Br. J. Pharmac. (1981), **74**, 563–569

THE ACTION OF LOPERAMIDE IN INHIBITING PROSTAGLANDIN-INDUCED INTESTINAL SECRETION IN THE RAT

JACQUELINE HARDCASTLE, P.T. HARDCASTLE, N.W. READ & J.S. REDFERN

Department of Physiology, The University, Sheffield S10 2TN

1 The mechanisms by which loperamide inhibits the intestinal secretion induced by prostaglandin E_2 were investigated in rat jejunum.

2 *In vivo* loperamide prevented prostaglandin-induced fluid secretion but did not reduce the associated rise in the transintestinal potential difference.

3 In intestinal sheets the electrical response to prostaglandin E_2 was enhanced in the presence of loperamide.

4 The ionic basis of these changes was determined by measuring Na^+ and Cl^- fluxes across intestinal sheets. Loperamide did not reduce the prostaglandin-induced increase in net Cl^- secretion, although it prevented the inhibition of mucosal-to-serosal Na^+ movement.

5 Loperamide does not alter cyclic adenosine 3′,5′-monophosphate (cyclic AMP) levels by a direct action at the enterocyte, since in isolated enterocytes neither basal nor prostaglandin-stimulated cyclic AMP levels were affected by the drug.

Introduction

For many years it was thought that the anti-diarrhoeal action of opiates resulted from their effects on intestinal smooth muscle (Vaughan Williams & Streeten, 1950), slowing down the passage of the luminal contents through the gut. However, recent studies have suggested that opiates might also alleviate diarrhoea by inhibiting intestinal fluid secretion (Coupar, 1978; Beubler & Lembeck, 1979), although the mechanisms by which opiates produce this effect have yet to be elucidated. Loperamide (Imodium, Janssen) is a commonly used anti-diarrhoeal agent (Niemegeers, Lenaerts & Janssen, 1974) whose effects are reversed by naloxone (Piercy & Ruwart, 1979), indicating its interaction with an opiate receptor. The present study set out to investigate the mode of action of loperamide on the fluid and electrolyte secretion induced by prostaglandin E_2 (PGE_2) in rat small intestine.

Methods

Experiments were carried out on male albino rats obtained from the Sheffield Field Laboratories and weighing 250–300 g. These were allowed free access to food (diet 86, Oxoid, London) and water. They were anaesthetized with sodium pentobarbitone (60 mg/kg i.p.). Four groups of experiments were conducted.

Measurement of fluid transport and transintestinal potential difference in vivo

Experiments were carried out using a 10 cm segment of mid-intestine which was cannulated at either end. Six ml of 154 mM NaCl containing 10 mg/ml polyethyleneglycol (PEG) 4000 labelled with [^{14}C]-PEG (0.005 μCi/ml) was introduced into the segment via the proximal cannula and excess fluid was allowed to flow out through the distal cannula, flushing out any endogenous fluid present. All the excess fluid was serially collected, weighed and its [^{14}C]-PEG activity determined with a liquid scintillation counter (Packard Tri-carb model 3375) using Bray's scintillation fluid (Bray, 1960). The [^{14}C]-PEG activity of the final aliquot to drain from the loop was within 2.7± 0.4% (n=15) of that of the original solution. The initial volume of fluid remaining in the gut was then calculated from the following equation:

$$\text{Initial volume} = \frac{(V \times [PEG])\ \text{introduced} - (V \times [PEG])\ \text{drained}}{[PEG]_a}$$

where $[PEG]_a$ = PEG concentration of final aliquot.

After an incubation period of 15 min the fluid remaining in the gut was collected and analysed and the final volume calculated from:

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$$\text{Final volume} = \text{Initial volume} \times \frac{[\text{PEG}]_a}{[\text{PEG}]_f}$$

where

$[\text{PEG}]_f$ = PEG concentration at end of incubation.

The difference between initial and final volumes represented the transport of fluid by the intestinal segment. A solution of 154 mM NaCl was then allowed to flow through the gut at 3 ml/min in order to wash out any remaining PEG. After 10 min the saline was blown out and the procedure for determining fluid transport was repeated in a second incubation.

