Ann. Rev. Pharmacol. Toxicol. 1983. 23:279–301

PHARMACOLOGY OF ANTIDIARRHEAL DRUGS

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INTRODUCTION

Diarrhea is a very common illness. The too frequent and too liquid stools of acute diarrhea are a sudden expression of digestive tract disease that generally lasts less than a week. In certain conditions the risk for such an attack is very high, affecting e.g. 25 to 50% of United States travelers to Mexico (75). In many cases acute diarrhea is more than an incidental change in bowel habits and imposes interruption of normal activities, confinement to bed, or even hospitalization to restore fluid and electrolyte balances. Obviously relief of the more severe diarrheal attack has long been pursued. Although many drugs can cause constipation in man (88), traditional medicine has selected opium preparations for effective relief of diarrhea and dysentery. The active compounds of these preparations produce effects that are either unnecessary for this therapeutic goal, such as analgesia, or even highly objectionable, such as respiratory depression and addiction. Antidiarrheal specificity is therefore a key concept in the pharmacology of drugs with antidiarrheal properties.

EARLY GASTROINTESTINAL PHARMACOLOGY OF OPIATES

The constipating activity of opiate preparations has long been known. Application of this action for the relief of diarrhea and dysentery preceded their use for analgesia and stimulated the study of their activity on the gastrointestinal tract. Opiates can change motility patterns in all parts of the gut with the net result of decreasing propulsion of its contents. This effect has been particularly clearly demonstrated by the study of peristaltic reflex of the guinea pig ileum (113). Filling of an isolated ileum segment by the inflow of fluid under low hydrostatic pressure distends the wall up to

the point that a series of coordinated contractions of the muscle layers cause expulsion of the intraluminal fluid. Low concentrations of morphine in the organ bath reduce the contraction intensity and the expelled fluid volume.

The local action of morphine has been traced to the intrinsic innervation of the intestinal wall. When the guinea pig ileum segment is stimulated electrically (coaxial or field stimulation), contractions are accompanied by the release of acetylcholine, a neurotransmitter that by itself produces similar contractions. Low concentrations of morphine were found to inhibit the electrically-induced contractions by reducing the acetylcholine release (89, 101). The consistent effect of morphine-like compounds on the intestinal wall is therefore best described as the inhibition of the first phase of the peristaltic reflex, the acetylcholine-dependent contraction of the longitudinal muscle layer. Such a mechanism of action has favoured the view that central actions of this class of compounds, such as analgesia, and peripheral actions, such as inhibition of gastrointestinal propulsion, result from a similar neuronal interaction. In fact an excellent correlation has been reported between the inhibitory effects on the coaxially stimulated guinea pig ileum and analgesia in man for a series of morphine-like compounds that vary in potency from 0.01 to 1000 times morphine (62).

THE PREFERENTIAL INTESTINAL ACTION OF DIPHENOXYLATE

In animal studies equianalgesic doses of morphine and of more potent synthetic compounds, such as dextromoramide, were found not to be equivalent on bowel function, morphine being much more constipating. Separation of these activities was, therefore, considered to be a realistic goal. New pethidine derivatives were systematically tested in mice for analgesic and intestinal effects using a modified hot plate method and the charcoal meal test (54). The orally administered charcoal suspension moves down the normal intestine at a rate such that the caecum contains black suspension within 2 h.

Many compounds of the diphenoxylate series, which are chemically related both to the analgesics of the pethidine type and to the anticholinergics of the isopropamide type (Figure 1), were found to be much more active in the charcoal test than in the hot plate test. High doses of 18 compounds from this series had no significant analgesic or mydriatic activity in mice; in the charcoal test the most potent ones, including diphenoxylate, were active at doses between 0.16 and 0.95 mg/kg (55). The ED_{50}-values of morphine, codeine, and atropine in this test were 9.0, 32.5, and 16.5 mg/kg respectively.

