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The effect of loperamide on the ion fluxes across the isolated rabbit colon

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Loperamide is a phenyl-piperidine derivative, structurally related to haloperidol, which is used to control diarrhoea.

Although loperamide is effective in inhibiting intestinal motility [1] it is also able to reverse diarrhoeas of a secretory origin as for example those due to prostaglandin E_2 [2] and to cholera toxin [3]. These agents act by increasing the activity of adenylate cyclase within the mucosal cells so raising cellular cAMP levels [4]. In the ileum the increased permeability of the apical membrane to Cl^-, which follows the rise in intracellular cAMP, effectively shorts out the high chloride resistance of the tight junctions and allows NaCl to leak out of the lateral intercellular spaces. This movement of solute is followed by water and a secretion of fluid into the lumen results [5].

Secretion may be reversed by several groups of drugs including δ-opiates [6], β-blockers [7], phenothiazines [8] and glucocorticoids [9] but they do not share the same mechanism of action. For example phenothiazines block the above process of chloride secretion by binding to and inactivating the complex between Ca^{2+} and the calcium dependent regulator protein, calmodulin [10] whilst the glucocorticoids increase the uptake of sodium by the tissue so that it is absorbed faster than it is secreted [9].

In an attempt to elucidate the mechanism by which loperamide abolishes secretion we have measured the unidirectional fluxes of sodium and chloride across the *in vitro* rabbit colon, in the presence of loperamide, before and after increasing tissue cAMP levels with theophylline.

The experiments were performed on lengths of descending colon removed from male NZW rabbits of 2–3 kg which had been killed by cervical dislocation and bleeding. The pellets were washed out and the mesenteric fat cleared from the serosal surface. A cut was made down one side of the colon which was then spread out, mucosa upwards on a sheet of perspex. The mucosa, submucosa and lamina propria were stripped from the underlying muscle using a pair of microscope slides. The stripped tissue was carefully mounted across the ports of a block of six flux chambers and the two halves of the block clamped together. The chambers were each filled with 8 ml of Kreb's bicarbonate Ringer solution and gassed with 5% CO_2 in O_2. The chambers were maintained at 38° by an integral water jacket and after mounting the preparation was left for 30 min to allow it to come to equilibrium before flux measurements were made.

The potential difference across the tissue was measured with calomel electrodes from saline–agar bridges placed close to the tissue and during fluxing the potential was clamped to zero by means of an automatic short circuit current apparatus. No correction was made for the resistance of the solution between the tips of the bridges as this did not exceed 3 per cent of the total resistance.

Fluxes were determined using ^{24}Na and ^{36}Cl as labels. These were added sometimes separately, sometimes together to one of the chambers. Mucosal to serosal (m–s) and serosal to mucosal (s–m) fluxes were measured in the same experiment. After two control periods of 15 min either theophylline (final concentration 1.5 mM) or loperamide (final concentration 2×10^{-5} M) was added to the serosal chamber and after two further periods of 15 min the other drug was added. Potential recordings showed that both drugs produced an effect within 30 sec of administration, so flux rates from the first and second periods were combined in the results. The mean rate for each unidirectional flux was calculated and the significance of the changes determined by a *t*-test between the means.

Theophylline was obtained from Sigma Chemical Co. (London, U.K.) and loperamide was the gift of Janssen Pharmaceuticals. ^{24}Na was obtained as the bicarbonate from the University of London Reactor and ^{36}Cl from the Radiochemical Centre (Amersham, U.K.).

It may be seen from Table 1 that under short circuit conditions the rabbit colon shows a net absorption of Na^+ and Cl^-. The unidirectional fluxes of Cl^- are higher than those of Na^+, in particular J^{Na} s–m, the low value of which reflects the high resistance of the leakage pathway in this tissue.

The effect of 1.5 mM theophylline on these fluxes is shown in part A of the table. There is an increase in J^{Na}m–s but the net movement of Na^+ is not significantly different from control because of the increase in J^{Na}s–m and this implies that there is now a leakage of Na^+ across the tight junctions.

Unidirectional Cl^- fluxes have also increased but J^{Cl}s–m

Table 1. Unidirectional fluxes of Na^+ and Cl^-*

	Treatment	$J^{Na}m$–s	$J^{Na}s$–m	$J^{Na}net$	$J^{Cl}m$–s	$J^{Cl}s$–m	$J^{Cl}net$
A	Control	2.2 ± 0.2 (14)	0.7 ± 0.1 (14)	1.5 ± 0.2	5.1 ± 0.3 (16)	4.5 ± 0.3 (14)	0.6 ± 0.3
	Theophylline	4.5 ± 0.5 (14)†	2.5 ± 0.5 (14)‡	2.0 ± 0.5	6.4 ± 0.2 (14)‡	7.8 ± 0.3 (13)†	−1.4 ± 0.3†
	Loperamide	7.1 ± 0.7 (12)‡	2.4 ± 0.5 (10)	4.7 ± 0.6‡	6.7 ± 0.4 (13)	4.8 ± 0.6 (12)†	1.9 ± 0.5†
B	Control	3.0 ± 0.4 (18)	1.0 ± 0.1 (16)	2.0 ± 0.3	5.5 ± 0.3 (16)	4.7 ± 0.3 (18)	0.8 ± 0.3
	Loperamide	4.2 ± 0.5 (18)	1.0 ± 0.1 (16)	3.3 ± 0.4	9.1 ± 1.0 (16)†	3.8 ± 0.4 (18)	5.3 ± 0.7†
	Theophylline	4.2 ± 0.5 (16)	2.2 ± 0.4 (16)§	2.0 ± 0.6	5.7 ± 0.3 (16)†	4.8 ± 0.5 (14)	0.9 ± 0.4†

