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Antisecretory Activities of Orally Administered Loperamide and Loperamide Oxide on Intestinal Secretion in Rats

ECKHARD BEUBLER, PRAMILA BADHRI AND ANDREA SCHIRGI-DEGEN

Department of Experimental and Clinical Pharmacology, University of Graz, Universitätsplatz 4, A-8010 Graz, Austria

Abstract—In-vivo experiments in the rat jejunum have been performed to compare the antisecretory effect of orally administered loperamide with the effect of its pro-drug, loperamide oxide. Both loperamide and loperamide oxide, administered orally, reduced the secretory effect of prostaglandin E_2 (32 ng min^{-1}, intra-arterially) in the jejunum and the colon. Differences between the two drugs as to time course and dose response can be seen. Loperamide oxide shows its antisecretory effect in the jejunum, and at a dose of 2 mg kg^{-1} also shows its effect in the colon 1 h after administration. The effect was maximal after 2 h and decreased after 4 h. A dose-response relationship was demonstrated at 2 h in the jejunum and the colon. In comparison, the effect of loperamide started later, and a good dose-response relationship was not observed in the jejunum or in the colon, higher doses always appearing less effective than lower doses.

The opiate loperamide is a well established, widely used and effective antidiarrhoeal agent. Its popularity results from a long and potent protection against diarrhoea, a high safety margin due to its low systemic bioavailability and the lack of action on the central nervous system. In an effort to make loperamide even safer, a pharmacologically inactive pro-drug of loperamide, loperamide oxide, was synthesized. The antidiarrhoeal effect of loperamide oxide was attributed to the conversion of loperamide oxide to loperamide in the gastrointestinal tract (Niemegeers et al 1986; Goldhill et al 1989). The reduction of loperamide oxide to loperamide by intestinal contents, red blood cells and liver microsomes has been demonstrated in-vitro (Lavrijsen et al 1984).

It is now generally accepted that loperamide has both antimotility and antisecretory properties that account for its antidiarrhoeal action (Beubler & Lembeck 1979; Niemegeers et al 1981). Administered intraluminally, both loperamide and loperamide oxide equally and dose-dependently reduced prostaglandin E_2 (PGE_2)-induced net fluid secretion in the jejunum and in the colon of the rat in-vivo (Beubler & Badhri 1990). In that study, loperamide oxide but not loperamide, both 2 mg kg^{-1}, inhibited PGE_2-induced secretion 2 h after oral administration, a difference which remained unexplained.

In this study, time course and dose response of the antisecretory effects of loperamide and loperamide oxide were investigated in jejunum and colon of the rat after oral administration of the two drugs.

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. Loperamide (0·008, 0·03, 0·125, 0·5, and 2 mg kg^{-1}) and loperamide oxide (equal doses) were given orally in the morning, so that PGE_2-infusion was always started at 1030 ± 0·5 h. After the appropriate time, the rats were anaesthetized with pentobarbitone sodium (65 mg kg^{-1}, i.p.). The abdomen was cut open and a polyethylene catheter (PE60) was placed in the jejunum about 5 cm distal to the flexura duodeno-jejunalis and fixed by ligation. The second ligation was made about 20 cm distal to the first ligation. A second catheter (PE60) was placed in the ascending colon before ligation within 0·5 cm distal to the caecum. To obtain comparable conditions, both jejunal and colonic loops were rinsed with 10 mL of body-warm saline.

One hour after the preparation, 2·0 mL 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 ligations were also tied off.

Administration of substances

PGE_2 (32 ng min^{-1}) or 0·9% NaCl (saline) was infused intra-arterially into a branch of the superior mesenteric artery (0·949 mL h^{-1}) using a perfusor (Braun-Melsungen, Germany) (Beubler et al 1986). In a previous work it had been shown that this infusion of PGE_2 affected fluid transfer both in the jejunum and the colon (Beubler & Badhri 1990). Loperamide and loperamide oxide (2 mL) or, in control experiments, the vehicles without drugs, were administered by gavage to the conscious rat at the appropriate time before anaesthesia, so that the time between administration and start of saline or PGE_2 infusion was constant.

Determination of net fluid transfer

Net fluid transfer rates were determined gravimetrically 30 min after the instillation of Tyrode solution for all 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.

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 the two-sample Student's *t*-test.

Correspondence: E. Beubler, Department of Experimental and Clinical Pharmacology, University of Graz, Universitätsplatz 4, A-8010 Graz, Austria.

