Loperamide has antisecretory activity in the human jejunum *in vivo*

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SUMMARY We investigated the possibility that loperamide might influence absorption and secretion in the human jejunum *in vivo*. Using a triple lumen tube perfusion technique in healthy normal volunteers we showed that loperamide did not affect net absorption of water or electrolytes under basal condition. When secretion was induced by prostaglandin E₂, however, loperamide significantly reduced that secretion and in three out of six subjects secretion was abolished. Loperamide was effective when it was given either before or after secretion had been initiated. The results lend support to the suggestion that the antidiarrhoeal activities of loperamide may include an antisecretory effect.

The efficacy of loperamide in the treatment of diarrhoea is usually attributed to its actions on intestinal motility.¹ Its use in secretory, usually infective, types of diarrhoea particularly in children, is controversial as it is commonly assumed that it does not influence intestinal secretion. Paralysis of the gut with pooling of fluid in the intestinal lumen are regarded as potentially dangerous consequences of its use in those types of diarrhoea.² Recent evidence suggests, however, that opiates, including loperamide, influence intestinal salt and water transport and can inhibit secretion *in vitro* and *in vivo*.³⁻⁵

In view of these observations it was important to know whether loperamide has antisecretory activity in man particularly as the theoretical objections to its use in secretory diarrhoeas have not been objectively examined. We report here studies of the influence of loperamide on basal absorption of salt and water in the human jejunum *in vivo* and on secretion induced by prostaglandin E₂.

Methods

PERFUSION STUDIES

Jejunal perfusion was carried out in 26 healthy volunteers by a triple lumen tube technique as previously described.⁶ After an overnight fast, the tube was swallowed and the infusion site positioned at the ligament of Treitz and checked by radiographic

screening. The mixing segment was 15 cm long, the test segment 30 cm and the infusion rate 10 ml/min. In basal studies the equilibration period was 40 minutes and the test period one hour. In experiments with prostaglandin E₂ (PGE₂) the equilibration period was 30 minutes and the test period 40 minutes. During test periods jejunal aspirate from the proximal end of the test segment was collected by suction with a hand held syringe at a rate of 1.5 ml/min. Aspirates from the distal end of the segment were collected by continuous low grade suction. At 15 minute intervals small samples of aspirate were withdrawn and used to measure pH and pCO₂ for the calculation of bicarbonate concentration.

Two perfusion solutions were used, one contained bicarbonate (Na, 135; K, 5; Cl, 105; HCO₃, 35; mmol/l) and the other was bicarbonate-free (Na, 135; K, 5; Cl, 140; mmol/l). Both perfusates contained polyethylene glycol 4000 (PEG) 2 gm/l and ¹⁴C PEG 0.5 µCi/l as a non-absorbable marker. Loperamide solution was administered intraluminally as a bolus of 4 mg or 8 mg followed half an hour later by perfusion with a solution containing loperamide (3 mg/l or 6 mg/l). In control periods the same volumes of a placebo solution were given. PGE₂ was administered intraluminally by adding it to the perfusate in a final concentration of 5 × 10⁻⁶M.

Transit time was assessed by noting the time taken for a 1.5 ml bolus of bromsulphthalein dye (BSP) to pass along the 30 cm segment as previously described.⁷ The infusion rate was measured at the beginning and end of the experiment.

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ANALYSIS AND CALCULATIONS

Infused test solutions and aspirates were assayed for ^{14}C PEG in a liquid scintillation counter (LKB 81000 Scintillation Counter; LKB Wallac). One millilitre samples were added to 10 ml of PCS Scintillation fluid (Amersham, UK) and counted for 10 minutes. Counts per minute were converted to disintegrations per minute from a standard curve using quenched standards. Na and K concentrations were measured by flame spectrophotometry (SP6 Spectrophotometer, Pye Unicam) and Cl concentrations using a chloride meter (Corning Eel). Bicarbonate in the form of CO_2 was calculated from the pCO_2 and free bicarbonate from the pH using the Henderson-Hasselbach equation. Water, electrolyte and bicarbonate movements were calculated by standard equations as previously described.⁸

PGE_2 was obtained from Upjohn Ltd, ^{14}C PEG from New England Nuclear, and loperamide from Janssen Pharmaceuticals.

