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Local and Systemic Actions of Loperamide on Fluid Transport and Transmural Potential Difference Across Rat Small Intestine

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Abstract—The ability of loperamide to influence intestinal fluid transport was assessed using a dual loop preparation. Loperamide was applied to the lumen of the oral, but not the aboral loop, yet basal and prostaglandin-stimulated fluid transport was affected in both, indicating that the drug can act systemically in addition to any local actions. Loperamide had both pro-absorptive and anti-absorptive effects, the latter only occurring when basal fluid absorption was high. The effects of loperamide were greater in the aboral loop to which it was available only via the systemic route. This difference may reflect the absence of counteracting local influences in the aboral loop or a variation in the sensitivity of different regions of the gut to loperamide.

Loperamide is one of the most popular anti-diarrhoeal agents in therapeutic use due to its long-lasting and effective protection against diarrhoea and its high safety margin resulting from a low systemic bioavailability (Stockbroeckx et al 1973). It has been shown to have both antimotility and antisecretory actions. In the guinea-pig, the latter is proposed to be mediated by binding to δ opioid receptors whilst the former has been shown to be mediated by μ opioid receptors (Ooms et al 1984), although μ-receptor binding can also have antisecretory effects in the rat (Coupar 1987). Opiate receptors have been identified in the myenteric and submucosal plexuses (Cooke 1987) and also at the tips of the villi (Dashwood et al 1986). Orally administered loperamide is rapidly taken up from the intestinal lumen but because the plasma concentration following absorption is low, it has been proposed that the anti-diarrhoeal effects of the drug are mediated locally as a result of diffusion to the various receptor sites within the gut wall following uptake by the epithelium (Awouters et al 1983). This investigation was designed to determine whether the systemic circulation plays a role in the antisecretory actions of loperamide. A dual loop technique was developed to test whether loperamide in the lumen of one loop could influence fluid transport or electrical activity in an adjacent loop.

Materials and Methods

Animals

Male Wistar rats, 230–250 g, obtained from the Sheffield Field Laboratories and allowed free access to food and water, were anaesthetized with sodium pentobarbitone (Sagatal, 60 mg kg^{-1}, i.p.).

The dual loop preparation

The trachea and the jugular vein were cannulated. Following a mid-line incision, a length of mid-intestine was identified and two incisions were made approximately 20 cm apart. The luminal contents were flushed out with 154 mM NaCl and residual fluid gently blown out. A wide-bore silicone tube was passed about 1 cm into the lumen through the distal incision and tied into place, providing a perfusate outflow. A further incision was made about 10 cm proximal to this point and a second length of silicone tubing was tied into the lumen at a point immediately distal to the mid-point incision thus forming the aboral loop. Two smaller cannulae had previously been fixed in place inside the proximal tubing using silicone sealant, one cannula being a narrow-bore perfusate inflow, whilst the second was a salt bridge electrode to enable the potential difference (PD) to be monitored. A second loop, the oral loop, was formed in a similar manner, with the wide-bore tubing being passed into the lumen immediately proximal to the mid-point incision and the dual cannula entering the loop through the most proximal incision. The tight ligatures forming the two loops would prevent communication between the loops via the enteric nervous system, with the blood supply remaining largely intact. The lengths of tubing passed from the peritoneal cavity via small incisions in the body wall. Core temperature was monitored using a rectal probe and maintained at $37 \pm 1{\cdot}5°C$ using a heating lamp.