Chemicals

Loperamide HCl and loperamide oxide were from Janssen Pharmaceutica (Beerse, Belgium). The following vehicle-containing stock solutions were prepared: 25 mg loperamide, 0·25 mL ethanol, 0·125 mL ethylene glycol, 4·625 mL distilled H_2O; 25 mg loperamide oxide, 0·25 mL ethanol, 2 mL cyclodextrin solution (200 mg cyclodextrin, 12 mL tartaric acid (10 mg mL^{-1})), 2·75 mL distilled H_2O. Before oral administration, the stock solutions were diluted with Tyrode solution to the appropriate concentrations. PGE_2 (Sigma Chemical Company, St Louis, MO) and pentobarbitone sodium (Abbott Laboratories, Chicago, IL). All other chemicals were of analytical grade.

Results

Time course. Jejunum

Fluid was absorbed in the jejunum of all control rats, which received the appropriate vehicle orally administered (Figs 1, 2). Close intra-arterial infusion of PGE_2 significantly reversed net fluid absorption into profuse net fluid secretion ($P < 0·01$). Neither loperamide nor loperamide oxide, when administered orally in doses of 0·125, 0·5 and 2·0 mg kg^{-1} affected net absorption in controls between 1 to 4 h after administration (Figs 1, 2). Loperamide was without effect after 1 h at all doses tested. A slight antisecretory effect was seen after 2 h, 0·5 mg kg^{-1} appearing a little more effective than 2·0 mg kg^{-1}. Four hours after oral administration, 0·125 and 0·5 mg kg^{-1} significantly inhibited PGE_2-induced fluid secretion whereas the dose of 2·0 mg kg^{-1} did not show any antisecretory effect (Fig. 1). In contrast, loperamide oxide already appeared to be antisecretory after 1 h at all three doses tested. The effect was maximal after 2 h and was still present after 4 h. No difference between the doses tested was detectable. As indicated in Fig. 2, the effect of loperamide oxide was significantly different from loperamide at 1 h (0·125 and 2 mg kg^{-1}), at 2 h (0·5 and 2 mg kg^{-1}) and at 4 h (2 mg kg^{-1}).

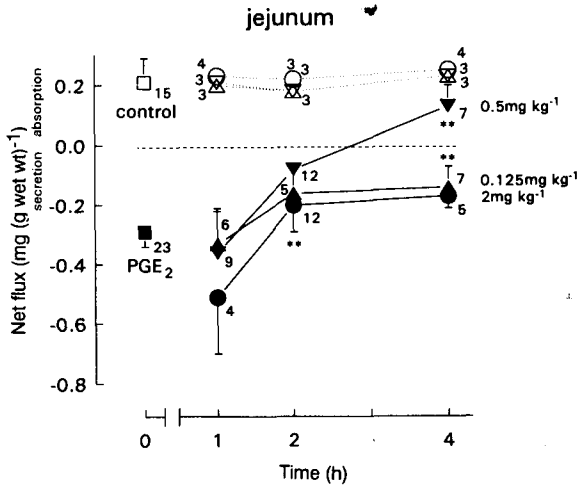

FIG. 1. Time-course of the effect of 0·125, 0·5, and 2·0 mg kg^{-1} of loperamide after oral administration on control absorption (open symbols) and on PGE_2-induced net fluid secretion (closed symbols) in the jejunum. Each point represents the mean ± s.e.m. The numerals indicate the number of experiments. $^{**}P < 0·01$ compared with PGE_2.

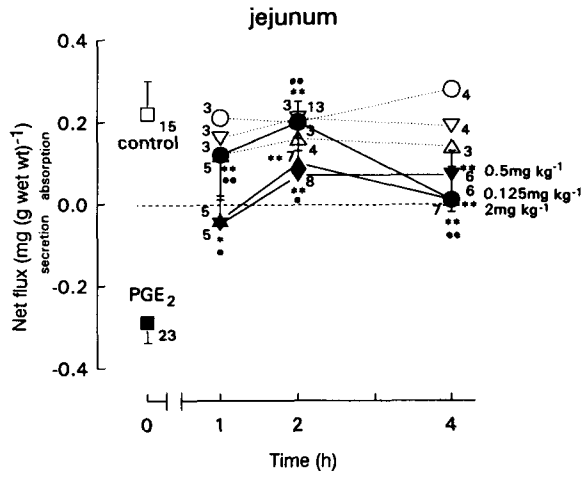

FIG. 2. Time-course of the effect of 0·125, 0·5, and 2·0 mg kg^{-1} of loperamide oxide after oral administration on control absorption (open symbols) and on PGE_2-induced net fluid secretion (closed symbols) in the jejunum. Each point represents the mean ± s.e.m. The numerals indicate the number of experiments. $^{*}P < 0·05$, $^{**}P < 0·01$ compared with PGE_2. $^{\bullet}P < 0·05$, $^{\bullet\bullet}P < 0·01$ compared with loperamide in Fig. 1.