STATISTICAL EVALUATION

All values are expressed as mean $\pm$ 1 SEM. The statistical significance of differences was determined by paired or unpaired Student's *t* tests as appropriate and considered significant if $p < 0.05$.

Results

BASAL STUDIES

After a one hour control period, four subjects were given a bolus dose of 4 mg loperamide and perfused with the bicarbonate containing test solution and 3 mg/l loperamide for a one hour test period. The experiment was repeated in a further five subjects using the bicarbonate free perfusate and prolonging the test period to two consecutive hour collections. All subjects absorbed water, Na, K and Cl in the control period and most subjects absorbed HCO_3^- . Subjects perfused with bicarbonate absorbed more water and electrolytes than those perfused with the bicarbonate free solution. In neither instance was

absorption influenced by loperamide during the test periods (Table 1). Mean transit time with bicarbonate free perfusate was $7.53 (\pm 3.34)$ minutes and this was not changed significantly by loperamide (5.85 ± 2.85 minutes).

INFLUENCE OF LOPERAMIDE ON PGE_2 INDUCED SECRETION

When 12 subjects were perfused with a test solution containing $5 \times 10^{-6}\text{M}$ PGE_2 they all secreted water, Na, Cl and HCO_3^- . Nine of the 12 secreted K. Mean transit time was 2.66 ± 0.42 minutes. After perfusion with PGE_2 , six of the subjects received a bolus of 4 mg loperamide and were perfused with a test solution containing both 3 mg/l loperamide and $5 \times 10^{-6}\text{M}$ PGE_2 . The other six subjects were perfused a second time with PGE_2 alone. All six subjects receiving a second perfusion with PGE_2 alone secreted less water, Na Cl and HCO_3^- than with their first perfusion (Figure; Table 2). Mean secretion of water fell by $51.7 (\pm 8.3)\%$, Na by $46.4 (\pm 7.7)\%$ and Cl by $50.1 (\pm 8.0)\%$. Three of the six subjects given loperamide with the PGE_2 reverted to absorption of water and Cl although control values of absorption were not restored (Figure; Table 2). The mean secretion of water for the six subjects was reduced by $95.1 (\pm 15.2)\%$ compared with their first perfusion with PGE_2 . Mean Na secretion fell by $73.8 (\pm 16.2)\%$ and Cl by $81.4 (\pm 14.9)\%$. The falls in mean water, Na and Cl secretion with loperamide plus PGE_2 were statistically greater than with PGE_2 alone ($p < 0.02$). Mean transit time was unaffected by loperamide (PGE_2 alone 2.43 ± 0.42 min; PGE_2 + loperamide 3.20 ± 0.21 min).

PRETREATMENT WITH LOPERAMIDE

Five subjects were pretreated with an 8 mg bolus of loperamide and then perfused with a solution containing 6 mg/l loperamide and PGE_2 ($5 \times 10^{-6}\text{M}$). Under these conditions one subject failed to secrete water and the mean secretion of

Table 1 Influence of loperamide, intraluminally, on absorption of water, Na^+ , K^+ , Cl^- and HCO_3^- in the human jejunum. $n=4$ for the HCO_3^- containing perfusate and $n=5$ for HCO_3^- free perfusate.

