Loperamide: studies on its mechanism of action

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SUMMARY The effects of loperamide on net solute and water absorption, and prostaglandin E₂ (PGE₂) and cholera toxin-induced secretion were studied in the rat jejunum using an *in vivo* steady-state perfusion technique. Loperamide stimulated absorption of fluid, electrolytes, and glucose and reversed PGE₂ and cholera toxin-induced secretion to absorption; this opiate analogue had no effect on cholera toxin stimulation of adenylate cyclase activity or the rise of tissue cyclic AMP (cAMP) concentrations. The opiate antagonist, naloxone, reduced the anti-secretory effects of loperamide without affecting tissue levels of cAMP. These results indicate that loperamide inhibits PGE₂ and cholera toxin-induced secretion, and that this phenomenon is independent of any direct effect that cholera toxin has on the adenylate cyclase system. The action of naloxone suggests, but does not prove, that loperamide exerts its effect *via* opiate receptors.

Loperamide, an opiate analogue, is a widely used anti-diarrhoeal agent and, until recently, its effects were attributed to an inhibitory action on smooth muscle tone and peristalsis mediated *via* both cholinergic and non-cholinergic systems.¹⁻³ Karim and Adaikan,⁴ however, observed that loperamide modified prostaglandin E₂ (PGE₂) induced secretion of fluid in the rat intestinal tract and they postulated that its anti-diarrhoeal action might be related not only to an effect on intestinal motility, but also on secretory processes.

We have recently been impressed by the beneficial effects of very large oral doses of loperamide (up to 4 mg/kg body weight) in some but not all infants with severe and life-threatening protracted diarrhoea (unpublished observations). On the basis of these clinical observations we suspected that the effects of the drug could not be explained solely on its action on smooth muscle, and that it was inhibiting small intestinal secretion of fluid and electrolytes. This prompted us to study the effects of loperamide, and its possible mechanism of action, on two well-established secretagogues, PGE₂ and cholera toxin, using a steady state perfusion technique in the rat jejunum *in vivo*.

The results of loperamide on cholera toxin-induced secretion have been briefly reported in a letter.⁵

Methods

PERFUSIONS

All experiments were performed on male Wistar rats weighing 220-280 g after an overnight fast, during which water was allowed *ad libitum*; the designs of the experiments are shown in the Figure. Two hours before laparotomy the animals received intragastric loperamide (4 mg/kg body weight) dissolved in 1 ml 5% (v/v) ethanol and water with omission of loperamide in controls. Anaesthesia was induced with intraperitoneal pentobarbitone (6 mg/kg), and maintained as necessary with intramuscular doses of 0.3 mg. The rectal temperature was maintained throughout the experiments at 37°C by overhead electric lamps. In the PGE₂ experiments the perfusions were essentially as described by Sladen and Harries.⁶ The abdomen was opened with a mid-line incision and 15-20 cm lengths of proximal jejunum (from the duodenojejunal junction) were prepared. An inlet catheter (vinyl tubing, bore 0.63 mm, diameter 1.4 mm, short hardness -70, portex) was inserted into the gut lumen through a 2 mm incision in the antimesenteric border of the gut and tied firmly in place with a circumferential ligature. Faecal material, if present, was removed from the loops by washing with isotonic saline at 37°C. A wider bore outlet catheter (bore 2 mm, diameter 3 mm, short hardness -70, portex) was similarly inserted into the distal end of the loop for the continuous collection of effluent and the

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Figure Experimental design: (1) effect of loperamide on absorption, and on prostaglandin E_2 (PGE_2) induced secretion; (2) effect of loperamide on cholera toxin (CT) induced secretion and of naloxone in this system.

loop was returned to the abdominal cavity. The solutions were infused continuously at 0.46 ml/min (Harvard 975 syringe pump), and were warmed by passage through the inlet catheter in a water bath at 37°C. After an equilibration period of 30 minutes, three consecutive 10 minute collections of effluent were obtained. The perfusate contained (mmol/l) NaCl (130), KCl (4), glucose (2), $NaHCO_3$ (25), and polyethylene glycol 4000 (3 g/l) with 5 μ Ci/l of 14 C polyethylene glycol; PGE_2 was added to the basic perfusate in a concentration of 0.2 mmol/l, the pH was adjusted to 7.0 with CO_2 and the osmolality of the solution was 290 mosmol/kg.

A low (2 mmol/l) concentration of glucose was chosen, as luminal disappearance will reflect active transport and/or mucosal metabolism, and thus tissue viability. In all the experiments the concentration of glucose in the effluent ranged from 1.3 to 1.6 mmol/l. The dose of PGE_2 was that which consistently induced secretion of water, as established in preliminary experiments. The mean percentage recovery of 14 C polyethylene glycol for all the perfusions was 100 (range 95–105). At the end of the three 10-minute collection periods the animal was killed by cardiac puncture.

