Inhibitory actions of loperamide on absorptive processes in rat small intestine

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SUMMARY Mucosal loperamide caused a dose dependent reduction in the absorption of actively transported hexoses and amino acids, together with the associated rise in short circuit current. Na^+ and fluid movement were also inhibited. Serosal application of the drug was without effect on these processes. The passive movement of fructose across the gut was not affected by loperamide which is therefore unlikely to act by reducing tissue permeability. In low Na^+ conditions the inhibitory actions of loperamide on glycine absorption were reduced. Loperamide reduced basal Na^+ transport although it did not affect the stimulation of Na^+ absorption caused by mannose. Loperamide had no effect on the total ATPase activity nor on the Na^+ , K^+ -ATPase activity of mucosal homogenates. The effects of loperamide were not mimicked by morphine nor were they antagonised by naloxone and hence do not seem to involve an opiate receptor. It is concluded that loperamide exerts its inhibitory effects by an interaction with the Na^+ sites of the nutrient carriers.

Loperamide (Imodium, Janssen) is an opiate agonist which is used as an antidiarrhoeal agent.¹ Its therapeutic efficacy appears to arise not only from an action on the motor functions of the intestine, which results in slowed intestinal transit and increased gut capacitance,² but also from its ability to inhibit intestinal secretion.³⁻⁶ The antisecretory actions of loperamide are observed in response to serosal application of the drug. Loperamide is, however, administered orally and will therefore come into contact with the luminal surface of the intestinal epithelium, which is the site of absorptive processes in the intestine. The present investigation indicates that loperamide causes an inhibition of hexose and amino acid absorption and the associated movement of Na^+ and fluid in everted sacs of rat small intestine.

Methods

RATS

Experiments were carried out on male albino rats weighing between 230 and 250 g. These were allowed free access to food (diet 86, Oxoid, London) and water. They were anaesthetised with sodium pentobarbitone (60 mg/kg ip).

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MEASUREMENT OF INTESTINAL TRANSPORT

The everted intestinal sac,⁷ was used to determine the transport of fluid, nutrients, and Na^+ . Two 10 cm sacs, taken from the midintestine, were made. These were filled with 0.5 ml fluid (serosal fluid) and incubated for 30 minutes at 37°C in 25 ml fluid (mucosal fluid). The incubation fluid was Krebs bicarbonate saline⁸ equilibrated with 95% O_2 /5% CO_2 and containing additions as indicated. In experiments where the Na^+ concentration was reduced to 25 mM this was achieved by replacing all the NaCl in the Krebs with isotonic Tris Cl. Fluid transport was measured gravimetrically and the volume of fluid taken up by the sac, mucosal fluid transport, is expressed as ml/g initial wet weight/30 minutes. The absorption of hexoses and amino acids was determined using ^{14}C -labelled compounds (1 $\mu\text{Ci}/100$ ml) which were added to the mucosal fluid containing unlabelled substrate at an initial concentration of 7.5 mM. At the end of the incubation period the serosal fluid was collected and the intestinal sac was deproteinised with 2/3N H_2SO_4 and 10% sodium tungstate, and homogenised. Samples were added to Bray's scintillation fluid⁹ and counted in a liquid scintillation counter (LKB, 1215 Rackbeta). Nutrient absorption was expressed in two ways: firstly as the amount taken up by the sac, expressed as $\mu\text{mol/g}$ initial wet weight/30 minutes, and secondly as the T/M ratio. This is the ratio of

nutrient concentration in the tissue water compared with that in the mucosal solution at the end of the incubation and is a measure of the concentrating ability of the intestine. Tissue concentration was calculated from the amount of nutrient in the tissue at the end of the incubation and the volume of tissue water (80% initial wet weight plus the volume of fluid taken up by the sac during incubation). Na^+ transport was measured by analysing the Na^+ content of the final serosal fluid and gut homogenate using a Corning flame photometer (Model 430). Substrate and Na^+ uptake by the sacs was assessed by adding together the amounts in the gut homogenate and serosal fluid at the end of the incubation. Uptake is expressed as $\mu\text{mol/g}$ initial wet weight/30 minutes.

