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Comparison of the Antisecretory Effects of Loperamide and Loperamide Oxide in the Jejunum and the Colon of Rats In-vivo

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Abstract—The antidiarrhoeal effect of loperamide is caused by its antimotility and antisecretory properties. In-vivo experiments in the rat jejunum and colon have been performed to compare the antisecretory effect of loperamide with the effect of its prodrug, loperamide oxide. Both loperamide and loperamide oxide administered intraluminally, equally and dose dependently (2 to 250 $\mu g\ mL^{-1}$) reduced PGE_2-induced net fluid secretion (32 ng min^{-1} i.a.) in the jejunum and colon. The antisecretory effect of both drugs is blocked by naloxone (1 mg kg^{-1} s.c.). It is concluded that loperamide oxide administered intraluminally is reduced to loperamide and has the same antisecretory potency as loperamide in jejunum and colon. The effect appears to be mediated via opiate receptors. The observation that loperamide cannot be detected in the colonic lumen two h after oral administration suggests that the drug is delivered from the blood stream to the site of action after absorption in the small intestine.

Loperamide is a widely used and effective antidiarrhoeal drug. Its antidiarrhoeal effect is partly due to a decrease in intestinal hyperperistalsis (Van Nueten et al 1974) and partly due to a decrease in intestinal hypersecretion (Beubler & Lembeck 1979). Orally administered loperamide is well absorbed, rapidly metabolized in the liver and does not enter the central nervous system. The low systemic bioavailability results in a high safety margin (Awouters et al 1983).

In an effort to make loperamide even safer, a pharmacologically inactive pro-drug of loperamide, loperamide oxide, was synthesized. This pro-drug was expected to be reduced to loperamide in the lumen and the wall of the intestine, so that it would become slowly available to the systemic circulation. This would prevent the undesired effect of overdosing.

In-vitro studies showed loperamide oxide to be active only after preincubation with intestinal contents. After preincubation, its effect was comparable to that of loperamide (Niemegeers et al 1986).

In-vivo studies, using orally treated rats, showed loperamide oxide to be equipotent to loperamide (Niemegeers et al 1986). Given intravenously or intraperitoneally, loperamide oxide was equieffective to loperamide only after preincubation with intestinal contents (Goldhill et al 1989).

To compare the antisecretory effects of loperamide and loperamide oxide, both drugs have been given intraluminally, in this study. This route of administration closely mimics the usual oral administration route, and more precisely allows the determination of the effective concentration of both drugs. In comparison with the subcutaneous and intraperitoneal routes (Beubler & Lembeck 1979; Beubler 1982; Goldhill et al 1989), a higher antisecretory activity of loperamide and loperamide oxide was expected.

Both the small intestine and colon have been shown to be target organs of the antisecretory effect of loperamide (Awouters et al 1983). Therefore in the present study both these organs were used to compare the two drugs. Since there have also been contradictory reports about the opiate-like nature of loperamide (Sandhu et al 1981; Beubler 1982; Hughes & Turnberg 1982) additional experiments were performed using the opiate antagonist, naloxone.

Intestinal fluid secretion was stimulated by low concentrations of prostaglandin E_2 (PGE_2) infused intra-arterially, since prostaglandins have been shown to be implicated in several types of secretory diarrhoea.

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Materials and Methods

Preparation of animals

Female Sprague-Dawley rats, 180 ± 20 g, were deprived of food for 20 h before the experiment, but had free access to water. The rats were anaesthetized with pentobarbitone sodium (65 mg kg^{-1} i.p.) and the abdomen opened. A polyethylene catheter (PE 60) was placed in the jejenum about 5 cm distal to the flexura-duodenojejunalis and fixed by ligation. The second ligation was made about 20 cm distal to the first. A second polyethylene catheter (PE 60) was placed in the ascending colon before ligation within 0·5 cm distal to the caecum. One hour after the preparation 2·0 mL of Tyrode solution was instilled into the jejunal loop and the colonic lumen after it had been tied off close to the rectum. The catheters were then withdrawn before the proximal ligation was also tied off.

Administration of substances

Prostaglandin E_2 (32 ng min^{-1}) or 0·9% NaCl (saline) was infused close intra-arterially into a branch of the superior mesenteric artery (0·949 mL h^{-1}) using a perfusor (Braun-Melsungen FRG) (Beubler et al 1986). Preliminary experiments showed that this infusion of PGE_2 affected fluid transfer both in the jejunum and the colon.

Loperamide and loperamide oxide were given intraluminally by adding various concentrations of the drug to the

Tyrode solution with which the respective jejunal or colonic loops were filled.

