·20
Heat-stable toxin + loperamide	14·27***	9·16	+5·11**	12·73	10·39	+2·95	5·85***	+3·08*
	±0·65	±0·43	±0·83	±0·99	±0·56	±1·07	±0·54	±0·94

Fluxes in μmol (μEq)/cm²/h. + = net absorption. − = net secretion. ms = mucosa to serosa flux. sm = serosa to mucosa flux. Isc = short-circuit current. J^RNet = residual ion flux.
***p<0·01. **p<0·02. *p<0·05.

loperamide in our study and partly inhibited its antisecretory effect in Sandhu's study[18]; this suggests that opiate receptors may be involved. The observation that loperamide binds to opiate receptors in both brain and myenteric plexus of the guinea-pig[24] supports this idea. We conclude that loperamide has opiate-like antisecretory effects when applied to mucosa stimulated by secretagogues, and that this effect on ion transport contributes to its antidiarrhoeal activity.

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