MEASUREMENT OF INTESTINAL ELECTRICAL ACTIVITY

Intestinal electrical activity was measured in paired sheets (tissue resistances did not vary by more than 20%) of rat midintestine from which the muscle layers had been removed. These were clamped between two Perspex chambers and incubated at 37°C in Krebs bicarbonate saline⁸ gassed with 95% $\text{O}_2/5\%$ CO_2 . The serosal solution contained 10 mM glucose and the mucosal solution 10 mM mannitol. The potential difference was measured by salt bridge electrodes connected *via* calomel half cells to a Vibron electrometer (Electronic Instruments Ltd, model 33B-2). Current was applied across the tissue using Ag/AgCl electrodes which made contact with mucosal and serosal solutions *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 and McColl.¹⁰ After setting up, tissues were left for 10 minutes by which time a stable short circuit current had been achieved. Readings were then taken every minute for five minutes before the addition of either D-galactose or L-alanine. The rise in short circuit current induced by these nutrients was taken as the difference between the maximum value attained in their presence and the value immediately before their administration. When present, loperamide was added to the mucosal solution as soon as the preparation had been set up. Control tissues received an equivalent volume of the ethanol vehicle.

MEASUREMENT OF ATPASE ACTIVITY

The middle 20 cm of small intestine was removed from an anaesthetised rat and washed through with 0.9% saline. The intestinal segment was laid on a glass slab and opened along the mesenteric line. The mucosal surface was then gently scraped using a

glass microscope slide to obtain a sample of mucosal cells. The ATPase activity of the unfractionated cell homogenates was measured using the method of Jorgensen¹¹ with minor modifications. The mucosal scrape was added to incubation buffer to give a protein concentration of 1.5 mg/ml, and homogenised by 10 strokes of a ground glass homogeniser. Protein content was determined by the method of Lowry *et al.*¹² The incubation buffer had the following composition; 10 mM MgCl_2 , 120 mM NaCl , 20 mM KCl , 1 mM EDTA, 60 mM imidazole and 60 mM Tris, pH 7.5. Triton X-100 was present at a concentration of 0.0375% because preliminary experiments had shown this to be the optimum detergent concentration for 'activation' of ATPase in the preparation used. The mucosal homogenate was incubated for 30 minutes at room temperature so that the Na^+ , K^+ -ATPase could be 'activated'. It has been suggested by Jorgensen¹¹ that the detergent activation of Na^+ , K^+ -ATPase is caused by exposure of latent enzyme sites in the preparation. After the 'activation' period, 25 μl aliquots of the solution were transferred to test tubes containing 1 ml of 3 mM ATP in incubation buffer for enzyme assay. The tubes were incubated at 37°C for 30 minutes and at the end of this time the reaction was terminated by the addition of 100 μl 50% trichloroacetic acid. Liberated inorganic phosphate was then assayed by Allen's¹³ modification of the Fiske and Subbarow¹⁴ method and enzyme activity was expressed as $\mu\text{mol Pi/mg protein/h}$. For each experimental condition the incubation was carried out in the absence and presence of 1 mM ouabain. Na^+ , K^+ -ATPase activity was taken as the difference between values obtained with and without ouabain. Loperamide was added to the incubation buffer to give the assay concentrations indicated. Preliminary experiments showed that the ethanol vehicle had no significant effect on either total ATPase activity or Na^+ , K^+ -ATPase activity ($p > 0.05$ in all cases).

EXPRESSION OF RESULTS

Results are expressed as mean values ± 1 SEM of the number of observations indicated. Significance was assessed using Student's *t* test, paired or unpaired as appropriate.