The jugular cannula was connected to an infusion pump (Braun Perfusor Secura), whilst the perfusate inflow cannulae were connected to a peristaltic pump (Quickfit Instrumentation type 10 PP 60). The wide-bore outflow tubes were placed in the mouth of fluid reservoirs which were capped to reduce evaporation. The perfusion circuits were completed by portex tubing extending from the bottom of the fluid reservoirs back to the perfusion pump. The fluid reservoirs were filled with known volumes (approx. 3·75 mL) of pregassed Krebs bicarbonate saline (KBS) containing the non-absorbable marker, polyethylene glycol 4000 (PEG 4000, 12·5 mM) to which was added 100 μL loperamide or 100% ethanol vehicle. This solution was labelled with [^{14}C]PEG 4000 (0·1 μCi mL^{-1}), and a 100 μL sample was added to 5 mL Optiphase Safe scintillation fluid and counted for ^{14}C in a

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liquid scintillation counter (LKB, 1215 Rackbeta) to determine accurately the initial activity. The contents of the fluid reservoirs were maintained at 37°C by water jackets.

Two differential input electrometers linked to two pairs of calomel half-cells monitored the transmural PD of the two loops using a common wick electrode as a reference and salt bridge electrodes in the lumen of each loop. The output from the electrometers was fed into an on-line BBC microcomputer to integrate PD with respect to time.

The peristaltic pump circulated fluid at a rate of 2–2·5 mL min^{-1} and the study was divided into three 15 min periods. At the end of each period a weighed volume (approx. 100 μL) of perfusate was sampled from each fluid reservoir, added to scintillation fluid and counted. Since the initial volume of perfusate and concentration of [^{14}C]PEG 4000 was known, changes in concentration over a particular time period could be determined and hence changes in fluid volume calculated. Preliminary experiments established that fluid transport and electrical activity of the intestinal loops had stabilized following surgery by the end of the first period, so fluid transport and PD were determined under basal and prostaglandin E_2 (PGE_2)-stimulated conditions during the second and third periods, respectively. PGE_2-stimulation was induced by intravenous infusion via the jugular cannula (40 $\mu g\ kg^{-1}\ min^{-1}$ at an infusion rate of 50 $\mu L\ min^{-1}$) throughout the third period. Fluid transport was calculated as μL fluid movement over each 15 min period and related to the wet weight of the drained loops determined at the end of the experiment. PD was monitored during each of these periods and expressed as total PD/15 min.

At the end of the experiment the perfusate was collected from both loops and the whole system was then flushed through seven times with 3·75 mL of unlabelled PEG 4000-supplemented KBS. Total label remaining at the end of the experiment was determined and, after accounting for sampling at the end of each of the three periods, recovery was calculated to exclude fluid leakage during perfusion as a cause of a change in luminal volume. Results were discounted if recovery fell outside the 100 ± 10% range.

Loops were then removed, blotted to remove residual fluid and weighed.

Measurement of the effects of loperamide on basal fluid transport and electrical activity

At the beginning of period 1, 100 μL of ethanol either alone or containing loperamide was administered into the oral fluid reservoir resulting in initial drug concentrations of 0, 10, 100 or 1000 μM. In each of the experiments, the same volume of ethanol vehicle was administered into the aboral fluid reservoir.

Fluid transport over period 2 was calculated and expressed as μL fluid transported/15 min (g wet weight)$^{-1}$, positive values indicating secretion and negative values indicating absorption. Electrical activity was expressed as total PD/15 min.

Measurement of the effects of loperamide on PGE_2-stimulated fluid transport and electrical activity

The effects of loperamide on PGE_2-stimulated fluid transport and electrical activity were investigated as a continuation of the experiments described above, with PGE_2 infusion starting at the beginning of period 3.

Fluid transport and electrical activity during period 3 were calculated and compared with those in period 2, the differences expressed as a change in μL fluid transported/15 min (g wet weight)$^{-1}$, with positive values representing a change towards secretion (either a decrease in absorption or an increase in secretion) and negative values representing a change towards absorption (either an increase in absorption or a decrease in secretion). Changes in electrical activity were expressed as an increase in total PD/15 min.

Chemicals

Loperamide was provided by Janssen Pharmaceutica, Beerse, Belgium. PGE_2 was obtained from Upjohn Limited, Crawley, Sussex, UK; PEG 4000 from BDH Limited, Poole, Dorset, UK, and [^{14}C]PEG 4000 from Amersham International PLC, Amersham, Bucks, UK.