In rats the pronounced intestinal action of these compounds was confirmed on the basis of daily faecal excretion. The decrease in the number

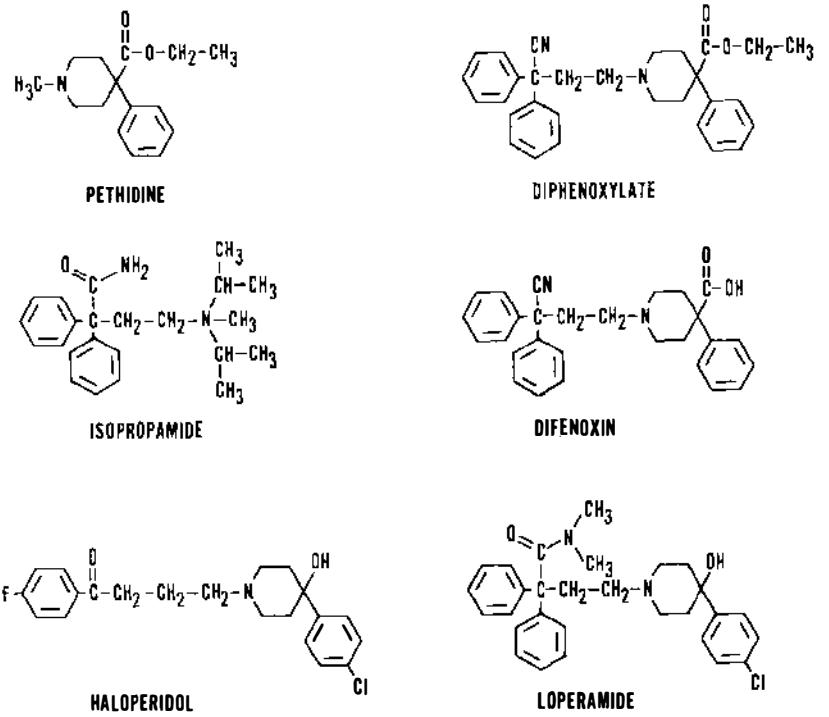

Figure 1 Chemical structures of key compounds in the development of synthetic antidiarrheal drugs.

of faecal pellets in the first day after injection of the compounds correlated well with their potency in the charcoal test, diphenoxylate being at least ten times as potent as morphine (55).

The further development of diphenoxylate (marketed in combination with a low atropine dose as Lomotil in the U.S. and as Reasec in most European countries) has confirmed the properties expected on the basis of these pharmacological data: effectiveness in the treatment of diarrhea and low incidence of central opiate actions. High doses of diphenoxylate, however, produce opiate-like CNS activity (102). Especially in infants and children, who have a varying degree of blood-brain barrier maturation, accidental overdosage can lead to unacceptable consequences and sometimes fatal intoxication (57, 90).

As it was clear that the relatively rare side effects of diphenoxylate were related to central opiate-like activity, an even larger dissociation between the intestinal and central actions was desirable. Both chemical and pharmacological innovation appeared necessary to obtain this result. The mouse hot plate test is appropriate to measure the analgesic potency of morphine-like compounds, but a great number of centrally acting compounds devoid

of narcotic analgesic properties can also prolong the reaction time. The TWR (Tail Withdrawal Reaction) test in rats is much more selective as typical neuroleptics and members of various centrally active drug classes are devoid of activity at high doses (56). Both the charcoal test in mice and the fecal pellet excretion test in rats measure constipating activity of the test compounds and are rather sensitive to drugs devoid of clinically useful antidiarrheal activity, such as the atropine-like drugs. The castor oil test in rats (84) gives a more direct measure of antidiarrheal activity, and quantitative evaluation of the tested drugs, including their potency and duration of action, is much easier to obtain.

ACTIVITY OF OPIATE-LIKE DRUGS IN THE CASTOR OIL AND TWR TESTS

Using diphenoxylate as a standard drug, the detailed results of Table 1 indicate that protection from castor oil-induced diarrhea for 1 h is obtained in 50% of the rats at the dose of 0.15 mg/kg. For more prolonged protection increasing doses are required and the calculated ED_{50}-values were 0.54 mg/kg at 2 h, 1.41 mg/kg at 4 h, and 4.77 mg/kg at 8 h. Oral administration of higher doses of diphenoxylate results in activity in the tail withdrawal reaction test. As shown in Table 2, the lowest effective dose that blocks the TWR (reaction time of more than 10 seconds) in 50% of the animals is 12.8

Table 1 Antidiarrheal activity of oral diphenoxylate in the caster oil test in rats