CHEMICALS

Glycine, L-alanine, D-galactose, D-mannose and D-fructose were obtained from BDH Chemicals Ltd, Poole, England. Ethylene diamine tetra-acetic acid, Tris base, imidazole, and naloxone were obtained from the Sigma Chemical Co, St. Louis, MO 63178, U.S.A. Loperamide was generously supplied by Janssen Pharmaceutical Ltd., Beerse, Belgium. D-[1- ^{14}C] galactose, [U- ^{14}C] glycine and D-[U- ^{14}C]

fructose were supplied by Amersham International PLC, Amersham, Bucks, England.

Results

EFFECTS OF LOPERAMIDE AND MORPHINE ON GLYCINE AND FLUID TRANSPORT

The presence of loperamide in the mucosal solution reduced the total amount of glycine taken up and the T/M ratio achieved by everted sacs of intestine in a dose-dependent manner (Table 1, Fig. 1). This effect was significant at 1.9×10^{-5} M. The effect of loperamide on fluid absorption in these experiments was less marked, no significant effect being observed until a concentration of 9.5×10^{-5} M was reached. Loperamide caused a 50% inhibition of glycine transport at a concentration of 1.2×10^{-4} M and a 50% inhibition of fluid transport at 3.1×10^{-4} M. In all cases the ethanol vehicle used had no significant effect on any of the indices measured ($p > 0.05$ in all cases). Serosal application of hyperamide (9.5×10^{-5} M) was without significant effect ($p > 0.05$) on glycine and fluid transport. In contrast with the actions of loperamide, mucosal morphine had no effect on fluid and glycine absorption (Table 1).

Fig. 1 Inhibition of fluid and glycine absorption by everted sacs of rat midintestine in the presence of varying concentrations of mucosal loperamide. Absorption in the presence of loperamide was compared with values obtained in control experiments where the mucosal fluid contained an equivalent volume of the ethanol vehicle, and a % inhibition calculated. Absolute values are given in Table 1. Each value represents the mean $\pm$ 1 SE of the mean of the number of observations indicated.

Table 1 Effect of varying concentrations of loperamide and morphine in the mucosal fluid on fluid and glycine absorption by everted sacs of rat mid-intestine. Glycine transport is expressed as the amount taken up during the incubation (glycine uptake) and as the tissue: mucosal fluid concentration ratio (T/M) achieved by the end of the incubation. Experiments were also conducted in which the mucosal surface of the sac was exposed to the appropriate concentration of the ethanol vehicle. This did not alter significantly ($p > 0.05$ in all cases) any of the indices measured. Results are expressed as mean values $\pm$ 1 SE of the mean of observations in parentheses. Significance was assessed using an unpaired t test.

Additions to mucosal fluid	Mucosal fluid transport (ml/g iww/30 min)	Glycine uptake (μ mol/g iww/30 min)	T/M
No additions (16)	0.53 $\pm$ 0.03	27.2 $\pm$ 1.8	2.5 $\pm$ 0.2
0.2 ml% ethanol (8)	0.52 $\pm$ 0.05	32.8 $\pm$ 3.2	3.0 $\pm$ 0.3
1.9×10^{-5} M loperamide (8)	0.46 $\pm$ 0.02	24.7 $\pm$ 0.9	2.2 $\pm$ 0.1
	NS	$p < 0.05$	$p < 0.05$
0.4 ml% ethanol (7)	0.61 $\pm$ 0.04	27.9 $\pm$ 1.7	2.5 $\pm$ 0.1
3.8×10^{-5} M loperamide (8)	0.52 $\pm$ 0.02	20.9 $\pm$ 1.0	1.8 $\pm$ 0.1
	NS	$p < 0.01$	$p < 0.001$
0.5 ml% ethanol (10)	0.60 $\pm$ 0.05	25.9 $\pm$ 1.7	2.2 $\pm$ 0.1
9.5×10^{-5} M loperamide (6)	0.43 $\pm$ 0.04	14.3 $\pm$ 1.4	1.3 $\pm$ 0.1
	$p < 0.05$	$p < 0.001$	$p < 0.001$
0.8 ml% ethanol (20)	0.48 $\pm$ 0.03	26.0 $\pm$ 1.4	2.4 $\pm$ 0.1
1.9×10^{-4} M loperamide (8)	0.28 $\pm$ 0.02	9.3 $\pm$ 0.6	1.0 $\pm$ 0.1
	$p < 0.001$	$p < 0.001$	$p < 0.001$
No additions (8)	0.54 $\pm$ 0.05	26.5 $\pm$ 2.5	2.4 $\pm$ 0.2
1.3×10^{-4} M morphine (8)	0.52 $\pm$ 0.03	25.0 $\pm$ 1.8	2.3 $\pm$ 0.2
	NS	NS	NS
No additions (8)	0.60 $\pm$ 0.02	29.6 $\pm$ 0.5	2.9 $\pm$ 0.1
10^{-3} M morphine (7)	0.55 $\pm$ 0.04	27.0 $\pm$ 2.0	2.7 $\pm$ 0.2
	NS	NS	NS