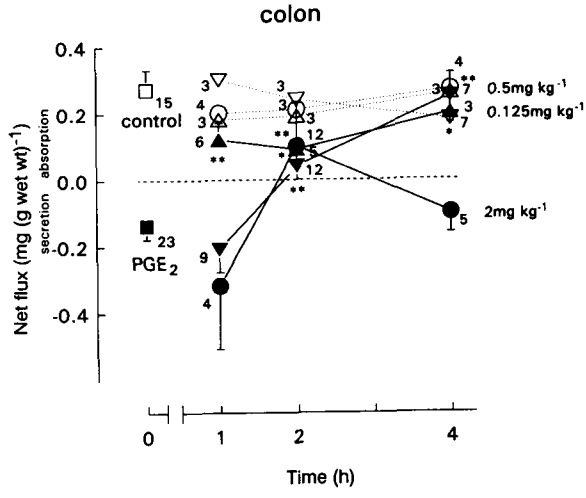

FIG. 3. Time-course of the effect of 0·125, 0·5, and 2·0 mg kg^{-1} of loperamide after oral administration on control absorption (open symbols) and on PGE_2-induced net fluid secretion (closed symbols) in the colon. Each point represents the mean ± s.e.m. The numerals indicate the number of experiments. $^{\bullet}P < 0·05$, $^{\bullet\bullet}P < 0·01$ compared with PGE_2.

Time course. Colon

Again, fluid was absorbed in all control rats (Figs 3, 4). Close intra-arterial infusion of PGE_2 significantly changed net fluid absorption into net fluid secretion ($P < 0·01$). Loperamide, at the lowest dose (0·125 mg kg^{-1}), developed an antisecretory effect within 1 h in the colon. This effect was unchanged over the whole observation period. The dose of 0·5 mg kg^{-1} was ineffective after 1 h. After 2 h the antisecretory effect began and after 4 h a total inhibition of net fluid secretion occurred. With 2·0 mg kg^{-1}, again no effect was seen after 1 h. After having reached a maximum at 2 h, the effect had disappeared by 4 h. More consistent results

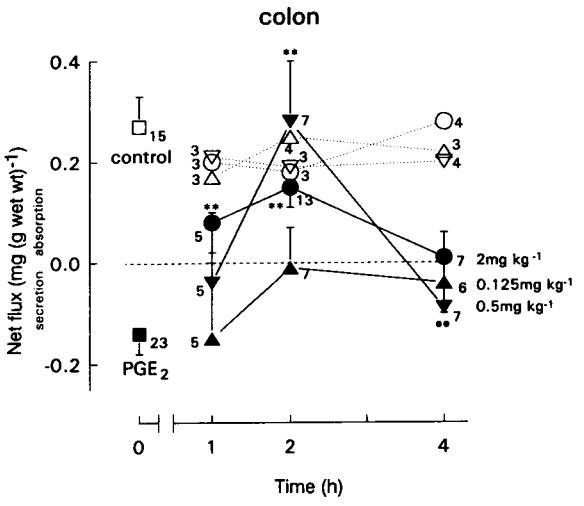

FIG. 4. Time-course of the effect of 0·125, 0·5, and 2·0 mg kg^{-1} of loperamide oxide after oral administration on control absorption (open symbols) and on PGE_2-induced net fluid secretion (closed symbols) in the colon. Each point represents the mean ± s.e.m. The numerals indicate the number of experiments. ** $P < 0.01$ compared with PGE_2. •• $P < 0.01$ compared with loperamide in Fig. 3.

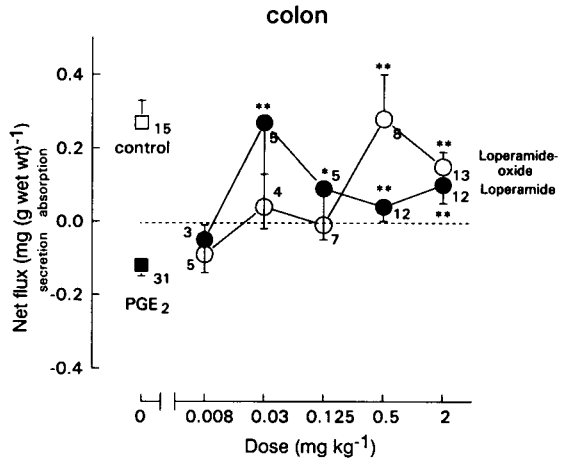

FIG. 6. Dose-response relationship of loperamide (●) and loperamide oxide (○) after oral administration on PGE_2-induced net fluid secretion in the colon. Each point represents the mean ± s.e.m. The numerals indicate the number of experiments. * $P < 0.05$, ** $P < 0.01$ compared with PGE_2.