Loperamide and loperamide oxide (2 mg kg^{-1} each) were administered (2 mL) by gavage to the unanaesthetized rat. The preparation of the animal was started in time to assure that PGE_2 infusion and measurement of net fluid transport, respectively, were started exactly 2 h after oral administration.

In some experiments naloxone at a dose of 1 mg kg^{-1} was administered subcutaneously 5 min before the loop was filled.

Determination of net fluid transfer

Net fluid transfer rates were determined gravimetrically 30 min after the instillation of Tyrode solution for all the experiments. Net fluid transport was expressed as mL/30 min g^{-1} wet weight of jejunum. Net absorption was indicated by a positive value and net secretion by a negative value.

Determination of loperamide and loperamide oxide in intestinal fluid

One or two hours after oral administration of loperamide (2 mg kg^{-1}) or loperamide oxide (2 mg kg^{-1}), respectively, the rats were killed by a blow on the neck and the small intestine and colon removed. Both were rinsed by flushing with saline, the fluid sampled and stored at −60°C. Loperamide and loperamide oxide were measured in the rinsing fluid by radioimmunoassay at Janssen Research Foundation, Beerse, Belgium.

Statistics

Experiments in each series were performed in balanced blocks. The results were given as the mean ± s.e.m. and the data were analysed by two sample Student's *t*-test.

Chemicals

Chemicals and reagents used were: loperamide HCl and loperamide oxide (Janssen Pharmaceutica, Beerse, Belgium), naloxone HCl (Knoll AG Ludwigshafen, FRG), PGE_2 (Sigma Chemical Company, St. Louis, MO) and pentobarbitone sodium (Abbott Laboratories, Chicago, IL). All other reagents were of analytical grade.

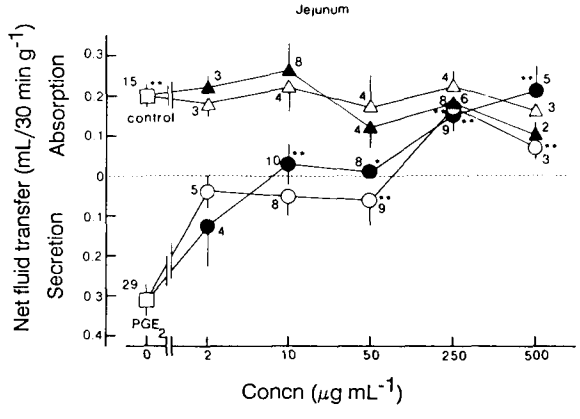

FIG. 1. Jejunum. Effects of loperamide (▲, ●) and loperamide oxide (△, ○) in controls and on PGE_2-induced net fluid secretion, respectively. Each point represents the mean ± s.e.m. The figures indicate the number of experiments. $*P<0.05$, $**P<0.01$ compared with PGE_2.

Results

Jejunum

Fluid was absorbed in the jejunum of all control rats (Fig. 1). Close intra-arterial infusion of PGE_2 (32 ng min^{-1}) significantly reversed fluid absorption into profuse net fluid secretion ($P<0.01$).

Loperamide and loperamide oxide; intraluminal. Neither loperamide nor loperamide oxide, when administered intraluminally in concentrations from 2–500 μg mL^{-1} (4×10^{-6} to 10^{-3} M) affected net absorption in the jejunum of controls. The secretory effect of PGE_2 (32 ng min^{-1}) was dose-dependently and significantly reduced equally by loperamide and loperamide oxide, showing a maximal effect at 250 μg mL^{-1} (5×10^{-4} M) (Fig. 1).

Loperamide and loperamide oxide; oral. When administered orally 2 h before the start of infusion of PGE_2, loperamide oxide (2 mg kg^{-1}), but not loperamide (2 mg kg^{-1}) inhibited PGE_2-induced net fluid secretion in the jejunum (Table 1).

Determination of loperamide and loperamide oxide. One and two hours after oral administration of 2 mg kg^{-1} loperamide or loperamide oxide, the intraluminal amount of both drugs was determined. Only about 1% of the orally administered loperamide was found in the intraluminal fluid after 1 or 2 h. After oral administration of loperamide oxide, 1·6% (1 h) and 1·3% (2 h) of the drug administered were found as loperamide. Only small amounts of unchanged loperamide oxide were detected (Table 2).

Colon

Again fluid was absorbed in all control rats. Close intra-arterial infusion of PGE_2 (32 ng min^{-1}) significantly changed net fluid absorption into net fluid secretion in the colon ($P<0.01$) (Fig. 2).

Loperamide and loperamide oxide; intraluminal. Neither loperamide nor loperamide oxide when administered intralu-

Table 1. Effect of loperamide (2 mg kg^{-1}) and loperamide oxide (2 mg kg^{-1}) 2 h after oral administration on PGE_2-induced net fluid secretion in colon and jejunum of rats.