EFFECTS OF LOPERAMIDE ON GALACTOSE AND FLUID TRANSPORT

Looperamide also reduced the absorption of galactose, a hexose that is not metabolised significantly by rat small intestine. At a concentration of 9.5×10^{-5} M in the mucosal fluid, loperamide reduced galactose uptake from 44.4 ± 2.3 [$n=7$] to 26.1 ± 2.4 [$n=7$] $\mu\text{mol/g iww/30 min}$ ($p<0.001$) and the T/M ratio from 4.4 ± 0.3 [$n=7$] to 2.5 ± 0.2 [$n=7$] ($p<0.001$). There was also a decrease in fluid absorption from 0.50 ± 0.03 [$n=7$] to 0.32 ± 0.02 [$n=7$] ml/g iww/30 min ($p<0.001$).

EFFECT OF LOPERAMIDE ON FRUCTOSE AND FLUID MOVEMENT

Under the experimental conditions used in this study there was no evidence of active fructose absorption since the T/M ratio was less than 1 (0.64 ± 0.02 [$n=8$]). Under control conditions the intestinal preparation took up 7.4 ± 0.6 [$n=8$] $\mu\text{mol fructose/g iww/30 min}$ and this was not altered significantly ($p>0.05$) by loperamide (9.5×10^{-5} M in the mucosal fluid). Similarly loperamide failed to affect the T/M ratio ($p>0.05$) in these experiments.

Fig. 2 Effect of mucosal loperamide (9.5×10^{-5} M) on glycine uptake in normal Krebs bicarbonate saline ($\text{Na}^+ = 143 \text{ mM}$) and when the mucosal Na^+ concentration was reduced to 25 mM (low Na^+) by partial replacement of Na^+ with Tris^+ . Control sacs were exposed to an equivalent volume of the ethanol vehicle. Each bar represents the mean ± 1 SE of the mean of the number of observations indicated. Significance of loperamide action was assessed using an unpaired *t* test.

Fig. 3 Effect of naloxone (10^{-4} M or 10^{-3} M) in the mucosal fluid on glycine and fluid transport in the absence and presence of loperamide (9.5×10^{-5} M in mucosal fluid). Experiments were carried out under control conditions (C), with naloxone (N), with loperamide (L) and with both loperamide and naloxone (L+N). Results are expressed as mean values ± 1 SE of the mean of the number of observations indicated. Significance of naloxone action was assessed using an unpaired *t* test.