are seen with loperamide oxide in the colon (Fig. 4). With the two higher doses tested, the antisecretory effect had already started after 1 h and was, as in the jejunum, maximal after 2 h and at the end of 4 h slightly reduced. No difference between the doses tested was seen. A difference of the effect of loperamide oxide compared with loperamide was seen at 4 h with the dose of 0·5 mg kg^{-1} ($P < 0.01$).

Dose response. Jejunum

Dose-response relationships were obtained 2 h after oral administration of loperamide or loperamide oxide. In the jejunum, loperamide reduced PGE_2-induced secretion at an oral dose of 0·008 mg kg^{-1}, 2 h after administration ($P < 0.05$). Higher doses, however, did not show any increased antisecretory effect. In contrast, loperamide oxide from 0·03 to 2 mg kg^{-1} reduced the secretory effect of PGE_2, the reduction being statistically significant at 0·125 mg kg^{-1} and higher. At 2 mg kg^{-1}, loperamide oxide completely inhibited fluid secretion (Fig. 5).

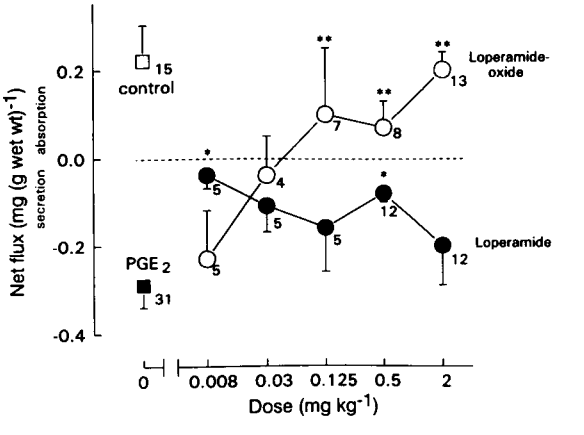

FIG. 5. Dose-response relationship of loperamide (●) and loperamide oxide (○) after oral administration on PGE_2-induced net fluid secretion in the jejunum. Each point represents the mean ± s.e.m. The numerals indicate the number of experiments. * $P < 0.05$, ** $P < 0.01$ compared with PGE_2.

Dose response. Colon

In the colon, loperamide totally inhibited PGE_2-induced fluid secretion at a dose of 0·03 mg kg^{-1}, 2 h after administration. Higher doses appear to be less effective. In contrast, loperamide oxide dose dependently from 0·008 to 0·5 mg kg^{-1} reduced PGE_2-induced fluid secretion (Fig. 6).

Discussion

In the present study, experiments were performed to compare the antisecretory effects of loperamide and loperamide oxide after oral administration. Both loperamide and loperamide oxide reduced PGE_2-induced net fluid secretion in the jejunum and the colon. In particular, however, certain differences between the two drugs can be seen. A distinct antisecretory effect of loperamide in the jejunum can only be demonstrated after 4 h and with 0·125 and 0·5 mg kg^{-1} loperamide, whereas 2·0 mg kg^{-1} remained without effect over the whole observation period, confirming the results of a former investigation (Beubler & Badhri 1990). The two effective doses are in the order of magnitude of the oral ED50 of the antidiarrhoeal action of loperamide in castor oil-induced diarrhoea (Niemegeers et al 1981). The observation that loperamide at 2 mg kg^{-1} does not affect control absorption excludes the possibility that it displays anti-absorptive properties in PGE_2-induced secretion. The effects of loperamide in the colon are similar to the effects in the jejunum. Again, the lower doses (0·125 and 0·5 mg kg^{-1}) are more effective than 2·0 mg kg^{-1}. One possible explanation for the lack of effect of high dose loperamide may be that at higher doses its antisecretory properties are countered by non-opiate anti-absorptive actions, a property not demon-

strated by loperamide oxide. The lack of effect on basal transport does not exclude this possibility, as loperamide may not exert any actions on the transport mechanisms that predominate in basal conditions.