Expression of results

Results are expressed as mean values ± s.e.m. of the number of observations indicated. Significance was assessed using Student's *t*-test, paired or unpaired as appropriate, with $P < 0{\cdot}05$ considered as significant.

Results

Basal fluid transport and PD

Under basal conditions fluid absorption was observed in both the oral (-43 ± 9 (20) $\mu L/15\ min\ g^{-1}$) and aboral loops (-46 ± 11 (20) $\mu L/15\ min\ g^{-1}$). In both cases a PD existed across the gut wall (59 ± 8 (20) mV/15 min in the oral loop and 82 ± 10 (20) mV/15 min in the aboral loop) with the lumen being negative with respect to the reference electrode. Whilst larger potential differences were generated across aboral compared with oral loops ($P < 0{\cdot}01$), for reasons that are unclear, fluid transport was similar in both loops ($P > 0{\cdot}05$).

Effects of loperamide on basal fluid transport and PD

Loperamide had a dose-dependent inhibitory effect on basal fluid absorption in the oral loop (Fig. 1a). At the highest dose loperamide reversed net fluid absorption to a net secretion (36 ± 17 (14) $\mu L/15\ min\ g^{-1}$), an effect which diminished to non-significant levels at lower doses. Compared with the oral loop, the aboral loop was more sensitive to loperamide, demonstrating a maximal effect at one-tenth the concentration. Net fluid secretion was induced at both 1000 μM (43 ± 18 (14) $\mu L/15\ min\ g^{-1}$) and 100 μM (55 ± 10 (4) $\mu L/15\ min\ g^{-1}$, $P < 0{\cdot}02$ compared with the oral loop in both cases). The anti-absorptive effect declined to non-significant levels at the lowest dose. In contrast to the anti-absorptive effects of loperamide, electrical activity was unaffected at any of the doses tested in either the oral or the aboral loops (Fig. 1b).

Effects of PGE_2 on fluid transport and PD

PGE_2 reversed fluid transport to a net secretion in both loops, causing changes of 57 ± 14 (9) and 68 ± 14 (9) $\mu L/15\ min\ g^{-1}$ in the oral and aboral loops, respectively. Further, PGE_2 increased the transmural PD in both the oral loop (41 ± 8 (9) mV/15 min) and the aboral loop (59 ± 9 (9) mV/15

