the other two agents in improving stool consistency and relieving urgency (Palmer et al., 1980).

Side effects. Diphenoxylate causes more side effects than either codeine phosphate or loperamide. Both codeine and diphenoxylate cross the blood–brain barrier and may cause central-nervous-system side effects such as nausea, dizziness, drowsiness and depression. Loperamide does not usually cross the blood–brain barrier and central-nervous-system side effects are therefore uncommon. The commonest cause for stopping loperamide in one study was abdominal pain and constipation! (Palmer et al., 1980).

Safety. The margin of safety when using a drug is usually assessed from the LD$_{50}$/ED$_{50}$ ratio, which is the relationship between the dose required to kill half the animals to the dose required to cure half of the animals. Greater ratios are associated with safer drugs. In tests of castor oil-induced diarrhoea in rats, the ratio is only 20:1 with codeine, 85:1 with diphenoxylate, and over 1000:1 with loperamide, i.e. the dose of loperamide required to kill half the animals is over 1000 times greater than the dose required to cure half of the animals of their diarrhoea. In man, lethal overdoses of codeine and diphenoxylate have both been reported on a number of occasions, and with codeine phosphate only two or three 30mg tablets ingested by a small child would be enough to exceed the usually lethal dose of 5mg/kg. No fatal overdoses of loperamide have been reported but several overdosed children have exhibited central-nervous-system depression requiring naloxone treatment. It may be that loperamide can cross an immature blood–brain barrier and this area requires further study. It would seem that loperamide, although the most expensive of the three opiates, is the safest choice.

Although the opiates have proved successful in treating acute and chronic diarrhoea in the developed world and appear safe in therapeutic doses, several anxieties exist about their use in third world countries for severe acute diarrhoeas. Several potential problems should be noted. Toxicity may be greater than hitherto appreciated as the doses required for severe secretory diarrhoeas may be higher than those usually recommended. The antimotility effect of these drugs may predispose to paralytic ileus, especially in hypokalaemic individuals, and pooling of fluid in the gut could lead to death from dehydration despite the apparent cessation of diarrhoea. Opiates have been incriminated in precipitating toxic megacolon in acute ulcerative colitis, although this complication can occur in patients with ulcerative colitis on no treatment, and therefore cannot definitely be attributed to the opiates. In one volunteer study, diphenoxylate enhanced symptoms and prolonged faecal excretion of Shigella organisms (Dupont & Hornick, 1973). This single, small study requires confirmation but it raises the possibility that opiate therapy could exacerbate infection with invasive organisms. Loperamide is undergoing field trials at the moment in a variety of doses and it remains to be seen whether the opiates prove as useful in controlling diarrhoea in third world countries as they have been in the developed world.

Conclusions

Detailed study of gastrointestinal physiology, and in particular the secretory process, has unearthed new anti-secretory agents, a few of which may constitute useful additions to our antidiarrhoeal armamentarium. Hopefully, with continued study in these areas, it will not be another 100 years before other useful drugs are added to this list.


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Opiate agonists and enterotoxins

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Natural opioids such as paregoric have been known to have antidiarrhoeal properties for over 2000 years. A number of opioid analogues have been synthesized more recently, but the mechanism of their antidiarrhoeal action is still not fully understood. We became interested in one of these compounds, namely loperamide, while treating children with severe, life-threatening protracted diarrhoea (Candy et al., 1981) who were passing large volumes of stool daily, even then having nothing by mouth. Loperamide (0.5–4mg/kg per day) dramatically reduced the daily stool volume of these children (Sandhu et al., 1983a). On the basis of these clinical observations, we suspected that the effects of the drug could not be explained solely by its action on intestinal motility (Van Nueten et al., 1974) and that it was inhibiting small intestinal secretion of water and electrolytes. This prompted us to examine the effects of loperamide, and its possible mechanism of action, on the well-established secretagogues CT and PGE$_2$ in an animal perfusion model in vivo (Sandhu et al., 1979, 1981). We have now extended these studies to examine the effects of loperamide on the secretion produced by the dehydroxy bile salt DCA and hypertonic solutions of mannitol (Sandhu et al., 1983b). The effects of an endorphin analogue, [(D-Ala)$_2$,Met]$\varepsilon$Enk, on the secretion induced by CT and PGE$_2$ were also examined in the same model (Sandhu et al., 1983c).