EFFECT OF LOPERAMIDE ON GLYCINE TRANSPORT IN LOW Na^+ CONDITIONS

Reducing the mucosal Na^+ concentration to 25 mM caused a significant ($p<0.001$) reduction in the uptake of glycine (Fig. 2). Under these conditions loperamide was much less effective in reducing glycine absorption, causing only a $20.6 \pm 5.1\%$ [$n=6$] inhibition, which compares with the $44.6 \pm 5.4\%$ [$n=6$] ($p<0.01$) observed when the Na^+ concentration was normal (Fig. 2). In addition, the Na^+ -dependence of glycine absorption was reduced significantly ($p<0.01$) in the presence of loperamide, since lowering the Na^+ concentration caused a $35.7 \pm 4.2\%$ [$n=6$] inhibition of glycine uptake in the presence of the drug and a $54.8 \pm 3.1\%$ [$n=6$] inhibition in its absence. These observations are

Table 2 Effect of loperamide on fluid and Na⁺ absorption by everted sacs of rat midintestine. Loperamide was present in the mucosal fluid at a concentration of 9.5×10^{-5} M while in control experiments an equivalent volume of the ethanol vehicle was added. When present, glycine was added to the mucosal fluid to give a concentration of 7.5 mM while mannose was added to the serosal fluid to give a concentration of 222 mM. Each value represents the mean $\pm$ 1 SE of the mean of the number of observations in parentheses. The significance of loperamide action was assessed using an unpaired t test.

	Mucosal fluid transport (ml/g iww/30 min)	Na ⁺ uptake (μ mol/g iww/30 min)	Glycine-dependent Na ⁺ transfer (μ mol/g iww/30 min)
Control (7)	0.40 $\pm$ 0.01	68.7 $\pm$ 1.7	
Loperamide (7)	0.33 $\pm$ 0.02	56.0 $\pm$ 4.2	
	p<0.01	p<0.05	
Glycine (7)	0.49 $\pm$ 0.03	90.5 $\pm$ 4.5	21.8 $\pm$ 4.5
Glycine+loperamide (7)	0.37 $\pm$ 0.02	65.9 $\pm$ 2.0	9.9 $\pm$ 2.0
	p<0.01	p<0.001	p<0.05
Mannose (7)	0.94 $\pm$ 0.05	132.0 $\pm$ 7.3	
Mannose+loperamide (8)	0.96 $\pm$ 0.05	133.3 $\pm$ 6.4	
	NS	NS	
Glycine+mannose (6)	1.34 $\pm$ 0.07	176.6 $\pm$ 10.3	44.6 $\pm$ 10.3
Glycine+mannose+loperamide (8)	1.13 $\pm$ 0.05	147.9 $\pm$ 8.5	14.6 $\pm$ 8.5
	p<0.05	p<0.05	p<0.05

consistent with an action of loperamide at the Na⁺ site of the nutrient carrier.

EFFECTS OF NALOXONE ON GLYCINE AND FLUID ABSORPTION

Naloxone alone had no effect on fluid or glycine transport, nor did it antagonise the inhibitory actions of loperamide (Fig. 3).

EFFECTS OF LOPERAMIDE ON Na⁺ AND FLUID TRANSPORT

In the absence of glycine, loperamide caused a small inhibition of fluid transport ($17.5 \pm 4.7\%$ [n=6]) and Na⁺ uptake ($18.5 \pm 6.1\%$ [n=6]) by the intestinal sacs (Table 2). These effects of loperamide on basal fluid and Na⁺ transport were less marked (p<0.01 in both cases) than the $44.6 \pm 5.4\%$ [n=6] inhibition of glycine absorption observed at the same concentration of the drug (Table 1). Glycine stimulated both fluid (p<0.05) and Na⁺ transport (p<0.01) significantly, but in the presence of loperamide (9.5×10^{-5} M), these effects were reduced. Thus as well as inhibiting glycine transport, loperamide also reduces the Na⁺ and fluid movement associated with nutrient absorption.