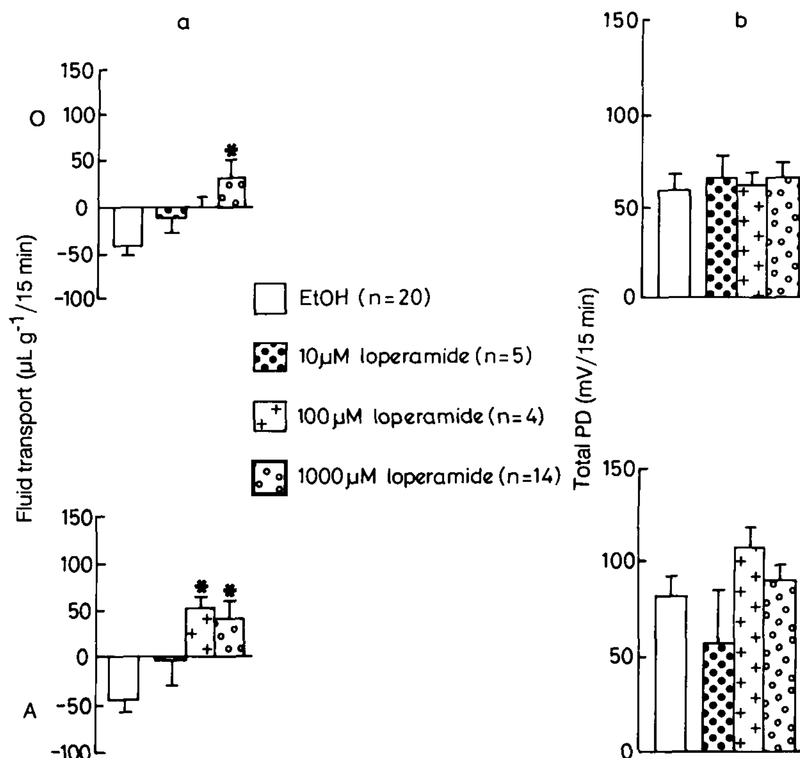

FIG. 1. Effects of loperamide on basal fluid transport (a) and electrical activity (b) in the oral (O) and aboral (A) loops. Loperamide was added to the fluid perfusing the oral loop to give concentrations of 10, 100 or 1000 μM. In control experiments an equivalent volume (2·7% v/v) of the ethanol vehicle was added. Aboral loops were exposed to the same concentration of the ethanol vehicle in all cases. Basal values were measured during period 2 as net fluid movement (positive values indicating secretion and negative values absorption) and electrical activity. Each bar represents the mean $\pm$ s.e.m. of the number of observations indicated and the significance of loperamide action was determined by an unpaired t-test with $^*P<0\cdot02$. Fluid transport data was excluded if PEG recovery was not $100\pm10\%$.

min). The magnitude of the effects of PGE_2 were similar in the two loops.

Effects of loperamide on PGE_2-stimulated fluid transport and PD

In the oral loop the presence of the lowest concentration of loperamide (10 μM) did not prevent PGE_2 from producing a significant secretory response (Fig. 2a). At the higher concentrations of loperamide (100 and 1000 μM) PGE_2 failed to cause a significant fluid secretion as the mean changes in fluid transport were not significantly different from zero ($P>0\cdot05$ in both cases). However, because the effects of loperamide were variable, there was no significant difference ($P>0\cdot05$ in both cases) between the mean values with and without the drug (Fig. 2a). In the aboral loop, loperamide was without effect on fluid transport at the lowest concentration, whilst at the highest dose it reduced PGE_2-induced fluid transport by 43%. At 100 μM the inhibitory action of loperamide ($P<0\cdot01$) was at a maximum. The effect of loperamide at this intermediary dose was significantly greater ($P<0\cdot05$) in the aboral loop compared with the oral loop. Loperamide did not affect electrical activity in either loop (Fig. 2b).

Discussion

The route by which orally administered loperamide reaches its site of action is uncertain, with both local diffusion from the lumen and systemic delivery following absorption being possible. The data presented indicate that loperamide action cannot be explained solely in terms of a local effect since the presence of loperamide in the oral loop influenced fluid transport not only in that loop but also in the aboral loop under both basal and PGE_2-stimulated conditions. Moreover, at 100 μM the effects of loperamide appear to be greater in the aboral loop than in the oral loop. Loperamide is rapidly absorbed by the mucosa and so diffusion to receptor sites in the nerve plexuses could explain a local mode of action. However, the distances over which diffusion must occur to reach the submucosal and myenteric plexuses are relatively large compared with the close proximity of the dense capillary network in the core of the villus, and thus a large proportion of absorbed drug may be taken up by the mucosal blood supply before it reaches the various nerve plexuses. Once in the systemic circulation, loperamide may be delivered along the whole length of the gut, reaching its site of action at opiate receptors in both the oral and aboral loops of the present study. This suggestion is at first in apparent contradiction to the observation that systemic levels of loperamide following oral administration are low and that orally applied loperamide is almost exclusively excreted in the faeces (Monbaliu et al 1988). However, the fact that portal levels of loperamide are up to 30 times greater than those in the systemic circulation (Monbaliu et al 1984, 1988) indicates that loperamide does reach the circulation but does not appear at high levels in the systemic circulation

