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The effect of loperamide oxide on prostaglandin-stimulated fluid transport in rat small intestine

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Abstract—The effects of loperamide and loperamide oxide on basal and prostaglandin E_2-stimulated fluid transport by rat small intestine have been investigated. In contrast to loperamide, loperamide oxide, when applied intraperitoneally, failed to inhibit either basal or prostaglandin E_2-stimulated fluid transport. However, intraperitoneal administration of loperamide oxide following its incubation with the contents of the intestinal lumen under aerobic conditions resulted in an effective inhibition of fluid secretion. The activating material was present in the essentially non-particulate 3000 *g* supernatant fraction of the luminal contents and was heat-stable.

The opiate loperamide is a well-established anti-diarrhoeal agent, its popularity resulting from a longer and more potent protection against diarrhoea than other agents (Stockbroeckx et al 1973), a high safety margin due to its low systemic bioavailability, and a lack of central action, which removes the potential for abuse (Niemegeers et al 1974).

It was thought originally that loperamide acts by decreasing gut motility since it inhibited acetylcholine-induced contractions of intestinal muscle in a concentration-dependent manner (Van Nueten et al 1974), but evidence has accumulated indicating that loperamide can also affect intestinal fluid transport (Hardcastle et al 1981; Sandhu et al 1981; Hughes et al 1982, 1984; McKay et al 1982). It is now generally accepted that loperamide has both antimotility and antisecretory properties that account for its anti-diarrhoeal action.

Despite the large safety margin of loperamide, there are occasional reports of overdosage (Friedli & Haenggeli 1980; Minton & Smith 1987). To overcome this problem, the *N*-oxide, loperamide oxide, a pro-drug of loperamide, has been developed. *N*-Oxides of alkaloids have previously been shown to be inactive (Bickel 1969), but oral administration of loperamide oxide inhibited caster oil-induced diarrhoea (Niemegeers et al 1986). This effect was attributed to the conversion of loperamide oxide to loperamide in the gastrointestinal tract since following oral administration of loperamide oxide, the parent drug loperamide appeared in the blood (Monbaliu et al 1984). The reduction of loperamide oxide to loperamide by intestinal contents, red blood cells and liver microsomes has been demonstrated in-vitro under anaerobic conditions (Lavrijsen et al 1984a, b). The aim of the present study was to investigate the ability of loperamide oxide to influence basal and prostaglandin E_2 (PGE_2)-stimulated fluid secretion and to compare its effects with those of loperamide.

Materials and methods

The in-vivo ligated loop technique was used to determine the effects of loperamide oxide and loperamide on fluid transport in the rat.

Animals. Male albino rats of the Sheffield strain, 200–250 g, were allowed free access to food and water. They were anaesthetized using sodium pentobarbitone (Sagatal, 60 mg kg^{-1}, i.p.), and body temperature was maintained at 37°C. The experimental procedure was divided into periods of pretreatment and treatment.

Pretreatment period. The ligated loop preparation has been described previously (Strombeck 1972). Briefly, the abdomen was opened and loperamide (40 μmol kg^{-1}), loperamide oxide (40 μmol kg^{-1}) or vehicle (ethanol, 100 μL) was applied i.p. After 5·5 min a 15 cm loop of mid-intestine was selected, incised at either end and the luminal contents washed out with 154 mM NaCl, care being taken to avoid contamination of the peritoneal cavity with the washings. Residual fluid was gently blown out and the distal end of the intestinal segment ligated. Approximately 0·5 mL 154 mM NaCl was injected into the proximal end of the loop from a syringe that was weighed before and after the addition and the loop was then closed by a second ligature.

Treatment period. Ten min after the beginning of the pretreatment period the animals received either PGE_2 (30 nmol kg^{-1}) or an equivalent volume of the vehicle (100 μL 154 mM NaCl) i.p. After a further 15 min the loop was removed and fluid transport determined gravimetrically.

To determine whether a luminal factor played a role in the activation of loperamide oxide, the effects of pre-incubating loperamide oxide and loperamide with various components of the intestinal contents were investigated.

Pre-incubation with the contents of the intestinal lumen. The luminal contents were obtained by removing the entire small intestine from a donor rat, cutting along the longitudinal axis and gently scraping off the contents. These were then mixed with either 1 mL ethanol, loperamide oxide or loperamide (both 100 μmol in 1 mL ethanol) and incubated in a shaking water bath at 37°C for 10 min under aerobic conditions. The incubate was washed into a centrifuge tube with 1 mL 154 mM NaCl and centrifuged at 3000 *g* for 5 min. A 200 μL sample of the supernatant was then used in the pretreatment period, the dose of loperamide oxide and loperamide being approximately 30 μmol kg^{-1} in 30% ethanol, and fluid transport determined in the presence of PGE_2 as before.