Diphenoxylate dose mg/kg orally	n	Rats protected from diarrhea at stated hours after caster oil			
		1 h	2 h	4 h	8 h
0.04	10	1	0	0	0
0.08	10	3	1	0	0
0.16	10	5	2	0	0
0.31	10	7	4	1	0
0.63	10	10	5	3	0
1.25	10	10	7	4	1
2.50	10	10	8	6	3
5.00	10	10	10	9	6
10.00	10	10	10	10	7
20.00	10	10	10	10	9
40.00	10	10	10	10	10
ED_{50} mg/kg		0.15	0.54	1.41	4.77
Lower limit		0.11	0.40	1.07	3.44
Upper limit		0.22	0.72	1.97	6.61

mg/kg. The relative antidiarrheal specificity is defined as the ratio of a nonspecific activity (in the case of diphenoxylate the analgesic activity) to the antidiarrheal activity; it remains well above unity for diphenoxylate, even when a prolonged antidiarrheal effect is considered. How long protection from diarrhea must last in order to be clinically useful is not immediately clear, but the dose of diphenoxylate affording protection for 8 h in rats is 4.77 mg/kg, which still has a relative antidiarrheal specificity of 12.8/4.77 = 2.68. Furthermore, such protection of long duration is safe, as the oral LD_{50} of diphenoxylate is 221 mg/kg. The relative safety margin, the LD_{50}/ED_{50} 8 h, is 221/4.77 = 46.3.

During metabolism of diphenoxylate, hydrolysis of the ester moiety leads to the formation of difenoxin (Figure 1). In the castor oil diarrhea test difenoxin was about five times as potent as diphenoxylate (84). Difenoxin was also more potent than diphenoxylate as an analgesic, but when the lowest effective dose in the TWR test is compared with the 8 h protective dose in the castor oil test, the relative antidiarrheal specificity is 4.5, or slightly higher than for diphenoxylate.

LOPERAMIDE IN COMPARISON TO OTHER ANTIDIARRHEAL COMPOUNDS

In an effort to detect more potent, longer acting, and especially more specific and safe antidiarrheal compounds, thousands of derivatives of diphenoxylate were investigated between 1956 and 1969 in the Janssen Research Laboratories. Some of these derivatives had interesting morphinomimetic,

Table 2 Analgesic activity of oral diphenoxylate in the tail withdrawal test in rats

Diphenoxylate dose mg/kg orally	n	Rats with blocked tail withdrawal reaction at stated hours			
		1 h	2 h	4 h	8 h
2.5	10	0	0	0	0
5.0	10	1	3	3	0
10.0	10	2	4	5	3
20.0	10	4	7	6	5
40.0	10	5	8	7	6
80.0	10	8	9	8	8
160.0	10	10	10	10	9
ED_{50} mg/kg		27.4	12.8	15.6	26.2
Lower limit		13.6	7.9	9.82	15.3
Upper limit		55.1	20.7	24.9	44.8

neuroleptic or anticholinergic activity, but as potential antidiarrheals they were inferior to diphenoxylate and difenoxin. In a new series of α,α-diphenylbutyramide derivatives, however, preliminary screening revealed the presence of compounds with potent activity in the castor oil test and devoid of analgesic activity (111). Several members of this series, including R 18 553 (loperamide, Figure 1), were selected for detailed studies.

In Table 3, the results obtained with loperamide in the castor oil test, the TWR test, and the acute toxicity test are compared to those of other compounds of interest. Codeine and morphine have an antidiarrheal specificity, for a duration of action of 2 h, of 5.24 and 6.45 respectively. An oral dose of codeine and morphine can thus be found with effective antidiarrheal but no analgesic activity. This is rather exceptional for narcotic analgesics; e.g. phenacozine and dextromoramide do not counteract diarrhea even for 2 h without inducing analgesia at the same time. Diphenoxylate and difenoxin, on the other hand, have high antidiarrheal specificity, and 8 h protection from diarrhea is still obtained at oral doses below the analgesic dose.