During each incubation period the transintestinal potential difference (PD) was measured by use of two salt bridge electrodes, one in contact with the luminal fluid and the other in contact, via a wick electrode, with fluid in the peritoneal cavity. These electrodes were connected via calomel half cells to a Vibron electrometer (Electronic Instruments Ltd, model 33B–2), the output of which was displayed on a chart recorder (Watanabe Multicorder). Readings were taken every minute during each 15 min incubation period and a mean PD calculated.

The first incubation period acted as a control, while at the beginning of the second incubation period PGE_2 was added to the peritoneal cavity at a dose of 20 μg/kg.

The effects of PGE_2 on fluid transport and PD were assessed in the presence and absence of loperamide. In experiments where loperamide was present the drug was administered intraperitoneally at a dose of 10 mg/kg, dissolved in ethanol, 30 min before the beginning of the first incubation period.

At the end of each experiment the intestinal segment was removed from the animal, dried and weighed.

Measurement of transintestinal potential difference and short-circuit current in vitro

The electrical activity of the intestine *in vitro* was measured using sheets of tissue taken from the mid-intestine. The muscle layers were retained in order to ensure the functional integrity of the neural elements within the gut wall, since it was possible that loperamide might be exerting its effects at this site. Each preparation was clamped between two perspex Ussing-type chambers and the area of exposed tissue was 1.925 cm^2. Mucosal and serosal solutions were Krebs bicarbonate saline (Krebs & Henseleit, 1932) and each was 5 ml in volume. In order to achieve the best possible oxygenation of the preparation both solutions were gassed continuously with 95% O_2 and 5% CO_2. The pH was 7.4 which is close to the luminal pH of the mid-intestine. The PD was measured by use of salt bridge electrodes connected via calomel half cells to a Vibron electrometer. Short-circuit current (SCC) and resistance (R) were measured by passing a current across the intestine by means of Ag/AgCl electrodes which made contact with mucosal and serosal fluids via wide-bore salt bridges. When short-circuiting the tissue, a correction was made for the resistance of the medium as described by Field, Fromm & McColl (1971). Holes drilled in the perspex chambers ensured that the electrodes for both PD and SCC measurements were fixed in a constant position with respect to the intestinal sheet. The resistance of the medium could therefore be measured to an accuracy of ± 1.3% in repeated determinations.

In these experiments paired sheets of tissue, taken from adjacent regions of the intestine, were used, one acting as a control. Before use, tissue resistance was determined by passing a current of 100 μA and measuring the PD change and tissue pairs were only accepted if their resistances varied by less than 25%. The initial resistance in control sheets (88.9 ± 4.0 (8) ohm/cm^2) was not significantly different ($P > 0.1$) from that in test sheets (84.1 ± 8.0 (8) ohm/cm^2).

After an initial stabilization period, open circuit PD and SCC were measured every min for 5 min. Loperamide (10 μg/ml, 1.9×10^{-5} M) was then added to both mucosal and serosal solutions of the test sheet. In the control sheet an equivalent volume of the ethanol/saline medium used to dissolve the drug was administered. PD and SCC measurements were taken for a further 10 min. Prostaglandin E_2 (PGE_2) was then added to the serosal solution of both sheets to give a final concentration of 5 μg/ml (1.4×10^{-5} M) and readings were taken for a final 10 min. Tissue resistance (R) was calculated from PD and SCC measurements.

Measurement of Na^+ and Cl^- fluxes in vitro

Na^+ and Cl^- fluxes were measured in the intestinal sheet preparation described above. The method for determining and calculating the unidirectional fluxes of Na^+ and Cl^- was as described by Corbett, Isaacs, Riley & Turnberg (1977). Na^+ and Cl^- fluxes were measured simultaneously in paired sheets of intestine, one sheet being used for the determination of mucosal-to-serosal Na^+ and Cl^- fluxes and the other sheet for the measurement of serosal-to-mucosal Na^+ and Cl^- fluxes. Net fluxes were calculated as the difference between the two unidirectional fluxes. Two 10 min incubations began 25 min after the addition of the isotope by which time steady-state fluxes had been achieved. The first period acted as a control while in the second period PGE_2 (5 μg/ml, 1.4×10^{-5} M) was present in the serosal solution. When present, loperamide (10 μg/ml, 1.9×10^{-5} M) was added to the mucosal and serosal solutions of

both sheets 10 min before the beginning of the first period of flux measurement. In control experiments an equivalent volume of the ethanol/saline vehicle was used. The total duration of the flux determinations was 45 min, much shorter than the 60–90 min used by other investigators (Munck, 1970; Taylor, Wright, Schultz & Curran, 1968).