Methods

The proximal 15cm of the jejunum of male Wistar rats weighing between 200 and 300g was perfused under intraperitoneal pentobarbitone anaesthesia after an overnight fast during which water was allowed ad libitum as previously described (Sladen & Harries, 1972).

The basic perfusate contained: NaCl (130mmol/l), KCl (4mmol/l), NaHCO$_3$ (25mmol/l), glucose (2mmol/l) and polyethylene glycol 4000 (3g/l) with 5μCi/l of $[^{14}$C]polyethylene glycol. To this basic solution PGE$_2$ (75μg/ml),
sodium deoxycholate (2.5 mmol/l) or mannitol (100 mmol/l or 200 mmol/l) were added as appropriate. The pH was adjusted to 7.0 with CO₂. The solutions were infused continuously at a rate of 0.46 ml/min. After an equilibration period of 30 min, three consecutive 10 min collections of effluent were made. The contents of the effluent were analysed, and using [¹⁴C]polyethylene glycol as a non-absorbable marker, absorption rates of fluid and solute were calculated from standard formulae (Powell & Malawer, 1968).

The animals received, 2 h before laparotomy, intragastric loperamide (4 mg/kg body weight) dissolved in 1 ml of 5% (v/v) ethanol and water, or the solvent alone. In order to study its effect on established PGE₁-induced secretion, some animals were given loperamide after the first hour of perfusion. In those experiments in which the effects of the competitive antagonist naloxone were observed, half the animals received naloxone 1.2 mg/kg body weight, and half received normal saline at half-hourly intervals during the perfusion, in random order.

In the CT experiments, the proximal 15–20 cm of jejunum was fashioned into a blind loop as described by Sladen & Harries (1972) and 75 μg of CT dissolved in 0.75 ml of perfusate was instilled into the loop. Two hours later, the loop was rinsed and prepared for perfusion.

At the end of the experiments, biopsies were taken for light and electron microscopy. Freeze-clamped full-thickness biopsies were taken after the CT experiments, stored at −70°C and cyclic AMP assayed later using the Radiochemical Centre kit. In the CT experiments, mucosal scrapings were also snap-frozen, stored at −70°C and adenylate cyclase activity measured later as described by Tripp et al. (1980).

**Results**

CT induced secretion of water, Na⁺ and Cl⁻ (P < 0.001), increased basal adenylate cyclase activity (P < 0.05) without affecting fluoride-stimulated activity, and increased tissue cyclic AMP level (P < 0.001) (Fig. 1). Loperamide reversed secretion of water, Na⁺ and Cl⁻ to absorption (P < 0.001) (Fig. 1), but had no effect on activation of adenylate cyclase or tissue cyclic AMP levels (Fig. 2). Naloxone inhibited the antisecretory effects of loperamide (P < 0.05) with no effect on tissue cyclic AMP (Figs. 1 and 2). Loperamide similarly

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**Fig. 1. Effect of nasogastric loperamide (L) on cholera toxin (CT)-induced secretion of water and the effect of subcutaneous naloxone (N) on this system**

+ , Net absorption; −, net secretion.

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**Fig. 2. Effect of cholera toxin (CT), loperamide (L) and naloxone (N) on tissue cyclic AMP levels**

C, Control.