At equal doses loperamide protects longer from diarrhea than diphenoxylate, and protection of 8 h is obtained at 1.81 mg/kg. The relative antidiar-

Table 3 Comparative pharmacological data of four narcotic analgesics and three antidiarrheals

Test		Orally administered compound						
		Phenazocine	Dextromoramide	Codeine	Morphine	Diphenoxylate	Difenoxin	Loperamide
Castor oil test								
Lowest ED_{50} mg/kg	A	6.65	1.80	2.85	1.52	0.15	0.04	0.15
ED_{50}, 2 h mg/kg	B	15.3	2.83	10.8	5.21	0.54	0.16	0.29
ED_{50}, 4 h mg/kg	C	23.0	5.58	28.8	30.9	1.41	0.31	0.61
ED_{50}, 8 h mg/kg	D	40.0	8.25	70.0	60.7	4.77	0.91	1.81
Tail withdrawal reaction test								
Lowest ED_{50} mg/kg	E	8.18	2.78	56.6	33.6	12.8	4.06	>160
Antidiarrheal specificity for action of								
2 h	E/B	0.53	0.98	5.24	6.45	23.7	25.4	>552
4 h	E/C	0.36	0.50	1.97	1.09	9.08	13.1	>262
8 h	E/D	0.20	0.34	0.81	0.55	2.68	4.46	>88.4
Acute toxicity LD_{50}, mg/kg	F	90.0	71.8	427	905	221	149	185
Safety margin for 8 h activity	F/D	2.25	8.70	6.10	14.9	46.3	164	102

rheal specificity of loperamide for 8 h activity is very high (> 88.4), since the oral dose of 160 mg/kg still does not block the tail withdrawal reaction in 50% of the rats. In fact the specificity of the intestinal action of loperamide is best weighed in comparison to the LD_{50} of acute toxicity, 185 mg/kg, a dose associated with neurotoxic symptoms that are not typical of opiate-like drugs (71). The safety margin of an 8 h-protecting dose against diarrhea is 104 (185/1.18) for loperamide.

The historical progression of drugs available for the treatment of diarrhea is well illustrated by Table 3. From opiate extracts, selected by traditional medicine for the treatment of diarrhea and dysentery, pure alkaloids can be prepared that already tend to act preferentially in the gastrointestinal tract when administered by the oral route, the only one indicated for the treatment of diarrhea. The intestinal action of the opiates is intensified in the newer synthetic compounds, whereas the unnecessary and even prohibitive actions of the opiates are reduced in diphenoxylate and absent in loperamide. Many aspects of loperamide's action have recently been reviewed (82, 103) but knowledge about its intestinal actions is rapidly growing and should be compared with what is known of other drugs.

NATURE OF THE ANTIDIARRHEAL ACTION OF LOPERAMIDE: MOTOR FUNCTIONS

The basic activity of loperamide was found by using the castor oil test in rats (85). The potent and prolonged action of loperamide in this model has been confirmed in studies from other laboratories (11, 26, 77, 106), and castor oil-induced diarrhea is also inhibited at low doses in mice (77, 106) and in monkeys (26). Activity of compounds in these studies is generally based on the time of occurrence of the first watery excretion after the oral administration of the oil. In the case of loperamide it is established that active doses do not only extend the diarrhea-free period, but they also decrease the wet weight of the excreted fecal material (4). Although it is established that the active ingredient of castor oil, ricinoleic acid, can change both motility patterns and water and electrolyte transport in the gut (3, 15, 50, 95, 110), detailed study of the effects of loperamide on these processes was indicated.

In vitro pharmacological studies on the peristaltic reflex activity of the isolated guinea pig ileum have clarified the effects of loperamide on the intestinal wall. In this preparation expulsion of the intraluminal content is the result of coordinated contractions of the longitudinal and circular muscle coat. This reflex activity is triggered by radial distension of the wall and is mediated by intramural ganglia and nerve endings located in the intestinal wall (63). Loperamide produces a rapid and sustained inhibition of the

peristaltic reflex at concentrations as low as 0.63 ng/ml. Detailed analysis of the contractile phases indicates first an inhibition of the slow cholinergic phase of the longitudinal muscle activity, followed by a depression of its rapid prostaglandin-sensitive phase; at the same time circular muscle activity is depressed; the net result of the effects is a marked reduction of the expelled volume (120, 122, 124). Quantitatively loperamide is about 47 times more potent than morphine in this preparation; the effects of loperamide have a fast onset and are long lasting. Loperamide differs from morphine by the persistence of its action: morphine is readily washed out from the preparation, whereas the effects of loperamide last for a long time after washing. A further qualitative difference is that loperamide depresses the fast prostaglandin-sensitive phase of the longitudinal muscle contraction (120). Protection from prostaglandin-induced diarrhea was therefore studied and loperamide was found effective against this type of diarrhea in both rat and man (58).