Estimation of cyclic AMP production in isolated intestinal epithelial cells

The small intestine was removed from rats killed by a blow to the head and cervical dislocation. Epithelial cells were isolated by the method of Hardcastle, Hardcastle & Redfern (1980). Cyclic AMP was assayed by the competitive binding protein method of Brown, Albano, Ekins, Sgherzi & Tampion (1971). Cyclic AMP production is expressed as pmol/mg protein in 15 min as determined by the method of Lowry, Rosebrough, Farr & Randall (1951), bovine serum albumin being used as standard.

Expression of results

Results are expressed as mean ± s.e.mean with the number of observations (n) in parentheses. Unless otherwise stated, the number of observations refers to the number of tissue preparations used, each of which was taken from a separate animal. Significance has been assessed by Student's t test, paired or unpaired as appropriate.

Chemicals

Loperamide was generously supplied by Janssen Pharmaceutical Ltd, Beerse, Belgium and PGE_2 by Dr J.E. Pike of the Upjohn Co., Kalamazoo, Michigan U.S.A. $^{22}Na^+$ and $^{36}Cl^-$ were obtained from the Radiochemical Centre, Amersham.

Results

Fluid transport and potential difference in vivo

Under control conditions the intestinal segment secreted a small volume of fluid (0.20 ± 0.06 (5) ml g^{-1} dry wt 15 min^{-1}) and generated a PD of 5.3 ± 0.9 (5) mV, the serosal side of the tissue being positive with respect to the mucosal side. Preliminary experiments showed that there was no significant difference ($P > 0.1$) in the volume of fluid transported and the PD generated during two consecutive control periods. The administration of PGE_2 caused a marked stimulation of fluid secretion in control animals and this was associated with a rise in the PD (Table 1). Loperamide did not significantly alter either fluid transport or the PD under basal conditions ($P > 0.1$ in both cases) but it prevented the increased fluid secretion induced by PGE_2. However, it did not affect the rise in PD observed in the presence of the prostaglandin.

Potential difference, short-circuit current and resistance

Figure 1 shows PD, SCC and R measurements in control and loperamide-treated tissues. At the end of the initial 5 min period PD, SCC and R values in the two sheets were not significantly different ($P > 0.1$ in all cases). The addition of loperamide to both mucosal and serosal fluids caused significantly greater reductions in PD ($P < 0.001$) and SCC ($0.05 > P > 0.01$) than those observed in the control tissues. However, the presence of loperamide did not alter R ($P > 0.1$). PGE_2 increased the PD and SCC in both preparations but these effects were significantly greater ($0.01 > P > 0.001$ in both cases) in the presence of loperamide (PD = 3.3 ± 0.3 (8) mV, SCC = 25.9 ± 3.8 (8) $\mu A/cm^2$) than in its absence (PD =

Table 1 Effects of prostaglandin E_2 (PGE_2 20 μg/kg i.p.) on jejunal fluid transport and transintestinal potential difference (PD) in untreated rats (control) and in those receiving 10 mg/kg loperamide i.p. (loperamide-treated).

		Fluid secretion (*ml g^{-1} dry wt. 15 min^{-1}*)	*PD* (*mV*)
Control	Control	0.20 ± 0.06	5.3 ± 0.9
($n = 5$)	+PGE_2	0.71 ± 0.08	6.8 ± 0.7
	PGE_2-induced change	0.52 ± 0.04 **	1.5 ± 0.4 *
Loperamide-treated	Control	0.04 ± 0.15	3.8 ± 1.2
($n = 5$)	+PGE_2	0.12 ± 0.08	6.0 ± 0.8
	PGE_2-induced change	0.08 ± 0.10 NS	2.2 ± 0.4 *

The significance of the effects of PGE_2 were assessed by Student's paired t test: NS = not significant; * = $0.01 > P > 0.001$; ** = $P < 0.001$.