In the cholera toxin experiments a blind loop was initially constructed from the proximal 15–20 cm of jejunum as described by Harries and Sladen,⁷ and cholera toxin (75 μ g dissolved in 0.75 ml perfusate) was instilled into the loop, which

was returned to the peritoneal cavity for two hours. The loop was then rinsed with perfusate and prepared for perfusion as in the PGE_2 experiments; the composition of the perfusate was identical with the basic perfusate used in the PGE_2 experiments. A second series of experiments with cholera toxin were carried out to study the effects of naloxone on the observed effects of loperamide on cholera toxin-induced secretion: naloxone was administered subcutaneously in two divided doses of 1.6 mg/kg at 4 and 4.5 hours as shown in the Figure. As with PGE_2 the dose of naloxone was chosen on the basis of preliminary experiments, and the timing of its subcutaneous injection was based on studies in rats showing a half life of 30 to 40 minutes.^{8,9} As all the animals were in a steady-state with respect to net water, electrolyte, and glucose absorption throughout the perfusion times, it was assumed that any effect of prolonged anaesthesia on absorption would be similar in control and test animals, and, therefore, comparisons are valid. In some experiments freeze-clamped, full-thickness biopsies of the perfused loops were obtained and stored at $-70^\circ C$ for cyclic AMP (cAMP) assay. In other experiments mucosal scrapings were snap-frozen and stored at $-70^\circ C$ for adenylate cyclase assay.

ANALYSES

The initial solutions and effluent were analysed for sodium and potassium by flame photometry

(Corning-E EL), chloride by coulometric titration (Corning-E EL), and glucose by hexokinase-G6PDH method (BCL, Lewes, UK); 500 μ l aliquots in duplicate were counted in Rialuma scintillant (LKB Instruments Ltd, Croydon, UK) for 14 C in an LKB (Wallac) scintillation counter. Loperamide was supplied by Janssen Pharmaceutical Ltd, Marlow, Buckinghamshire, PGE₂ obtained from Upjohn Ltd, Crawley, Sussex, and CT Batch VT 2210 supplied by Wellcome Research Laboratories, Beckenham, Kent.

Adenylate cyclase activity was measured as described by Tripp *et al.*¹⁰ with the following modifications: the media were preincubated at 37°C for five minutes, 100 μ l of mucosal homogenate was incubated in 500 μ l of medium, the basal activity assay being incubated for 10 minutes and the fluoride stimulated assay for five minutes.

cAMP was assayed using a kit (Radiochemical Centre, Amersham, Buckinghamshire) by homogenising the freeze-clamped biopsies in 0.5 ml EDTA (4 mM) buffer at 4°C and measuring cAMP by protein binding assay after precipitating

homogenate protein by boiling. Protein concentration of the homogenate was measured using a Coomassie blue dye binding method.¹¹

CALCULATIONS

Using 14 C polyethylene glycol as a non-absorbable marker absorption rates of fluid and solute were calculated from standard formulae,¹² and in each rat a mean value was obtained from the three 10-minute collection periods. The significance of the difference between mean values was assessed by the unpaired Student's *t* test.

Results

EFFECT OF LOPERAMIDE ON ABSORPTION AND ON PGE₂ INDUCED SECRETION (Table 1)

Pre-treatment with loperamide itself enhanced absorption of water ($P < 0.05$), sodium ($P < 0.05$), chloride ($P < 0.005$), and glucose ($P < 0.05$). PGE₂ induced secretion of water ($P < 0.001$), sodium ($P < 0.001$), potassium ($P < 0.001$), and chloride

Table 1 Effect of loperamide on absorption and on PGE₂ induced secretion

	Net absorption rates (units/min/g wet weight of gut)				
	Water (μ l)	Sodium (μ mol)	Potassium (μ mol)	Chloride (μ mol)	Glucose (μ mol)
Controls (8)	+28.3 $\pm$ 2.3 $P < 0.05$	+4.4 $\pm$ 0.3 $P < 0.05$	+0.06 $\pm$ 0.01 NS	+1.9 $\pm$ 0.9 $P < 0.005$	+0.03 $\pm$ 0.02 $P < 0.05$
Loperamide-treated (8)	+40.1 $\pm$ 4.7	+6.3 $\pm$ 0.8	+0.08 $\pm$ 0.02	+5.3 $\pm$ 0.02	+0.40 $\pm$ 0.04
PGE ₂ perfusion (8)	-7.3 $\pm$ 1.1 $P < 0.001$	-1.15 $\pm$ 0.29 $P < 0.001$	-0.11 $\pm$ 0.02 $P < 0.01$	-1.37 $\pm$ 0.62 $P < 0.02$	+0.18 $\pm$ 0.01 NS
Loperamide-treated + PGE ₂ perfusion (8)	+7.4 $\pm$ 2.4	+1.16 $\pm$ 0.4	-0.02 $\pm$ 0.02	+0.71 $\pm$ 0.5	+0.23 $\pm$ 0.02

+ Net absorption; - net secretion; values are given as mean $\pm$ SEM. Figures in parentheses denote number of animals in each group. NS: not significant. *P* values refer to values immediately above and below.