	HCO_3^- containing perfusate		HCO_3^- free perfusate		
	Control	Loperamide	Control	Loperamide (1st hour)	Loperamide (2nd hour)
H_2O (ml/h/30 cm)	120.3 $\pm$ 15.5	126.2 $\pm$ 20.2	62.2 $\pm$ 11.0	78.0 $\pm$ 16.3	61.9 $\pm$ 24.5
Na^+ (mEq/h/30 cm)	15.4 $\pm$ 1.8	15.16 $\pm$ 2.6	7.03 $\pm$ 1.6	9.92 $\pm$ 2.0	8.18 $\pm$ 3.2
K^+ (mEq/h/30 cm)	0.75 $\pm$ 0.1	0.8 $\pm$ 0.1	0.38 $\pm$ 0.1	0.37 $\pm$ 0.2	0.31 $\pm$ 0.2
Cl^- (mEq/h/30 cm)	8.87 $\pm$ 1.2	9.89 $\pm$ 2.4	6.75 $\pm$ 1.7	10.39 $\pm$ 1.9	9.16 $\pm$ 3.2
HCO_3^- (mEq/h/30 cm)	6.91 $\pm$ 2.9	5.81 $\pm$ 4.3	0.24 $\pm$ 0.2	0.12 $\pm$ 0.2	0.05 $\pm$ 0.2

Table 2 Effect of (a) two consecutive perfusions with PGE₂ containing fluid and (b) of loperamide given during the second of two consecutive PGE₂ perfusions on water and electrolyte transport in the human jejunum. -ve sign indicates secretion; +ve sign indicates absorption. Water movement in ml/h/30 cm ± SEM and electrolytes in mEq/h/30 cm

	(a)		(b)	
	PGE ₂ (1st perfusion)	PGE ₂ (2nd perfusion)	PGE ₂ (1st perfusion)	PGE ₂ + loperamide (2nd perfusion)
Water	-91.6±10.9	-43.7±9.1	-89.1±18.9	-8.6±15.6*
Sodium	-13.88±1.1	-7.33±1.0	-14.42±2.6	-3.83±2.3*
Potassium	-0.11±0.2	-0.7±0.1	-0.36±0.2	+0.13±0.13*
Chloride	-12.42±0.9	-6.19±1.0	-13.07±2.8	-2.73±1.9*
Bicarbonate	-1.73±0.5	-0.83±0.3	-1.55±0.4	-0.61±0.4

±SEM. *p<0.02. n=6 for each of studies (a) and (b).

water for all five subjects was 36.3 (±13.3) ml/30 cm/h compared with a mean secretion of 90.3 (±10.4) ml/30 cm/h in the 12 subjects perfused with PGE₂ without loperamide pretreatment (Table 3). The difference was statistically significant (p<0.02). Similar trends were seen with Na and Cl although these differences were not statistically significant. Mean transit time was not affected by pretreatment

with loperamide (PGE₂ alone 2.43±0.42 min, pretreated with loperamide 3.28±0.4).

Discussion

The well established efficacy of opiates as anti-diarrhoeal agents has usually been attributed to their effects on gastrointestinal motility.⁹ Recent studies in animals, however, have indicated that a variety of opiate agonists can influence intestinal secretion.^{4,5,10} Morphine, enkephalins and loperamide have been shown to enhance absorption or inhibit secretion induced by a variety of secretagogues *in vitro* in ileal mucosa from rabbit or guinea pig. Studies with closed loops of rat intestine *in vivo* suggest that opiates also have anti-secretory activity *in vivo*.³

In view of the controversy surrounding the use of opiates in secretory diarrhoeas, it was important to investigate the influence of loperamide on the absorption and secretion of salt and water in the human intestine. Loperamide was chosen because of its wide usage as an anti-diarrhoeal agent and because of its apparent safety.¹¹

Loperamide did not influence absorption of electrolytes or water when the jejunum was perfused with an isotonic saline solution, or when

Figure Net water transport in human jejunum in vivo. Secretion induced by prostaglandin E₂ (PG) is shown above zero line and was significantly less during second of two consecutive perfusion periods (left hand panel). Influence of loperamide, given during second of two PGE₂ perfusions is shown in right hand panel. Fall in secretion induced by loperamide is significantly greater than that which occurred spontaneously in its absence.