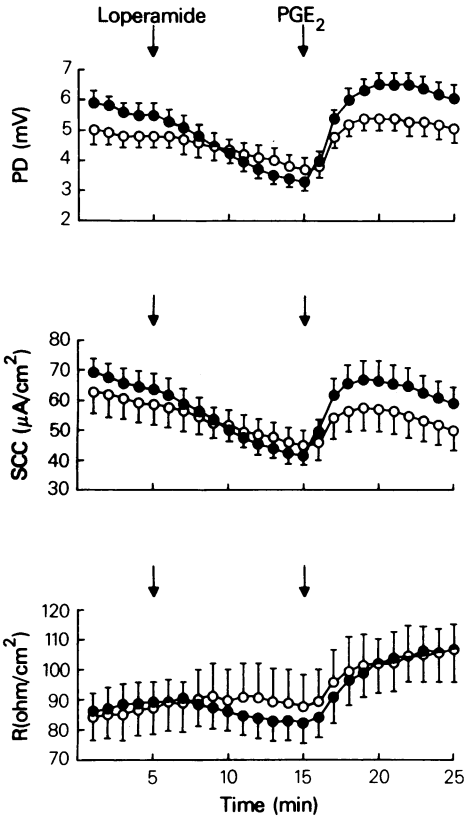

Figure 1 Effect of loperamide on potential difference (PD), short-circuit current (SCC) and resistance (R) in paired sheets of rat jejunum. Loperamide was added to both mucosal and serosal solutions of one sheet (●) to give a final concentration of 10 μg/ml while the control sheet (○) received the same volume of the ethanol saline mixture used to dissolve the drug. Prostaglandin E_2 (PGE_2) was added to the serosal solutions of both preparations to give a final concentration of 5 μg/ml. Each point represents the mean of 8 observations; vertical lines show s.e.mean.

1.9 ± 0.3 (8) mV, SCC = 12.7 ± 2.9 (8) $\mu A/cm^2$). In contrast, the increased R caused by PGE_2 was the same in control and loperamide-treated tissues ($P > 0.1$).

Na^+ and Cl^- fluxes across sheets of intestine

Na^+ and Cl^- fluxes in control and loperamide-treated tissues are shown in Table 2. Under control conditions there was no net movement of Na^+ across the intestinal sheet. Administration of PGE_2 did not significantly affect the net Na^+ flux although an examination of the individual values (Figure 2) showed that the prostaglandin tended to reduce net Na^+ movement, an effect that was no longer consistently observed in the presence of loperamide. These changes were reflected in statistically significant alterations in the mucosal-to-serosal Na^+ fluxes where the PGE_2-induced inhibition was blocked by loperamide (Table 2).

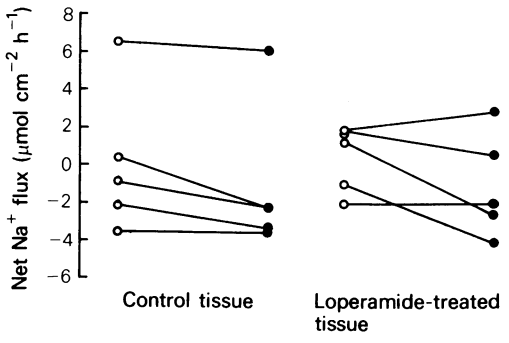

Figure 2 Effects of prostaglandin E_2 (PGE_2, 5 μg/ml in serosal solution) on net Na^+ fluxes across sheets of rat jejunum in the absence (control tissue) and presence of 10 μg/ml loperamide in mucosal and serosal solutions (loperamide-treated tissue). Each individual experiment is represented and values for net Na^+ fluxes obtained in the control period (○) and in the presence of PGE_2 (●) are given.