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**Fig. 3. Effect of subcutaneous ([d-Ala]₂Met]Enkephalinamide (E) on cholera toxin (CT)-induced secretion of water and the effect of subcutaneous naloxone (N) on this system.**

+ , Net absorption; −, net secretion.
reversed PGE2- and DCA-induced secretion (P<0.001) and was also effective in inhibiting established PGE2-induced secretion (P<0.001). Loperamide had no significant effect on water secretion induced by hypertonic mannitol at (a) 390 mosmol/l; (b) 490 mosmol/l.

[(D-Ala)2, Met]Enk reversed net secretion of water induced by CT (P<0.001) (Fig. 3) and PGE2 (P<0.001) but had no significant effect on basal absorption. This antisecretory action was inhibited by naloxone (Fig. 3). The effect of [(D-Ala)2, Met]Enk on small intestinal secretion appears to be similar to that of loperamide, both qualitatively and quantitatively. Histological studies show that loperamide partially reverses alterations in endothelial permeability produced by CT but has no significant effect on interepithelial spaces (Phillips et al., 1983).

Discussion

Opioids have long been used in the treatment of diarrhoeal diseases in man. Their effect on intestinal motility is well established (Jaffe & Martin, 1980) and hence traditionally it was assumed that the antidiarrhoeal action of opioids, including loperamide, was due to their effect on intestinal motility (Van Nueten et al., 1974). Karim & Adler (1977) noted that loperamide could prevent prostaglandin-induced diarrhoea. Clinical observations led us to suspect that loperamide may have an antisecretory action, and we subsequently showed that loperamide inhibits CT-induced secretion of water and electrolytes in the rat jejunum in vivo (Sandhu et al., 1979). There are now several studies, in vivo and in vitro, which show that opioids affect fluid and electrolyte transport, and this may be in part the basis of their antidiarrhoeal action (Beubler & Lembeck, 1979; Dobbins et al., 1980; Hardcastle et al., 1981; Sandhu et al., 1981; McKay et al., 1982). However, the exact mode of their action is still not fully understood. (Editorial, 1981.)

A number of studies have shown a close relationship between adenylate cyclase activity, tissue cyclic AMP concentrations and the onset of secretion in the small intestine treated with CT (Guerrant et al., 1972; Parkinson et al., 1972; Kimberg et al., 1974; Frizzel, 1977; Field, 1978). In our studies, the antisecretory effect of loperamide in the CT-treated intestine does not appear to be mediated via an effect on adenylate cyclase activity or on tissue cyclic AMP concentrations but is blocked by naloxone (Fig. 2). This suggests that loperamide may act via opiate receptors, separate from the adenylate cyclase/cyclic AMP pathway in the secretory process. Our studies are in keeping with previous studies which have shown that the action of loperamide is opiate receptor mediated (Piercey & Ruwart, 1979; Mackerer et al., 1976; Clay et al., 1977) and not dependent on alteration in tissue cyclic AMP concentration (Hardcastle et al., 1981; Farack et al., 1981).

[(D-Ala)2, Met]Enk also inhibits intestinal secretion induced by CT and PGE2, and this antisecretory effect is blocked by naloxone. It appears that the effect of [(D-Ala)2, Met]Enk on intestinal transport, like that of loperamide, is also not mediated via an effect on tissue cyclic AMP content (Dobbins et al., 1980). Studies with morphine have, however, shown variable and conflicting effects on tissue cyclic AMP formation in the intestine (McKay et al., 1981; Hardcastle et al., 1981; McKay et al., 1982). In brain tissue morphine appears to inhibit adenylate cyclase activity and tissue cyclic AMP formation (Collier & Roy, 1974; Klee et al., 1975; Sharma et al., 1977). This discrepancy may be associated with differences in binding to specific opiate receptors. Morphine is largely a μ-opiate-receptor agonist and loperamide and enkephalins may be predominantly δ-opiate-receptor agonists. It has been postulated that δ-opiate-receptors may be more concerned with intestinal transport and less with motor activity of intestinal smooth muscle, whereas the reverse may be true for the μ-opiate-receptor (Kachur et al., 1980).