* In $\mu Eq \cdot hr^{-1} \cdot cm^{-2}$ across the short-circuited rabbit colon under control conditions and after sequential treatments with 1.5 mM theophylline and 2×10^{-5} M loperamide. Values are mean ± S.E. (n). †, ‡ and § indicate a significant difference from the preceding treatment with $P < 0.001$, < 0.005 and < 0.01, respectively.

has increased more than $J^{Cl}m$–s and the tissue now secretes Cl^-. A similar change in net Cl^- flux has been shown by Frizzell *et al.* [11] after exposing the tissue to cAMP.

Addition of 2×10^{-5} M loperamide to theophylline-stimulated tissue results in a further rise in $J^{Na}m$–s, no changes in $J^{Na}s$–m or $J^{Cl}m$–s but a fall in $J^{Cl}s$–m, back to control values. Thus loperamide abolishes the net secretion of Cl^- induced by theophylline and enhances Na^+ uptake as well. These changes, presumably are the basis of its antisecretory activity.

Although these results show that loperamide is able to reverse the effects of theophylline they give no indication of its mechanism of action. In order to investigate this, the response of control fluxes to loperamide was determined and is shown in part B of Table 1.

2×10^{-5} M Loperamide appears to cause a small rise in net uptake of Na^+ and a fall in $J^{Cl}s$–m but these are not significant. However, there is a marked increase in $J^{Cl}m$–s so the uptake of Cl^- rises significantly.

If theophylline is now added to the preparation net uptake of Na^+ returns to control values due to an increase in $J^{Na}s$–m suggesting again that Na^+ is leaking across the tight junctions. Cl^- uptake also return to control values but this is due to the abolition of the loperamide-induced increase in $J^{Cl}m$–s and not an increase in $J^{Cl}s$–m. Hence, theophylline can reverse the effects of loperamide on Cl^- movement just as loperamide can reverse the effects of theophylline and this means that loperamide does not act by blocking stimulus–secretion coupling. Indeed loperamide may have no effect on the permeability changes brought about by theophylline since $J^{Na}s$–m remains elevated.

According to the model of Frizzell *et al.* [12] Cl^- is accumulated within the cells at a concentration higher than that in the bathing medium due to the action of NaCl cotransport system at the serosal border and a $Cl^-:HCO_3^-$ exchange process at the mucosal surface. Theophylline, by increasing the mucosal border permeability to Cl^- allows it to leak out of the cell so lowering the gradient against which it must enter across the serosal border. Thus there is an increase in $J^{Cl}s$–m.

A similar explanation could apply to the increase in $J^{Cl}m$–s produced by loperamide. If serosal permeability to Cl^- were raised, the reduced gradient would assist the exchange of Cl^- for HCO_3^- across the mucosal border enhancing $J^{Cl}m$–s. When theophylline is present, loperamide would produce an alternative pathway for Cl^- to leave the cell and since this would be in the direction of the electrical gradient, the effective driving force for Cl^- secretion would be removed.

Evidence for such an increase in serosal Cl^- permeability in the rabbit ileum has recently been given by Ilundain and Naftalin [13]. By measuring the rates of exchange across the mucosal and serosal borders they have shown that the permeability of the latter was increased by loperamide which has no effect on the theophylline-increased permeability of the mucosal surface.

The mechanism by which loperamide brings about a change in $J^{Na}m$–s is more difficult to understand. The lower concentration of Cl^- within the cell would, by increasing the activity of the NaCl cotransport system, raise the $[Na^+]$ within the cells so reducing $J^{Na}m$–s [14]. Thus, unless the co-transport system is inhibited by loperamide it would seem that the drug has an effect on Na^+ that is independent of its action on Cl^- permeability.

The results of this study of loperamide may be compared with studies on other opiates. Our findings are similar to those of Dobbins *et al.* [15] for the action of D-Ala_2-Met-enkephalin on ion transport in the rabbit ileum, although these authors report an increase in $J^{Na}s$–m. This would not be expected from the above explanation but the lower resistance of the tight junctions in this tissue may be responsible for the difference.