There was a net secretion of Cl^- ions under control conditions and this was enhanced by PGE_2. Loperamide did not significantly alter the net movement of Cl^- ions either in the absence or presence of PGE_2 ($P > 0.1$ in both cases). However, the actions of PGE_2 on the unidirectional fluxes seemed to be different in the presence of the drug. In control tissues the primary effect of the prostaglandin was to reduce mucosal-to-serosal Cl^- movement while with loperamide there was a smaller decrease in the mucosal-to-serosal flux together with an increase in the serosal-to-mucosal flux. Under all conditions net Na^+ and Cl^- fluxes entirely accounted for the SCC.

Cyclic AMP production in isolated enterocytes

In the absence of loperamide, cyclic AMP production was markedly elevated by PGE_2 (Figure 3). The presence of the drug, at concentrations from 0.005 to 50 μg/ml, did not significantly alter ($P > 0.1$ in all cases) either basal or prostaglandin-stimulated cyclic AMP production.

Discussion

The present study has demonstrated that loperamide abolishes the intestinal fluid secretion induced by prostaglandins *in vivo* (Table 1) and this confirms earlier studies with the drug (Beubler & Lembeck,

Table 2 Effects of prostaglandin E_2 (PGE_2 5 μg/ml in serosal solution) on Na^+ and Cl^- fluxes measured across sheets of rat jejunum in the absence (control tissue) and presence of loperamide 10 μg/ml in mucosal (M) and serosal (S) solutions (loperamide-treated tissue)

		Na^+ fluxes ($\mu mol\ cm^{-2}\ h^{-1}$)			Cl^- fluxes ($\mu mol\ cm^{-2}\ h^{-1}$)		
		M to S	*S to M*	*Net*	*M to S*	*S to M*	*Net*
Control tissue	Control	10.8± 1.4	10.8± 1.2	0.0±1.8	7.6± 0.9	11.4± 0.8	−3.8± 1.0
($n = 5$)	+PGE_2	9.2± 1.6	10.2± 1.0	−1.0± 1.8	5.0±0.6	11.8± 1.0	−6.8± 0.6
		**	NS	NS	*	NS	*
Loperamide-treated	Control	10.8± 1.4	10.4± 1.4	0.4± 0.8	6.8± 0.4	10.0± 1.6	−3.4± 1.2
tissue ($n = 5$)	+PGE_2	10.2± 1.2	11.2± 1.8	−1.0± 1.2	6.0±0.6	11.8± 1.6	−5.8± 1.0
		NS	NS	NS	*	*	*

The significance of the effects of PGE_2 (i.e. control vs. PGE_2) was assessed by Student's paired t test: NS = not significant; * = $0.05 > P > 0.01$; ** = $0.01 > P > 0.001$.

1979). However, the effects of loperamide on fluid secretion were not accompanied by a reduction in the electrical response to PGE_2 (Table 1). In fact *in vitro* studies showed that this effect was enhanced when loperamide was present (Figure 1). Thus the opiate has dissociated the effects of PGE_2 on intestinal fluid transport and electrical activity. Hence the electrical activity of the small intestine does not necessarily mirror its secretory behaviour.

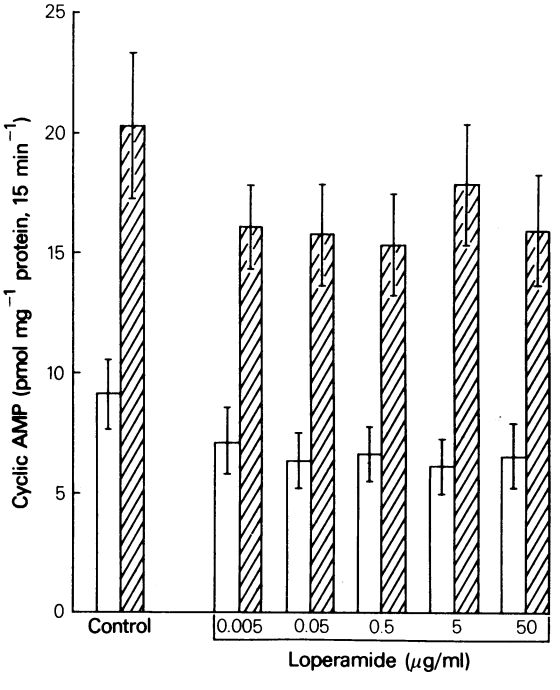

Figure 3 Effect of loperamide on cyclic AMP production in isolated enterocytes in the absence (open columns) and presence of PGE_2 (4 μg/ml, hatched columns). Each column represents the mean of 4 experiments carried out in triplicate: vertical lines show s.e.mean.