The increased Na⁺ transport associated with nutrient absorption takes place via an electrogenic pathway.^{15 16} In sheets of rat midintestine, the increased short circuit current generated by this Na⁺ movement in the presence of alanine and galactose was reduced significantly when loperamide (9.5×10^{-5} M) was added to the mucosal solution (Fig. 4). In these experiments alanine was used instead of glycine. Alanine is absorbed by the same transport systems as glycine but it produces a larger increase

in short-circuit current. The reason for this is not known.

Loperamide did not alter significantly the marked stimulation of fluid and Na⁺ absorption induced by

Fig. 4 Effect of loperamide (9.5×10^{-5} M in the mucosal fluid) on the rise in short circuit current (Δ SCC) observed with L-alanine and D-galactose (7.5 mM in the mucosal fluid) in sheets of rat midintestine. Experiments were carried out with paired tissues and the control preparation received an equivalent volume of the vehicle (ethanol). Each bar represents the mean $\pm$ 1 SE of the mean of the number of observations indicated. Significance was assessed using a paired t test.

the metabolisable hexose, mannose (Table 2). This sugar is known to increase the absorption of Na^+ by a neutral pathway¹⁷ and it appears that this mechanism is not susceptible to inhibition by loperamide. In the presence of mannose, glycine stimulated significantly the absorption of both fluid ($p < 0.001$) and Na^+ ($p < 0.01$) and these effects were reduced by loperamide (Table 2).

EFFECT OF LOPERAMIDE ON THE KINETICS OF NUTRIENT ABSORPTION

The electrical technique was used to assess the effects of loperamide on the kinetics of nutrient absorption, as the rise in short circuit current induced by nutrients is directly related to their rates of transport.^{15 16} The rise in short circuit current caused by serial additions of alanine or galactose to the mucosal fluid exhibited saturation kinetics (Fig. 5). The $J_{\max}$ (maximum rise in short circuit current) and apparent K_t (apparent Michaelis constant) were calculated in two ways: firstly from a Lineweaver-Burk plot of the data and secondly from a direct linear plot.¹⁸ These two methods produced values that did not differ significantly from one another ($p > 0.05$ in all cases). Loperamide reduced the rise in short circuit current induced by both alanine

and galactose (Fig. 5) and this was reflected in lower $J_{\max}$ values in the presence of the drug (Table 3). Loperamide also reduced the apparent K_t for both nutrients (Table 3). It has been pointed out, however, that changes in $J_{\max}$ can create artefactual alterations in the apparent K_t ¹⁸, and so the decreased values for apparent K_t observed in these experiments are likely to be a consequence of the reduced $J_{\max}$ rather than a change in the affinity of the carriers for nutrients.

EFFECT OF LOPERAMIDE ON Na^+ , K^+ -ATPASE ACTIVITY

The total ATPase activity was 42.2 ± 2.3 [$n=9$] $\mu\text{mol Pi/mg protein/h}$ and Na^+ , K^+ -ATPase activity was 14.9 ± 1.0 [$n=9$] $\mu\text{mol Pi/mg protein/h}$. Neither of these values was affected significantly by loperamide ($p > 0.05$, $n=4$ in both cases).

Discussion

The small intestine possesses both absorptive and secretory mechanisms. The net movement of fluid depends on a balance between these absorptive and secretory processes. Normally absorption exceeds

Fig. 5 Effect of mucosal loperamide (1.9×10^{-4} M) on the kinetics of alanine and galactose absorption. Experiments were carried out on paired sheets of rat mid-intestine and nutrient absorption was monitored as a rise in short-circuit current (ΔSCC). Loperamide was added to the mucosal fluid of test sheets (●) while an equivalent volume of ethanol (1 ml%) was added to the mucosal fluid of control sheets (○). Each value represents the mean ± 1 SE of the mean of the number of observations indicated.