	Net fluid transfer mL/30 min g^{-1}			
	n	Colon	n	Jejunum
Control	4	+0·38 ± 0·10	4	+0·20 ± 0·13
Control + LOP	3	+0·41 ± 0·07	3	+0·26 ± 0·03
Control + LOP OX	3	+0·32 ± 0·08	3	+0·30 ± 0·08
PGE_2	3	−0·08 ± 0·05*	3	−0·26 ± 0·09*
PGE_2 + LOP	10	+0·11 ± 0·05**	10	−0·20 ± 0·10*
PGE_2 + LOP OX	10	+0·14 ± 0·05**	10	+0·18 ± 0·05**

PGE_2, prostaglandin E_2, LOP, loperamide, LOP OX, loperamide oxide; values are mean ± s.e.m.: positive values represent absorption, negative values represent secretion; n refers to number of animals studied. $*P<0.01$ compared with control, $**P<0.01$ compared with PGE_2.

Table 2. Intraluminal contents of loperamide and loperamide oxide after oral administration of 2 mg kg^{-1} of the two drugs in the small intestine and the colon of the rat.

	Amount recovered (μg)		
	Small intestine		Colon
	1 h	2 h	2 h
Loperamide, 0·4 mg/rat administered			
loperamide recovered after loperamide 0·4 mg/rat	4·4 ± 1·4 (3)	3·9 ± 0·9 (4)	−(4)
Loperamide oxide 0·4 mg/rat administered			
loperamide recovered after loperamide oxide 0·4 mg/rat	6·6 ± 1·2 (4)	5·2 ± 1·2 (3)	−(3)
loperamide oxide recovered after 0·4 mg/rat	0·16 ± 0·01 (4)	0·09 ± 0·001 (3)	−(3)

Values are mean ± s.e.m.; numbers in parentheses refer to number of animals studied.

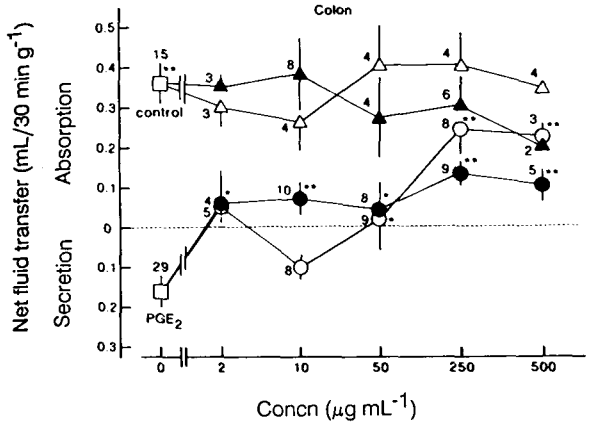

FIG. 2. Colon. Effects of loperamide (▲, ●) and loperamide oxide (△, ○) in controls and on PGE_2-induced net fluid secretion, respectively. Each point represents the mean ± s.e.m. The figures indicate the number of experiments. * $P < 0.05$, ** $P < 0.01$ compared with PGE_2.

Table 3. Effect of naloxone (1 mg kg^{-1}) on the inhibitory effect of loperamide and loperamide oxide (250 μg mL^{-1}) in colon and jejunum of rats on PGE_2-induced net fluid secretion.

		Net fluid transfer mL/30 min g^{-1}	
	n	Colon	Jejunum
Control	15	+0·36 ± 0·05	+0·19 ± 0·03
PGE_2	29	−0·16 ± 0·04*	−0·31 ± 0·04*
NAL	4	+0·22 ± 0·10	+0·23 ± 0·10
PGE_2 + NAL	3	−0·23 ± 0·11	−0·34 ± 0·08
PGE_2 + LOP	9	+0·13 ± 0·03**	+0·15 ± 0·06**
PGE_2 + LOP OX	8	+0·24 ± 0·06**	+0·17 ± 0·06**
PGE_2 + LOP + NAL	6	−0·14 ± 0·05 n.s.	−0·20 ± 0·10 n.s.
PGE_2 + LOP OX + NAL	9	−0·17 ± 0·11 n.s.	−0·15 ± 0·06 n.s.

PGE_2, prostaglandin E_2; NAL, naloxone, LOP, loperamide; LOP OX, loperamide oxide; Values are mean ± s.e.m.: positive values represent absorption, negative values represent secretion; n refers to the number of animals studied. * $P < 0.01$ compared with control; ** $P < 0.01$ compared with PGE_2; n.s.: not significantly different from PGE_2.

minally affected fluid absorption in controls in concentrations from 2 to 500 μg mL^{-1}.