Loperamide appears to be able to inhibit intestinal secretion induced by agents with differing mechanisms of action, but has no effect on mannitol-induced osmotic diarrhoea. We have previously shown that it inhibits small intestinal secretion in infant mice induced by Escherichia coli heat-stable toxin, whose action is mediated via cyclic GMP (Watt et al., 1982). It also inhibits secretion induced by 2.5 mM DCA (Sandhu et al., 1983) in the rat jejunum. In the small intestine DCA does not affect adenylate cyclase activity or tissue cyclic AMP concentration (Taub et al., 1977; Simon et al., 1981; Gaginella et al., 1978) and may act by inhibiting Na+/K+-ATPase activity (Guiraldes et al., 1975). Alternatively, bile acid might alter luminal permeability and act as a calcium ionophore (Binder, 1980). Our finding that loperamide inhibits DCA-induced secretion supports the notion that loperamide acts separately from the adenylate cyclase/cyclic AMP pathway.

In addition to cyclic AMP and cyclic GMP, calcium acts as a second messenger in mediating intestinal secretory processes. Ilundain & Naftalin (1979) suggested that calcium ions may stimulate secretion through the calcium-calmodulin complex. Calcium-calmodulin activation increases the C1- permeability across the serosal border, but has no effect on mannitol-induced osmotic diarrhoea. We have previously shown that it inhibits small intestinal secretion in infant mice induced by Escherichia coli heat-stable toxin, whose action is mediated via cyclic GMP (Watt et al., 1982). It also inhibits secretion induced by 2.5 mM DCA (Sandhu et al., 1983) in the rat jejunum. In the small intestine DCA does not affect adenylate cyclase activity or tissue cyclic AMP concentration (Taub et al., 1977; Simon et al., 1981; Gaginella et al., 1978) and may act by inhibiting Na+/K+-ATPase activity (Guiraldes et al., 1975). Alternatively, bile acid might alter luminal permeability and act as a calcium ionophore (Binder, 1980).

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α-Adrenergic agonists and enterotoxins

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The physiological effects of LT and CT are generally considered to arise from the enzymic activity of their α subunits, leading to increased intracellular concentrations of cyclic AMP. However, no LT- or CT-induced increase in cyclic AMP (or cyclic GMP) could be detected in pig ileum (Hamilton et al., 1978; Newsome et al., 1978a).

ST’s are a family peptide which induce ileal secretory activity similar to that of LT and CT, but are too small to function as an enzyme and do not elevate intracellular cyclic AMP concentrations. ST is known to raise cyclic GMP concentration in the ileum (Hughes et al., 1978; Newsome et al., 1978b), but cellular receptors for ST have not been described.

Since the intestinal wall contains as many neurons as the spinal chord it seems probable that normal fluid balance in the intestine is at least partly under neuronal control and that enterotoxins may disturb this control. Indeed the first evidence for such control was perhaps the work of Bernard (Bernard, 1859), who stimulated secretory diarrhoea by cutting sympathetic nerve fibres. Recently Cassuto and co-workers have suggested that a neuronal mechanism is involved in choleraic secretion and have shown that agents such as tetrodotoxin and lidocaine inhibit the response to CT (Cassuto et al., 1981, 1982).

Pesti & Gordon (1977) found that preparations containing ST relaxed noradrenaline-contracted rabbit aorta in vitro and behaved like phenolamine in some smooth muscle preparations. Noradrenaline has been shown to stimulate electrolyte absorption in stripped rabbit ileum (Field & McColl, 1973) and in rat jejunum in vivo and in vitro (Levens et al., 1979; Cotterell et al., 1983a) and α₁ and α₂-receptors have been identified on enterocytes (Cotterell et al., 1983b). α₂-Agonists have been shown to antagonize fluid secretion caused by STA or β-methanolic cyclic GMP in orally challenged infant mice (Newsome et al., 1981). CT-treated rat intestinal loops (Gullikson et al., 1983), ST- or theophylline-