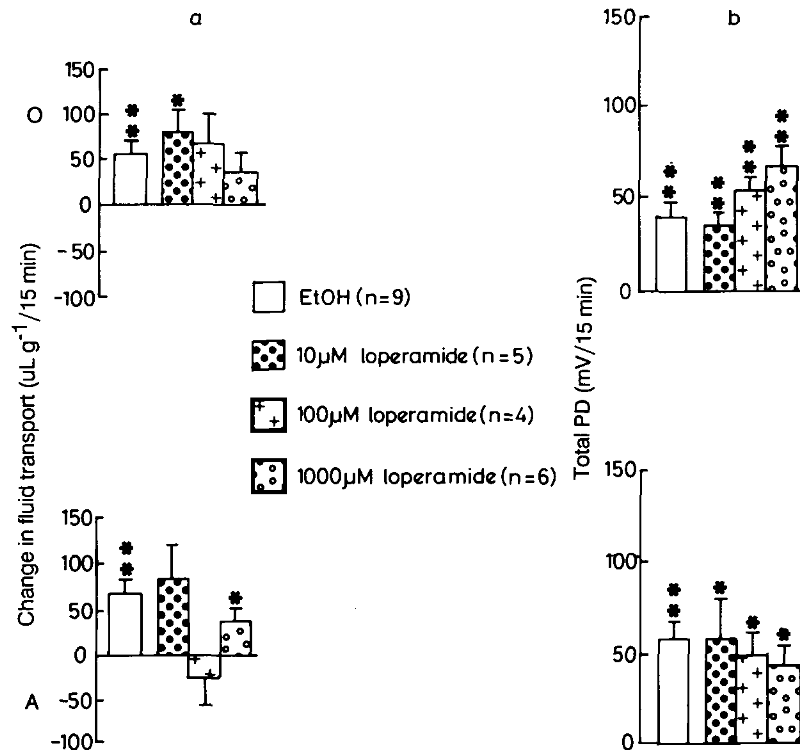

FIG. 2. Effects of loperamide on PGE_2-stimulated fluid transport (a) and electrical activity (b) in the oral (O) and aboral (A) loops. Loperamide was added to the fluid perfusing the oral loop to give concentrations of 10, 100 or 1000 μM. In control experiments an equivalent volume (2·7% v/v) of the ethanol vehicle was added. Aboral loops were exposed to the same concentration of the ethanol vehicle in all cases. Changes in fluid transport and electrical activity induced by PGE_2 were calculated as the difference between the values obtained in periods 2 and 3 in the same experiment. Positive values for net fluid movement indicate a change towards secretion and negative values a change towards absorption. Each bar represents the mean ± s.e.m. of the number of observations indicated and the significance of PGE_2 action was determined by a paired t-test ($*P < 0{\cdot}05$, $**P < 0{\cdot}01$). Fluid transport data was excluded if PEG recovery was not 100 ± 10%.

due to its secretion back into the intestinal lumen in the bile. The present data is in agreement with reports demonstrating the importance of the blood supply in mediating the anti-diarrhoeal effects of loperamide (Beubler & Badhri 1990).