In further agreement with these observations it was found that naloxone reverses the loperamide inhibition of acetylcholine release, but not that of prostaglandin release (132). Naloxone is a much poorer antagonist of loperamide than of morphine (131). The observed partial antagonism may indeed imply that interaction of loperamide with opiate receptors of the gut is essential for its activity, but it has also been noted that naloxone antagonizes the inhibitory effects on intestinal motility of nonnarcotic substances, such as adenine nucleotides (123).

Further studies have shown that both loperamide and morphine inhibit the peristalsis of small intestinal and colonic loops (107). The motor activity of human colonic strips is also inhibited by loperamide (119). Loperamide is active in some other classical tests for the intestinal effects of opiates, such as the electrically induced contractions of the guinea pig ileum (108) and of its longitudinal muscle-myenteric plexus preparation (130). The direct smooth muscle-stimulating action of exogenous acetylcholine, $BaCl_2$, bradykinin, calcium, histamine, nicotine, PGE_1, PGE_2, and serotonin can be inhibited by loperamide but generally at concentrations much higher than those that block the contractile responses mediated by endogenous agonists (58, 69, 79, 80, 108, 122).

The constipating action of morphine in vivo is attributed for one half to delayed gastric emptying and for the other to inhibition of propulsive contractions in the small and large intestine (53). Loperamide, in general, increases motility and muscle tone throughout the gastrointestinal tract of guinea pigs and dogs but at the same time prevents the propagation of contraction waves (80). The effects of codeine, diphenoxylate, and loperamide were compared in volunteer physicians. The three drugs induced rhythmic activity in the small intestine that was of highest amplitude and

duration after codeine; only codeine, however, evoked rhythmic activity in the antrum (36). It is therefore likely that the inhibition of propulsive contraction in the small and large intestine accounts for the largest part of loperamide's action. Furthermore, increased sphincter pressures of the colon after administration of loperamide to patients with diarrhea and incontinence were found to result in effective retention of saline and feces (94).

The intestinal motility pattern is changed by loperamide and by the opiate antidiarrheals. The most characteristic change is increased segmentation activity, which is nonpropulsive. This action, by itself, may favour epithelial absorption of solutes and water. The relation between motor activity and epithelial transport in vivo, however, is very complex and requires consideration of numerous factors such as gastric emptying, contact time, contact area, reflex secretion, mixing, villous movements, and blood flow (93). It is, therefore, of great interest to determine whether drugs affect mucosal transport in the virtual absence of smooth muscle activity.

NATURE OF THE ANTIDIARRHEAL ACTION OF LOPERAMIDE: TRANSPORT OF FLUID AND ELECTROLYTES

The epithelial lining of the digestive tract has an enormous capacity to take up water, electrolytes, and soluble digestion products. Net uptake is the sum of two opposite fluxes: absorption, mainly located in the villi, and secretion, mainly from the crypt cells. In man seven or eight litres of water enter the duodenum and only about 100 ml per day are voided in normal stool (27, 114, 115). Most water absorption occurs in the jejunum by a passive response to the osmotic pressure generated by the absorption of soluble digestion products. In the ileum and colon active electrolyte transport, especially of sodium and chloride, is followed by passive water absorption.

Transport across the intestinal mucosa may become markedly insufficient, resulting in diarrhea, by several mechanisms. An excess of unabsorbed solutes may cause osmotic diarrhea; a decreased mucosal permeability to water and small solutes may play a major role in coeliac disease; the damage to villous tips by invading infectious agents may greatly reduce absorption; or, finally, a series of stimuli such as bacterial toxins, prostaglandins, and serotonin may cause hypersecretion. In principle, agents useful against diarrhea can be thought to act in three general ways. Firstly, a decreased propulsion of the intestinal contents would allow more time for absorption. This is the classical view on the action of morphine-like antidiarrheals. Secondly, effective agents could act on the

intestinal microcirculation and lower the hydrostatic pressure in favour of water absorption. There is no good evidence for this proposition. Thirdly, useful agents may act directly on mucosal transport processes and so reduce fluid accumulation in the intestinal lumen. In this context the "antisecretory" action of drugs has recently been studied more intensively.