The antisecretory effect of loperamide oxide can already be detected 1 h after administration in the jejunum and the colon. This indicates that the drug has to be absorbed and exerts its activity, at least in the colon, from the blood side, since within 1 h, a test solution administered into the rat stomach would not even be transported as far as the caecum (Beubler et al 1985). The maximal effect was seen after 2 h, being similar to the antisecretory effect of loperamide (0·125 and 0·5 mg kg^{-1}) after 4 h. No difference between the effect of the different doses occurred.

It has been shown previously that loperamide oxide must be reduced to loperamide by intestinal contents to produce an effect (Lai & Shearman 1981). The present experiments indicate that despite this metabolic step, loperamide oxide is better absorbed, which may cause a better bioavailability and therefore exert its effect earlier than loperamide. Better absorption and better distribution may also be the reason for the fact that loperamide oxide, in higher concentrations, does not lose its effectiveness like loperamide.

The dose response experiments show that in the jejunum only loperamide oxide reveals a dose-dependent antisecretory effect 2 h after oral administration, whereas loperamide shows the maximal effect at 0·008 mg kg^{-1}. After administration of higher doses of loperamide, the antisecretory effect is declining. This is in contrast to the inhibitory effect of loperamide and loperamide oxide after intraluminal administration. This local administration caused a concentration-dependent inhibition of PGE_2-induced secretion both for loperamide and loperamide oxide (2–500 $\mu g\ mL^{-1}$) (Beubler & Badhri 1990). One explanation may be that loperamide is slowing down gastric emptying. This would also explain the delay in the onset of action of loperamide compared with loperamide oxide, which is only activated in the gut lumen and therefore passes the stomach unrestrained. The same effect, though not equally pronounced, can be demonstrated in the colon. Loperamide oxide again shows a reasonable dose-response curve, whereas loperamide reveals a maximal and total inhibition after 0·03 mg kg^{-1}, higher doses being less effective.

In conclusion, it can be stated that both loperamide and loperamide oxide, administered orally reduced the secretory effect of PGE_2 in the jejunum and the colon of the rat in-vivo. However, certain differences with regard to time course and dose response can be seen. Loperamide oxide shows its antisecretory effect in the jejunum and after 2 mg kg^{-1} also in the colon within 1 h. The effect was maximal after 2 h and slightly decreased after 4 h. A dose-response relationship of loperamide oxide can be demonstrated at 2 h in the jejunum and in the colon. In comparison, the effect of loperamide started later, and both in the jejunum and in the colon higher doses appeared to be less effective than lower doses.

Acknowledgements

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References

Beubler, E., Badhri, P. (1990) Comparison of the antisecretory effect of loperamide and loperamide oxide in the jejunum and the colon of rats in-vivo. J. Pharm. Pharmacol. 42: 689–692

Beubler, E., Lembeck, F. (1979) Inhibition of stimulated fluid secretion in the rat small and large intestine by opiate agonists. Naunyn-Schmiedebergs Arch. Pharmacol. 306: 113–118

Beubler, E., Dirnhofer, R., Ranner, G. (1985) Parathion and gastrointestinal transit in the rat. Arch. Toxicol. 57: 72–73

Beubler, E., Bukhave, K., Rask-Madsen, J. (1986) Significance of calcium for the prostaglandin E_2-mediated secretory response to 5-hydroxytryptamine in the small intestine of the rat in vivo. Gastroenterology 90: 1972–1977

Goldhill, J., Hardcastle, J., Hardcastle, P. T. (1989) Effect of loperamide oxide on PGE_2-stimulated fluid transport in rat small intestine. Z. Gastroenterol. 5: 292

Lai, H., Shearman, G. T. (1981) A comparison of the antidiarrheal and some other pharmacological effects of clonidine, lidamidine, and loperamide in the rat. Drug Dev. Res. 1: 37–41

Lavrijsen, K., Meuldermans, W., Hendrickx, J., Swysen, E., Heykants, J. (1984) The reductive in vitro metabolism of loperamide *N*-oxide by rat liver homogenates, red blood cells and gut contents. Arch. Int Pharmacodyn. Ther. 270: 174–175

Niemegeers, C. J. E., Colpaert, F. C., Awouters, F. H. L. (1981) Pharmacology and antidiarrheal effect of loperamide. Drug Dev. Res. 1: 1–20

Niemegeers, C. J. E., Awouters, F. H. L., Lenaerts, F. M., Artois, K. S. K., Vermeire, J. (1986) Antidiarrheal specificity and safety of the *N*-oxide of loperamide (R 58,425) in rats. Drug Dev. Res. 8: 279–286