Table 2 Effects of cholera toxin (CT), loperamide, and naloxone on water absorption, tissue adenylate cyclase activity and cyclic AMP levels

	Water absorption (μ l/min/g/wet/weight)	Adenylate cyclase (pmol/mg protein/min)		Cyclic AMP (pmol/mg protein)
		Basal	Fluoride-stimulated	
Controls	+27.9 $\pm$ 3.0 (6) $P < 0.001$	4.56 $\pm$ 0.77 (6) $P < 0.05$	20.3 $\pm$ 1.9 (6) NS	3.06 $\pm$ 0.13 (5) $P < 0.001$
CT treated	-24.0 $\pm$ 2.3 (8) $P < 0.001$	8.58 $\pm$ 2.03 (6) NS	15.16 $\pm$ 2.21 (6) NS	6.09 $\pm$ 0.29 (4) NS
Loperamide + CT treated	+12.3 $\pm$ 3.5 (9) $P < 0.005$	9.84 $\pm$ 1.77 (6)	16.97 $\pm$ 2.37 (6)	6.01 $\pm$ 0.38 (5) NS
Loperamide + CT + naloxone treated	-11.7 $\pm$ 5.3 (5) NS			5.80 $\pm$ 0.18 (5) NS
CT + naloxone treated	-17.5 $\pm$ 3.4 (5)			5.57 $\pm$ 0.2 (5)

+ Net absorption; - net secretion; values are given as mean $\pm$ SEM. Figures in parentheses denote the number of animals in each group; NS: not significant. *P* values refer to values immediately above and below.

($P<0.005$) and inhibited glucose absorption ($P<0.001$). Pre-treatment with loperamide reversed secretion of water ($P<0.001$), sodium ($P<0.001$), and chloride ($P<0.02$) to absorption, though rates of absorption did not return to normal control values; secretion of potassium was reduced ($P<0.01$). Glucose absorption was enhanced but the values were not statistically different from the PGE_2 group not given loperamide.

EFFECTS OF CT, LOPERAMIDE AND NALOXONE ON WATER ABSORPTION, TISSUE ADENYLATE CYCLASE ACTIVITY, AND cAMP LEVELS (Table 2) Cholera toxin induced secretion of water ($P<0.001$), and increased basal adenylate cyclase activity ($P<0.05$) without affecting fluoride-stimulated activity; cholera toxin had a similar effect on tissue cAMP levels ($P<0.001$). Loperamide reversed secretion of water to absorption ($P<0.001$) but had no effect on adenylate cyclase activity or cAMP levels. Naloxone reduced the antisecretory effects of loperamide ($P<0.005$), absorption of water reverting to secretion without any effect on tissue cAMP.

Discussion

This study clearly demonstrates that in large doses loperamide markedly inhibits small intestinal secretion induced by cholera toxin and PGE_2 , and supports our clinical observations that the effects of the drug in patients with severe protracted diarrhoea may in part be due to its antisecretory properties. This is in keeping with our findings that the small intestine is in a secretory state in such children.¹³

Previous studies have shown a close temporal relationship between tissue cAMP concentrations, adenylate cyclase activity and the onset of secretion after exposure of the small intestine to cholera toxin, as well as to other agents which increase tissue cAMP, suggesting that the secretory process is intimately linked to the cAMP system.¹⁴⁻¹⁶ Prostaglandin-induced secretion closely mimics that produced by cholera toxin in terms of tissue cAMP changes and electrical phenomena^{14,17}; there are differences, however, in the secretion¹⁴ suggesting that the two secretagogues act via different or additional mechanisms.

In the present experiments loperamide inhibited cholera toxin induced secretion without any effect on the increased activity of adenylate cyclase or the tissue concentrations of cAMP. Moreover, naloxone which reduced the anti-secretory effects

of loperamide also had no effect on tissue cAMP levels. These observations suggest that loperamide acts at a point distal to the increase in tissue cAMP, in modifying the secretory process. The finding that PGE_2 inhibited glucose absorption is in agreement with previous reports,^{18,19} and is in marked contrast to the lack of any effect of cholera toxin on glucose absorption, which is also in agreement with both our⁵ and other previous studies.^{18,21} In addition, loperamide itself enhanced absorption of water, electrolytes, and glucose from an isotonic buffer containing glucose, and partially reversed the effects of PGE_2 on water and electrolytes transport.

Although this study has not defined the precise mechanism(s) by which loperamide modifies cholera toxin and PGE_2 induced secretion, the results suggest that the effects of the opiate analogue do not result from a direct effect on the cyclic AMP system. The data indicate that the antisecretory actions of loperamide occur via a naloxone sensitive mechanism, and as naloxone is an opiate antagonist, this in turn suggests that the effects of loperamide may be mediated by opiate receptors. However, only one concentration of the agonist and antagonist was investigated in the present study and additional studies directed towards demonstrating a dose-related effect of antagonist and agonist activity would add support to the notion that opiate receptors are involved.

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