In a preparation that is independent of contractions and changes in blood flow, sheets of intestinal mucosa are clamped between two chambers filled with a salt solution [Ussing chamber (118)]. Addition of isotopes allows to calculate the rate of transfer of solutes between the two compartments. Basal fluxes can be changed to a "hypersecretory" pattern by agents such as prostaglandins (92), theophylline, choleragen and A 23187 (48) or serotonin (43). Mucosal transport has also been studied in vivo by measuring accumulation of material in ligated intestinal loops or following changes in the composition of the perfusion fluid of such loops. Secretory responses can further be studied in unanesthetized animals by "enteropooling" (97) or continuous perfusion of the intestine (38).

Table 4 is a survey of the antisecretory effects of various drugs, but does not consider the effects of endogenous substances, such as catecholamines, somatostatin, and enkephalins. Many of these drugs, i.e. the corticosteroids, aspirin-like drugs, and morphine-like drugs, inhibit the synthesis or the action of prostaglandins. Endogenous prostaglandin production is important, both in in vitro preparations of intestinal tissue and in diarrhea (92). Propranolol, verapamil, and the phenothiazines may act primarily at the level of the intracellular messengers, calcium, and cAMP. The antisecretory activity of atropine is consistent with the general effects of muscarinic receptor blocking agents, but mianserin appears to have a peculiar ability, which was not found with cyproheptadine or methysergide, to antagonize the secretory action of serotonin (87).

Studies on intestinal mucosal transport have established the physiological basis for the use of oral glucose-salt solutions in diarrhea, to compensate for the dehydration and for the electrolyte loss in stools. Responses to secretory stimuli and the inhibitory effects of various drugs have greatly contributed to differentiate epithelial transport of the intestine from that in other tissues. It is hoped that further studies with drugs will open new approaches for the treatment of diarrhea, but at this time all agents known to reverse net secretion into net absorption lack the necessary specificity of intestinal action (27, 91, 116).

Pharmacologically, loperamide is the drug with the highest antidiarrheal specificity and appears to be the first exception to the general rule. Studies on the effects of loperamide on intestinal mucosal transport are summarized in Table 5. In general basal absorption of water and electrolytes is not

Table 4 "Antisecretory" effects of various drugs

Drug	Effect	Possible mechanism	References
Methylprednisolone	Reduced intestinal secretion in response to cholera enterotoxin	Glucocorticoids stimulate absorption, via induc-induction of Na^+/K^+-ATPase	19
Dexamethasone	Increased sodium and water absorption		12
Acetylsalicylic acid and other inhibitors of prostaglandin biosynthesis	Reduction of cholera toxin-induced intestinal secretion in cat and rat	Inhibition of the formation of prostaglandins from endogenous arachidonic acid or a direct epinephrine-like effect	33, 34, 51 126
Furosemide	Inhibition of secretion when applied to the serosal side	Blockade of NaCl cotransport of the basolateral membrane of crypt cells	37
Propanolol	Inhibition or reversal of the secretory effects of bile acids, ricinoleic acid or cholera toxin	Blockade of cAMP production or "membrane stabilization"	13, 24, 28
Chlorpromazine	Inhibition of cholera-induced intestinal secretion	Interference with calcium mobilization, probably by binding of the phenothiazine with the calcium-dependent regulator protein	46
Trifluorperazine and other neuroleptics	Inhibition of secretion induced by various stimuli		48, 105
Verapamil	Reduction of electrically induced chloride secretion	Calcium antagonism	47
Atropine	Inhibition of intestinal fluid increase in response to PGE_2 or colchicine	Blockade of cholinergic receptors	10
Mianserin	Antagonism of serotonin-induced chloride secretion of the rat jejunum	Unknown, but the effect is not obtained with cyproheptadine or methysergide	43
Morphine and morphine-like drugs	Antisecretory effect on different types of stimulated secretion in small and large intestine	Peripheral action related to inhibition of prostaglandin-mediated increase in cAMP-levels	8, 9, 25, 65, 72