Preincubation with "particulate-free" luminal contents. The following procedure was adopted to collect intestinal contents that were essentially free of particulate matter. A 25 cm segment of intestine starting 10 cm from the pylorus was washed out with 154 mM NaCl and the residual fluid blown out. Approximately 2 mL prewarmed 154 mM NaCl was added and the loop was tied off. After 15 min the loop was removed and the fluid contents were drained and then centrifuged at 3000 *g*. A 500 μL sample of the supernatant was mixed with an equal volume of ethanol, loperamide oxide or loperamide (200 μmol mL^{-1} ethanol for both drugs) and the mixture incubated for 10 min in a shaking water bath at 37°C under aerobic conditions. Animals were then pretreated with 100 μL of this solution injected i.p., the final dose

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of the opiate being 40 μmol kg^{-1}, and its effects on PGE_2-stimulated fluid secretion determined.

Preincubation with heat-treated "particulate-free" intestinal contents. The heat-stability of any activating material was tested by collecting the "particulate-free" 3000 *g* supernatant fraction of the intestinal contents, placing it in a boiling water bath for 5 min and, after cooling, incubating it with the drugs as before.

Expression of results. Fluid transport is expressed as μL g^{-1}/15 min with fluid movement related to the wet weight of the empty loop of intestine. Values are given as the mean ± s.e.m., with the number of observations in parentheses. Significance was assessed using a two-tailed, unpaired Student's *t*-test, with $P<0.05$ considered significant.

Chemicals. Loperamide oxide and loperamide were supplied by Janssen Pharmaceutica, Beerse, Belgium. PGE_2 was obtained from Upjohn Limited, Crawley, Sussex.

Results

Under basal conditions the ligated loops accumulated fluid in the lumen (110 ± 11 μL g^{-1}/15 min, n = 6). This accumulation was reduced ($P<0.01$) by loperamide (40 μmol kg^{-1} i.p.) to a value of 30 ± 22 μL g^{-1}/15 min (n = 6), although loperamide oxide at the same dose was without significant effect.

PGE_2 caused a significant ($P<0.001$) stimulation of fluid accumulation to 182 ± 8 μL g^{-1}/15 min (n = 6). Pretreatment with loperamide reduced the fluid accumulation with PGE_2 while the same dose of loperamide oxide was without significant effect (Fig. 1A).

After preincubating loperamide oxide with the contents of the intestinal lumen it became activated, decreasing PGE_2-stimulated fluid secretion to a level that was similar to that obtained with loperamide (Fig. 1B).

The activating material was present when the "particulate" fraction of the intestinal contents was absent, since pre-incubation of loperamide oxide with the "non-particulate" 3000 *g* supernatant fraction reduced the PGE_2-stimulated fluid secretion to an extent which was similar to that obtained with loperamide (Fig. 1C).

Even after heat-treatment of the "non-particulate" 3000 *g* supernatant fraction of the intestinal contents, loperamide oxide was still activated to reduce PGE_2-stimulated fluid accumulation to a value not significantly different from that observed with loperamide (Fig. 1D).