Table 3 Effect of mucosal loperamide (1.9×10^{-4} M) on the $J_{\max}$ (maximum rise in short-circuit current – $\mu\text{A}/\text{cm}^2$) and apparent K_i (apparent Michaelis constant – mM) for alanine and galactose. Experiments were carried out in paired stripped sheets of rat mid-intestine and the mucosal surface of the control tissue was exposed to an equivalent volume of the ethanol vehicle. Kinetic parameters were calculated using a Lineweaver-Burk plot (first values) and a direct linear plot (values in parentheses). Each value represents the mean ± 1 SE of the mean of the number of tissue pairs indicated. The significance of loperamide action was assessed using a paired *t* test.

		Control	+ Loperamide	P
Alanine (7)	$J_{\max}$	256 $\pm$ 45 (223 $\pm$ 35)	61 $\pm$ 14 (60 $\pm$ 14)	<0.01 (<0.001)
	Apparent K_i	18.2 $\pm$ 3.0 (16.9 $\pm$ 2.1)	7.0 $\pm$ 0.5 (7.0 $\pm$ 0.8)	<0.05 (<0.01)
Galactose (9)	$J_{\max}$	408 $\pm$ 61 (413 $\pm$ 64)	149 $\pm$ 23 (146 $\pm$ 24)	<0.01 (<0.01)
	Apparent K_i	26.4 $\pm$ 2.8 (26.9 $\pm$ 3.5)	14.6 $\pm$ 2.6 (15.2 $\pm$ 3.0)	<0.01 (<0.01)

secretion and there is a net movement of fluid into the blood. If, however, this balance is reversed, diarrhoea can result. Such an imbalance can be either because of a stimulation of secretion or an inhibition of absorption. The antidiarrhoeal efficacy of loperamide is due, at least in part, its ability to inhibit secretory processes in the small intestine. In addition, it now appears that loperamide can also influence absorptive mechanisms in the gut. Thus the overall effect of loperamide on net fluid movement will be determined by its relative antisecretory and antiabsorptive actions as well as on the initial balance between absorption and secretion.

Exposure of the luminal surface of the intestine to loperamide reduced the absorption of glycine and the Na^+ and fluid transport associated with it, in everted sacs of rat mid-intestine (Tables 1 and 2). Galactose transport was similarly affected by mucosal administration of the drug. In contrast, serosal application of loperamide was without effect on these processes. The inhibitory actions of mucosal loperamide seem to be directed primarily towards the mechanisms for active nutrient absorption, because basal fluid and Na^+ transport were much less affected by the drug. In addition, the movement of fructose, a hexose which was not actively absorbed in these experiments, was unaltered by the drug. How the loperamide concentrations used in the present study relate to the luminal concentration in the intestine after oral ingestion of a typical dose (4 mg) is not known, as no values have been reported. Loperamide has, however, been perfused intraluminally in man¹⁹ at concentrations that are comparable with those used in this investigation.

It has been shown in the colon that loperamide reduces paracellular permeability.²⁰ It is unlikely, however, that the inhibition of active nutrient

absorption observed in this study can be attributed to this cause, as the passive movement of fructose was unaffected by the drug.

The active absorption of nutrients by the small intestine utilises the energy from the electrochemical gradient for Na^+ across the luminal membrane. This is harnessed by means of carriers which have two sites – one for the nutrient and one for Na^+ . When both sites are occupied the carrier crosses the membrane and the nutrient and Na^+ enter the enterocyte. In order to maintain the Na^+ gradient, this Na^+ is removed by an electrogenic Na^+ pump situated on the basolateral membrane.²¹ The increased activity of this pump is responsible for the rise in short circuit current associated with nutrient absorption (Fig. 4). This change was reduced in the presence of luminal loperamide (Fig. 4), reflecting the observed inhibition of Na^+ transport associated with nutrient absorption (Table 2). In rabbit ileum mucosal loperamide was seen to have no effect on the short circuit current even though glucose was present in the bathing solution.²² Close examination of the data, however, does suggest a small inhibition of electrical activity after 40 minutes exposure to the drug which was present at a concentration (10^{-6} M) that was lower than that used in the present study.