Table 3 Effect of pretreatment with loperamide on PGE₂ induced secretion in the human jejunum. -ve sign indicates secretion; water in ml/h/30 cm; electrolytes in mEq/h/30 cm; PGE₂ alone n=12; pretreated with loperamide n=5

	PGE ₂ alone	Loperamide + PGE ₂
Water	-90.3±10.4	-36.3±13.3*
Sodium	-14.4±1.4	-8.08±1.2
Potassium	-0.23±0.1	-0.44±0.4
Chloride	-12.75±1.4	-5.70±2.3
Bicarbonate	-1.64±0.3	-1.18±0.3

* p<0.02.

absorption was stimulated by the presence of bicarbonate in the perfusate. These results resemble the behaviour of rabbit ileal mucosa *in vitro* in which loperamide did not influence electrolyte transport under basal conditions.⁴ PGE₂-induced secretion, however, was inhibited by loperamide. This inhibition, although incomplete, was noted when the loperamide was administered either before or after secretion had been started.

The observation that the effects of prostaglandin alone diminished with time suggests some form of tachyphylaxis. The importance of its demonstration here lies in the necessity to exclude a spontaneous decrease in secretion as a cause of the apparent loperamide effect. Loperamide, however, caused a significantly greater fall in secretion than was observed spontaneously in the second of two prostaglandin perfusion periods. In three of the six subjects given loperamide, secretion was abolished and a small amount of absorption was observed. Furthermore, a comparison of the effect of prostaglandin alone with that of loperamide, given during an initial perfusion with prostaglandin-containing fluid, indicates that the loperamide reduced the response to PGE₂.

In these studies PGE₂ shortened the transit time measured by the dye-dilution curve technique. It is of interest that loperamide, in this large dose, failed to influence transit time measurements either in the basal state or after PGE₂ stimulation. These transit time determinations may not be a good way of detecting motility changes but the results do not suggest that the antidiarrhoeal activity of loperamide is mediated through an effect on motility, at least in the jejunum.

The dose of loperamide used in these experiments was large compared with the usual therapeutic dose. Much larger doses, of up to 1 mg/kg body weight, however, have been used and shown to be effective in controlling severe secretory diarrhoea in infants.¹²

These results contrast with those of Schiller *et al*¹³ who studied the effects of codeine in man. Intramuscular codeine reduced stool volume in subjects in whom diarrhoea was induced by intragastric fluid infusions, but did not influence absorption in the gut as a whole or in segments of jejunum, ileum, or colon perfused separately. In addition the reduced absorption of salt and water produced by VIP in the ileum was unaffected by codeine. Transit rates in the jejunum were slowed by codeine. Several reasons may account for the discrepancies between the studies with codeine and loperamide. Firstly, the doses of loperamide we studied would be equivalent to some three to five times more than the dose of codeine used by Schiller *et al*. Secondly, the

loperamide was administered intraluminally, that is directly at the epithelium rather than intramuscularly. Thirdly, different secretagogues were used in the two studies and fourthly it is conceivable that loperamide has different effects from those of codeine. Our failure to influence transit time measurements with high doses of loperamide is of interest in this respect. Finally, the possibility has to be considered that the loperamide reacted in some way with the PGE₂ in the lumen and rendered it inactive. We cannot exclude this possibility at present but believe it to be unlikely.

The epithelial effects of opiates are probably mediated through δ receptors while smooth muscle effects occur *via* μ receptors.¹⁰ It is conceivable that the differences between the effects of codeine and loperamide described above are because of the modest differences in receptor selectivity between these agents. Indeed it might be possible to improve antidiarrhoeal drug design by tailoring them to specific needs, a δ agonist for secretory types of diarrhoea and a μ agonist where a motor effect is more desirable.

The antidiarrhoeal actions of loperamide must clearly depend on its effects on the ileum and colon as well as those studied here in the jejunum. Colonic responses may be the most important determinants of clinical responsiveness in many diarrhoeal diseases. The observation, however, that secretion in the jejunum, a site from which many infective, secretory, diarrhoeas arise, can be reduced by loperamide suggests that this drug may have some potential value in the treatment of secretory diarrhoea. The results should encourage the initiation of well controlled clinical trials of its therapeutic efficacy in this type of diarrhoea.

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