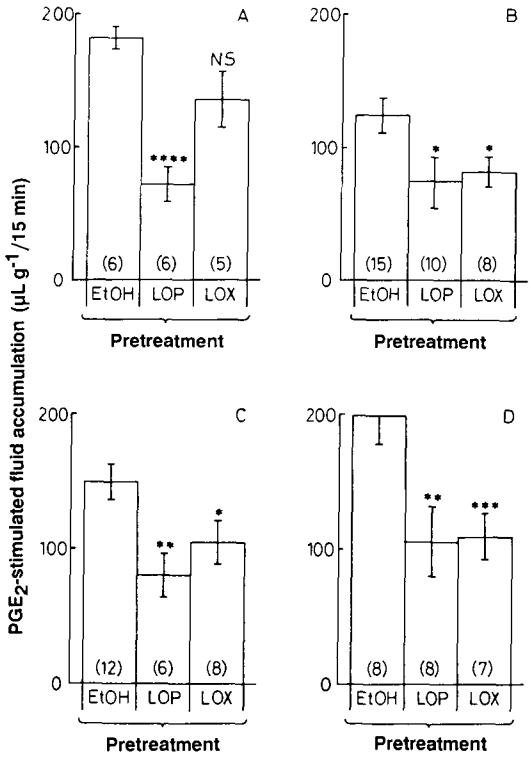

FIG. 1. Effect of loperamide (LOP) and loperamide oxide (LOX) under a variety of conditions on PGE_2-stimulated fluid accumulation in ligated loops of rat mid-intestine in-vivo. (A) Untreated drugs (40 μmol kg^{-1}). (B) Drugs pre-incubated with the contents of the intestinal lumen giving a final concentration of 30 μmol kg^{-1}. (C) Drugs (40 μmol kg^{-1}) pre-incubated with the "particulate-free" 3000 *g* supernatant fraction of the intestinal contents. (D) Drugs (40 μmol kg^{-1}) pre-incubated with the heat-treated "particulate-free" 3000 *g* supernatant fraction of the intestinal contents. The drugs were applied 10 min before the beginning of fluid transport measurement (pre-treatment period), with control animals receiving an equivalent volume of the vehicle (ethanol, EtOH). Fluid accumulation was determined over 15 min (treatment period) following the administration of PGE_2 (30 nmol kg^{-1} i.p.). Each bar represents the mean ± s.e. of the mean of the number of animals indicated and significance was assessed using a two tailed unpaired *t*-test, with $P<0.05$ considered significant. The significance of the difference between test and control is denoted by *($P<0.05$), **($P<0.02$), ***($P<0.01$) and ****($P<0.001$). N.S. indicates no significance ($P>0.05$).

Discussion

The present study has shown that in both non-stimulated and PGE_2-stimulated animals, the intraperitoneal administration of the parent drug loperamide inhibited fluid accumulation whereas the prodrug loperamide oxide had little effect (Fig. 1A).

Some modification of the loperamide oxide is necessary to produce an anti-secretory effect and the understanding of this process is important for effective therapeutic use. It has already been reported that liver microsomes and intestinal contents can convert loperamide oxide to loperamide under anaerobic conditions, with the microsomal conversion process being inhibited in the presence of oxygen (Lavrijsen et al 1984a, b). In the present study the ability of the intestinal contents to activate loperamide oxide has been confirmed (Fig. 1B); but in contrast to liver microsomes the intestinal activation occurs under aerobic conditions. Thus there appear to be at least two mechanisms for loperamide oxide activation: an oxygen-sensitive reduction in liver microsomes and an intestinal mechanism capable of operating in the presence of oxygen. The reduction of loperamide oxide is similar to the reduction of other *N*-oxides which may be either oxygen-sensitive or insensitive, heat-labile or stable and enzymatic or non-enzymatic (Bickel 1969), depending on the *N*-oxide and the environment. Although red blood cells have been shown to reduce loperamide oxide to loperamide (Lavrijsen et al 1984a, b) this type of conversion must play a minor role in comparison to the conversion by intestinal factors, as loperamide oxide not previously exposed to any intestinal factors is not effective in reducing PGE_2-induced fluid accumulation (Fig. 1A). The present study shows that the intestinal material responsible for activating loperamide oxide is present in the "particulate-free" phase of the luminal contents. The source of this activating material is unknown but it may be either secreted by the intestinal epithelium, bound to the luminal wall or derived from aged cells extruded from the villous tips. It has been suggested that the gut flora within the intestinal contents may be responsible for the conversion of loperamide oxide (Lavrijsen 1984a). As the activation of loperamide oxide is

evident in "particulate-free" fractions of the luminal contents (Fig. 1C) it would appear that significant bacterial presence may not be necessary for the conversion process. In further contrast to the heat-labile reduction of loperamide oxide by liver microsomes (Lavrijsen 1984b), activation by the luminal material is not inhibited by heat treatment (Fig. 1D) and must therefore be non-enzymatic in nature. This is similar to the reduction of some other *N*-oxides such as trimethylamine oxide where the process was found to be mimicked by ferrous ions (Bickel 1969). Ferrous ions are also implicated in the reduction of imipramine oxide and in this case the reaction is greatly enhanced by EDTA. Both ferrous ions and their chelating agents are found to be associated with the luminal membrane of the small intestine (Bothwell et al 1979), and although neither are secreted, the use of the ligated loop technique to collect "particulate-free" luminal contents may allow their movement into the lumen. Ferrous ions bound to the luminal wall may also be important in the intestinal activation of loperamide oxide.

Although untreated loperamide oxide which has not been exposed to the contents of the intestinal lumen is ineffective in inhibiting either basal or PGE_2-stimulated fluid accumulation, once exposed to luminal contents it becomes, in this study, as effective as loperamide in inhibiting fluid accumulation. As loperamide oxide is intended for oral administration then some activation will ensue, but further investigation of this process may lead to a more effective therapeutic use of loperamide oxide.

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