Because nutrient absorption depends on the maintenance of the Na^+ gradient across the luminal membrane, loperamide action could be caused by an inhibition of the Na^+ pump. This seems unlikely, however, for two reasons. Firstly, loperamide failed to inhibit the absorption of Na^+ stimulated by mannose (Table 2). In this process Na^+ is absorbed in association with Cl^- by a neutral pathway²³ that also utilises energy from the Na^+ gradient.²⁴ As this pathway was unaffected by loperamide the Na^+ gradient must be normal. These experiments also

indicate that the metabolic energy supply of the cell was unaffected by the drug. Secondly, loperamide did not reduce the activity of Na^+ , K^+ -ATPase, the enzyme that is associated with the Na^+ pump. Thus loperamide does not appear to influence Na^+ pump activity. It is therefore more likely that loperamide acts at the nutrient carriers situated on the luminal membrane. Sugars and amino acids use different carriers but these all have a common requirement for Na^+ . As loperamide inhibits the absorption of both types of nutrient it may therefore exert its effect at the Na^+ sites of the carriers as has been postulated for harmaline.²⁵ The fact that loperamide was much less effective when the Na^+ gradient was reduced (Fig. 2) is consistent with this view. An action of loperamide at the Na^+ site could prevent the nutrient carrier from utilising the energy of the Na^+ gradient and this would explain the decreased J_{max} observed in the presence of the drug (Table 3).

Some studies in man using segmental jejunal perfusion, have indicated that the antidiarrhoeal effects of loperamide may result from an inhibition of secretion.²⁶ Schiller *et al.*,² however, have reported that the action of loperamide is solely because of a change in motor function and not to an alteration in the rate of transport. These experiments were performed by infusing a balanced electrolyte solution into the stomach and hence the lack of effect of loperamide on absorption is consistent with the data reported here, where loperamide acted primarily on the transport functions associated with nutrient absorption.

The inhibitory effect of loperamide was not a general action shown by other opiate agonists, because morphine, an agonist for the μ -receptor, did not produce any inhibition of nutrient absorption (Table 1), even though it has similar antisecretory actions to loperamide.²⁷ The opiate antagonist naloxone has been reported to block the antisecretory actions of loperamide,⁶ but in the present investigation it did not antagonise the effects of loperamide on fluid and nutrient absorption at either of the doses used (Fig. 3). There appear to be multiple opiate receptors in existence in the small intestine and most agonists and antagonists are capable of interacting with more than one type of receptor.²⁸ Although this complicates the determination of the receptor involved in any particular physiological response, the μ -receptor has been reported to be primarily involved with motility while the δ -receptor is principally involved with transport.²⁹ Naloxone interacts with both these receptor types although it has a greater affinity for the μ -receptor.³⁰ At the concentrations used in the present study, however, both these receptors would be affected and hence the lack of effect of naloxone

alone on fluid and nutrient absorption (Fig. 3) suggests that endogenous opiates do not influence these transport processes in rat small intestine *in vitro*. This contrasts with the *in vivo* observations of Fogel and Kaplan³¹ who found that intraluminal naloxone decreased basal ion and water absorption in rat jejunum and ileum. It therefore appears that any endogenous opiates released *in vivo* are no longer present in *in vitro* preparations. Because naloxone did not prevent the inhibitory actions of loperamide on nutrient transport (Fig. 3), it seems unlikely that the antiabsorptive effects of loperamide involve an opiate receptor. Other workers³² have speculated that the antisecretory actions of loperamide might also involve a non-opiate-receptor-mediated process.

The ability of loperamide to reduce excessive intestinal secretion is well known, but its inhibitory influence on active nutrient absorption has not been described before. The data presented in this paper are consistent with the view that loperamide acts at the Na^+ sites of the nutrient carriers, preventing the utilisation of the energy available from the Na^+ gradient. This would account for the decreased absorption of nutrients together with the associated reduction in fluid and Na^+ transport.

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