Both loperamide and loperamide oxide dose-dependently inhibited the effect of PGE_2. Except for the fact that loperamide oxide appeared to be non-active at 10 μg mL^{-1}, there was no difference between the effects of the two drugs in the colon (Fig. 2).

Loperamide and loperamide oxide; oral. When administered orally 2 h before the start of infusion of PGE_2, both loperamide and loperamide oxide inhibited PGE_2-induced net fluid secretion in the colon (Table 1).

Determination of loperamide and loperamide oxide. Two hours after oral administration of either loperamide or loperamide oxide (2 mg kg^{-1} each) neither drug was detected in the colonic luminal fluid (Table 2).

Effect of naloxone

The morphine antagonist, naloxone (1 mg kg^{-1} s.c.) was without any influence on basal net fluid absorption in the jejunum and the colon. Naloxone, however, significantly blocked the antisecretory effects of both loperamide and loperamide oxide at their maximally effective dose of 250 μg mL^{-1} (Table 3). This effect was similar both in the colon and in the jejunum.

Discussion

In this study experiments were performed to compare the antisecretory effects of loperamide and loperamide oxide on intestinal fluid secretion induced by PGE_2 in the jejunum and the colon of the rat in-vivo. Although PGE_2 appears to cause less pronounced net fluid secretion in the colon, the absolute effect, i.e. the difference between the net fluid secretion and the control absorption, is the same in both organs (Figs 1, 2). Both loperamide and loperamide oxide, administered intraluminally, dose dependently reduced PGE_2-induced net fluid secretion in the jejunum and in the colon. The results show that in the jejunum the two drugs appear to be equipotent at all concentrations tested. In the colon, loperamide oxide only at the dose of 10 μg kg^{-1} appears to be less potent in inhibiting PGE_2-induced secretion. No explanation can be given for this difference. The effective concentrations of both drugs are comparable with the concentrations used in in-vitro studies and in in-vivo studies when the drugs have been given orally (for ref. see Awouters et al 1983) but are below the doses used in in-vivo studies when the drugs are given intraperitoneally or subcutaneously (Beubler & Lembeck 1979; Beubler 1982; Goldhill et al 1989).

Preliminary experiments have shown that loperamide oxide is completely ineffective if the jejunal or the colonic lumen is rinsed with saline before the administration of the

drug (data not shown). This is in agreement with other studies which have shown that loperamide oxide has to be reduced by intestinal contents to produce an effect (Goldhill et al 1989).

The oral administration of either drug (2 mg kg^{-1}) resulted in a similar inhibitory effect on PGE_2-induced secretion in the colon as caused by 250 $\mu g\ mL^{-1}$ administered locally. In the colonic lumen, however, no loperamide or loperamide oxide could be detected after 2 h. In contrast 85% of loperamide given orally is recovered from the gastrointestinal tract (Heykants et al 1974). It can therefore be suggested that the antisecretory effect in the colon is caused by the drug being delivered to the colon via the blood flow to the site of action after absorption in the small intestine. In other words, the drug is not acting "locally" in the sense that the drug acts from the intestinal lumen, but acts after delivery from the blood stream. This is in agreement with the observation from in-vitro experiments, that the serosal addition of loperamide caused a quicker and more pronounced fall in short-circuit current than the mucosal addition (Hughes & Turnberg 1982).

The fact that locally administered loperamide in-vivo is as equally effective as loperamide in-vitro, together with the findings that most of the drug is recovered from the gastrointestinal tract after oral administration and the observation that loperamide does not enter the central nervous system (Heykants et al 1974), confirms the hypothesis that the antidiarrhoeal properties are mediated by local mechanisms. This local action of loperamide appears to be mediated by opiate receptors since both the inhibition of the propulsive activity (Awouters et al 1983) and the antisecretory potency of loperamide can be blocked by naloxone. This is in agreement with former studies where the effects of loperamide in-vivo given orally (Hughes & Turnberg 1982), or the effects of loperamide in-vitro (Niemegeers et al 1986), have been shown to be blocked by naloxone. The finding that high doses of loperamide given systemically are not counteracted by naloxone (Beubler 1982) does not contradict the opiate-nature of loperamide but may be explained by low antagonistic properties of naloxone against loperamide effects compared with those against morphine (Wuester et al 1976).

In conclusion it can be stated that both loperamide and loperamide oxide, administered intraluminally dose dependently and equally, reduced the secretory effect of PGE_2 on net fluid transfer in the jejunum and the colon of the rat in-vivo. Since loperamide cannot be detected in the colonic lumen 2 h after oral administration it is suggested that the drug is delivered from the blood stream to the site of action after absorption in the small intestine. The effects both in the jejunum and the colon appear to be mediated via opiate receptors.

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