Under basal conditions loperamide reduced net fluid absorption in both the oral and aboral loops, the effect being more pronounced in the latter (Fig. 1a). This may seem surprising since the oral loop is exposed to both luminal and systemic loperamide whilst the aboral loop receives loperamide only via the circulation. Although loperamide could diffuse into the lumen of the aboral loop from the circulation, the levels achieved are likely to be low. It is possible that loperamide may exert different effects when present either luminally or systemically, with the effects in the oral loop representing a balance between local and systemic actions and those in the aboral loop primarily demonstrating systemic effects. Another factor which may contribute to the difference in loperamide action in the two loops is a possible variation in the sensitivity of different regions of the gut to the drug. The effects of opiates on peristalsis in the intestine demonstrate a regional variation since naloxone, the opiate antagonist, causes a progressively greater stimulation of peristalsis on descending the gut (Kromer 1988). Conversely opiates inhibit peristalsis and, like naloxone, this effect would be expected to increase towards the distal gut. The same author suggests the "gradient of the intestine" to be a causative factor in the decreasing frequency of peristaltic waves and sensitivity to distension on descending the gut. These actions on basal fluid transport are in contrast to a number of studies which have shown loperamide to have no effect on basal fluid absorption in either man (Hughes et al 1984; Kachel et al 1984) or rat (Beubler & Lembeck 1979; Hardcastle et al 1981). This may reflect differences in the techniques employed, the concentrations used and the routes of administration. In the present study loperamide was applied intraluminally at concentrations which were in the same range as these found to abolish PGE_2-induced fluid secretion into loops of rat jejunum (Beubler & Badhri 1990). The concentration of loperamide achieved in human intestine following oral administration has not been determined, but in the interdigestive state a 4 mg dose may well lead to a luminal concentration in the upper gut that is similar to those used in this investigation.

The mechanism by which high doses of loperamide may have an anti-absorptive effect is unclear. Under basal conditions the small intestine absorbs fluid, a process which in the absence of luminal nutrients is predominantly dependent on the uptake of sodium chloride. Inhibition of this uptake or stimulation of electrogenic chloride secretion causes a decrease in net fluid absorption and, as loperamide does not alter electrical activity, it is likely that it produces its anti-absorptive effect by an inhibition of sodium chloride

uptake. In a number of species, including the rat, this uptake mechanism involves two brush-border exchange proteins, the bicarbonate/chloride and the sodium/proton co-transporters (Murer et al 1976). The sodium/proton exchanger is directly inhibited by loperamide in brush-border membrane vesicles (Balkovetz et al 1987) so this protein may be involved in the anti-absorptive effects observed in the present study. These observations could explain the paradoxical diarrhoea seen in toxicological studies carried out in dogs (Janssen Pharmaceutica 1989).

PGE_2 induces a net secretion of fluid into the intestine by reducing sodium chloride absorption and stimulating chloride secretion (Hardcastle et al 1981), and this effect was observed in both the oral and aboral loops as a fluid secretion accompanied by a rise in the transintestinal PD (Fig. 2). When loperamide is administered intraperitoneally, it inhibits PGE_2-induced fluid secretion without affecting the electrical response, as it prevents the inhibition of sodium chloride absorption but does not influence the stimulation of chloride secretion (Hardcastle et al 1981). In the present study loperamide in the lumen of the oral loop did not affect the PGE_2-induced elevation of the transmural PD in either the oral or the aboral loop (Fig. 2b). It did however, inhibit the fluid secretion in response to PGE_2-stimulation, an effect that was significant in the aboral loop (Fig. 2a). The ability of loperamide in the lumen of the oral loop to inhibit PGE_2-induced fluid secretion in the aboral loop is further evidence that loperamide can act via the systemic circulation. The inhibitory effects of loperamide on the fluid secretory response to PGE_2 were more marked in the aboral loop suggesting once again that either there is a gradient of sensitivity to the opiate along the length of the intestine or that loperamide has an additional local effect on transport. As the PGE_2-inhibited sodium chloride co-transport system is restored, the possibility exists that it may become susceptible to inhibition by higher doses of loperamide which can directly inhibit the transport proteins (Balkovetz et al 1987). Thus the pro-absorptive actions of loperamide may be self-limiting and this could explain why loperamide is less effective in reducing the secretory response to PGE_2 in the oral loop which is exposed to higher levels of the opiate.

This study provides evidence that the effects of loperamide are not mediated exclusively through local diffusion from the lumen, but that the drug can also reach its sites of action via the systemic circulation. Such an effect would enable loperamide to influence fluid movement throughout the intestinal tract and not only in the regions where it was present in the luminal fluid.

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