The fluid secretion and increased electrical activity induced by prostaglandins in rabbit and human small intestine result from an inhibition of Na^+ absorption together with a stimulation of Cl^- secretion (Al-Awqati & Greenough, 1972; Matuchansky, Mary & Bernier, 1972; Bukhave & Rask-Madsen, 1980). To investigate the ionic basis of the action of loperamide on the prostaglandin-induced changes in rat jejunum, Na^+ and Cl^- fluxes were measured *in vitro*. The results of these experiments suggested that the abolition by loperamide of PGE_2-induced fluid secretion was not related to an inhibition of active Cl^- secretion but was instead possibly associated with a restoration of Na^+ absorption (Table 2, Figure 2). This latter conclusion was based primarily on the finding that loperamide blocked the prostaglandin-induced inhibition of mucosal-to-serosal Na^+ movement, since it is not possible to demonstrate any significant net Na^+ transport in sheets of rat jejunum (Munck, 1970; Taylor *et al.*, 1968). This is because the passive Na^+ channels are particularly leaky in the jejunum and, therefore, any Na^+ absorbed tends to leak back into the mucosal fluid via the paracellular shunt pathway (Krejs & Fordtran, 1973). Cl^- does not suffer from these restrictions as the tight junctions are much less permeable to anions (Krejs & Fordtran, 1973). The action of loperamide in altering the prostaglandin-induced changes in Na^+ and Cl^- fluxes cannot be attributed to an effect on tissue R since this was the same in control and loperamide-treated preparations (Figure 1). The fact that active Cl^- secretion was unchanged may explain why the electrical response to PGE_2 was not diminished by loperamide (Table 1, Figure 1). As active Cl^- secretion is believed to originate from the crypts, while absorptive processes occur in the villi (Field, 1976), it seems likely that loperamide primarily influences processes occurring in the villous enterocytes.

The effects of prostaglandins on intestinal ion transport are known to be mediated by a rise in mucosal cyclic AMP concentrations (Kimberg, Field,

Gershon & Henderson, 1974). Many actions of opiates are associated with a reduction in the concentration of this nucleotide (Klee, 1976) and so it seemed possible that their anti-secretory effects in the intestine might also be mediated in this way. In the present study isolated enterocytes were used to assess this possibility. PGE_2 caused a marked stimulation of cyclic AMP levels in these cells but this was unaffected by loperamide over a wide range of concentrations (Figure 3). There are two possible explanations for this lack of effect of loperamide on the PGE_2-induced rise in cyclic AMP levels: opiate action in this system may not involve changes in cyclic AMP production, or alternatively, the opiate receptor may not be located on the enterocytes. There is evidence that opiate receptors are confined to nervous tissue (Pert & Snyder, 1973) and in the intestine opiates have been found to interact with receptors in the nerve plexuses (Mackerer, Clay & Dajani, 1976), causing a decrease in neuronal firing (Dingledine, Goldstein & Kendig, 1974). It is therefore possible that loperamide acts within the intestinal nerve plexuses and influences enterocyte activity through an intermediary pathway. This could explain why opiates have been found to inhibit PGE_1-stimulated cyclic AMP levels in mucosal scrapes taken from rats pretreated *in vivo* with morphine and PGE_1 (Beubler & Lembeck, 1980), while in isolated enterocytes loperamide was without effect on the production of this nucleotide (Figure 3).

In summary, it has been shown that loperamide abolishes intestinal secretion induced by PGE_2 by blocking the inhibition of Na^+ absorption without affecting the stimulation of Cl^- secretion. If loperamide exerts its effects solely through an alteration of cyclic AMP concentrations it seems that the receptors involved are not located on the enterocyte.

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(*Received February 23, 1981.*
Revised June 15, 1981.)