In contrast, Mackay *et al.* [16] have found that morphine does not affect Na^+ fluxes in the rabbit ileum nor does it cause such an increase in $J^{Cl}m$–s. However, it does produce a significant reduction in $J^{Cl}s$–m. It also increases the residual flux, normally taken to be a measure of HCO_3^- transport. If this has increased then a rise in Cl^- uptake across the mucosal border should have occurred and this fits well with the increase in $J^{Cl}m$–s which we have shown. However, the fact that $J^{Cl}m$–s does not change significantly and $J^{Cl}s$–m falls with this drug implies that the pathway for Cl^- efflux across the mucosal border has become saturated and this would point to the stimulation of HCO_3^- production as the primary effect of morphine.

In summary we can say that loperamide's anti-diarrhoeal action can be explained in terms of its causing changes in electrolyte transfer across the intestinal mucosa. Specifically the the reversal of stimulated Cl^- secretion which process is consistent with the drug producing an increase in the Cl^- permeability of the serosal border. There is also evidence of greater Na^+ absorption and this would further enhance the anti-secretory effect.

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On developmental changes in pulmonary and hepatic monooxygenase activities of the rat

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Evidence accumulated from several studies suggests that the controls of monooxygenase activities in the various organs of the rat are probably tissue-differentiated. Among these studies are those of Oppelt *et al.* [1], Lake *et al.* [2], and Litterst *et al.* [3] which demonstrated that phenobarbital pretreatments of rats increase hepatic and nephric monooxygenase activities but produce no change in the corresponding pulmonary activities. Histologically differentiated effects similar to those produced by phenobarbital pretreatments of rats have also been shown for pretreatments with methyltestosterone, spironolactone or pregnenolone-16 α-carbonitrile [4]. More lately, Al-Turk *et al.* [5–8] have shown that gonadectomy, estrogen-pretreatments, hypophysectomy or streptozotocin-induced diabetic states produce histologically differentiated effects on monooxygenase activities in rats.

In the present communication, we provide data which seem consistent with these earlier observations. Monooxygenase activities of liver and of lung microsomes from rats aged 1–10 weeks were studied using DME*, ethylmorphine and aniline as substrates in order to compare temporal changes in hepatic and pulmonary activities. This study was stimulated by the apparently striking observation that the ability of female rat liver enzymes to form an oxidation product, designated M_2, from DME retrogresses to undetectable limits before adulthood while the corresponding activity for lungs is retained. Earlier reports upon this substrate (DME) have shown that the metabolite M_2 is the major product formed by adult male rat liver enzymes and that the ability of the female rat liver to form the same product appears to be lost during adolescence [9–11].

Methods

Sprague–Dawley rats (C.D. Strain) aged 1–10 weeks were killed by decapitation. Liver and lung microsomes were prepared as described by Litterst *et al.* [12] except that we used KCl where the previous authors used sucrose. Microsomal protein was determined by the method of Lowry *et al.* [13].

Ten microlitres of ethanolic solution containing 250 nmoles DME were incubated with shaking (0.8 c/s) at 36° for 4 min (liver) or 6 min(lung) in 2.5 ml aqueous medium (pH 7.2) containing 200 μmoles sodium phosphate buffer, 300 μmoles KCl, 20 μmoles G-6-P, 2 μmoles NADP, 1.6 units glucose-6-phosphate dehydrogenase and 0.4 mg liver microsomal protein or 1 mg lung microsomal protein. The reactions were stopped by the addition of 1 ml acetone. The mixtures were then extracted twice with *n*-hexane and the amount of each product formed was estimated by g.l.c. [10].

Assays involving aniline were performed as follows: 15 μmoles aniline were incubated at 36° with shaking (0.8 c/s) for 10 min (liver) of 30 min (lung) with 3 mg microsomal protein in 3 ml of aqueous reaction mixture containing 300 μmoles sodium phosphate buffer (pH 7.2), 300 μmoles KCl, 30 μmoles G-6-P, 3 μmoles NADP, and 2 units glucose-6-phosphate dehydrogenase. Aniline hydroxylase activity was determined by the method of Imai *et al.* [14] in which *p*-aminophenol production is monitored.

Incubation mixtures for the assay of ethylmorphine demethylase activity contained the following components in a final volume of 6 ml: 60 μmoles ethylmorphine, 5 mg microsomal protein, 600 μmoles sodium phosphate buffer (pH 7.2), 600 μmoles KCl, 50 μmoles semi-carbazide, 30 μmoles G-6-P, 3 μmoles NADP and 2 units glucose-6-phosphate dehydrogenase. Incubations at 36° with shaking (0.8 c/s) were for 10 min (liver) or 20 min (lung). The demethylase activity was determined by estimation of formaldehyde formed using the method of Nash [15].

In all the assays described above, the reactions are linear with time for the periods stated.

Results and discussion

Temporal changes in hepatic formation of *p*-aminophenol and metabolite M_1 from aniline and DME respectively are broadly similar in pattern, and they show no marked sex differences (Table 1). Developmental patterns in the corresponding pulmonary activites also compare well between the reactions and between the sexes. However,

* Abbreviations: DME, 1,2,3,4,9,9-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-6,7-dimethyl-6,7-epoxy-1,4-methanonaphthalene. M_1, M_2: two monohydroxylated derivatives formed from DME.