Table 5 Effects of loperamide in relation to intestinal mucosal transport

Test	Dose or conc. of loperamide	Effects of loperamide	References
Intestinal fluid of the rat stimulated by PGE_2	0.25–1.0 mg/kg	Dose-dependent inhibition	58
Same, but stimulated by 16,16-dimethyl-prostaglandin E_2	1.2 μmol/kg	Dose inhibiting maximal fluid accumulation by 50%	134
Deoxycholic acid-induced sodium and water secretion of the rat cecum	0.6 mg/kg	Inhibition of sodium and water secretion and prevention of histological changes	40
Cholera toxin stimulation of the in vivo perfused rat jejunum	4 mg/kg	Reversal of water, sodium and chloride secretion to absorption; reduced potassium secretion	98, 99
Increased intestinal contents of the rat after PGE_1	10 mg/kg	Pronounced inhibition of prostaglandin (but not of carbachol) hypersecretion	9
Bisacodyl stimulation of the rat colon			
Mucosa of the rabbit colon; stimulation by theophylline	$2 \cdot 10^{-5}$ M	Increased sodium uptake; theophylline-induced chloride secretion is abolished	6
Normal rat colon transport	10^{-6} M	Increased water absorption	14
Normal rabbit ileal mucosa	10^{-5} M	Increased sodium and chloride absorption, also in the presence of naloxone	
Cholera toxin-increased secretion and cAMP of the rat colon loop	4 mg/kg	Pronounced reduction of secretion without effect on cAMP	32
Secretion in segment of rat mid-intestine after PGE_2	10 mg/kg	No effect on potential difference, decrease of fluid secretion and increase of sodium absorption	41, 42
Theophylline-stimulated mucosa of the rabbit ileum	10^{-5} M	Prevention of the theophylline-induced chloride secretion	49
Perfused rat jejunum stimulated by cholera toxin or PGE_2	4 mg/kg	Antagonism of the cholera toxin and PGE_2-induced effects, except for the increase of cAMP-levels	98, 100
Permeability of the colonic mucosa of guinea pigs treated with 1,8-dihydroxyanthraquinone	0.01–2.5 mg/kg	Dose-dependent abolition of the increased permeability	125

significantly changed in the presence of the drug. The net secretion or permeability increase induced by various stimuli, such as prostaglandins, cholera enterotoxin, theophylline, and laxatives, is, however, markedly reduced or abolished by the administration of loperamide in vivo or its presence in the medium. Reversal of the increase in cAMP produced by stimuli such as PGE_2 and cholera enterotoxin apparently is not required for the antisecretory action of loperamide, which is, however, practically abolished by naloxone. The results of these studies support the view that the antisecretory action of loperamide requires persistent interaction with intestinal opiate receptors.

This may not be the only significant site of interaction. Loperamide also inhibits calmodulin activation of phosphodiesterase (74) and calcium-dependent binding of [^{3}H]-trifluoperazine to calmodulin (134). Considering the role of calmodulin in the activation of secretion (78), these properties of loperamide are of great interest, since they are independent of opiate agonist action (74) and are observed at much lower concentrations than for other calmodulin-binding drugs such as chlorpromazine and promethazine (134).

The antisecretory activity of loperamide may have important therapeutic implications. In contrast to the specific antagonism of cholinergic secretion by atropine-like drugs or the specific prevention of prostaglandin biosynthesis, loperamide has been found effective against a wide range of secretory stimuli. This confirms the therapeutic efficacy of loperamide: a fairly uniform dose rapidly restores normal bowel function in most cases of diarrhea of widely varying etiology (2, 44, 96).

It is likely that both normalization of mucosal transport and inhibition of excessive propulsive activity contribute to the overall effect, perhaps in different proportions according to the nature of the etiological agent (35). This is difficult to establish exactly, since in vivo all known secretory agents are also spasmogens and affect local blood flow (18). Bacterial toxins too affect mucosa and muscle (7, 16, 70), but their potent and persistent action may require higher than the common therapeutic doses of loperamide.

The minimum requirement for an effective antisecretory agent is a reduction of fluid and electrolyte loss in vivo. In castor oil diarrhea of the rat this result is obtained from the lowest dose of loperamide that increases the diarrhea-free period (4). Aspirin-like drugs, in contrast, produce delay of diarrhea without a decrease in total watery excretion (5). In horses, the antidiarrheal activity of loperamide, but not of other drugs, is expressed by a significant reduction of water and electrolyte excretion (1). The clinical improvement of ileostomy patients is also reflected in a marked reduction of water and electrolyte losses (60, 117).

ABSOLUTE OR RELATIVE FREEDOM OF UNDESIRABLE EFFECTS

The antidiarrheal specificity of loperamide, in comparison to other clinically useful drugs, has been based on two well-established pharmacological tests in the rat (82). The question of the absence of narcotic effects and of secondary effects of any other type has been studied in much more detail.

Subtoxic dose levels of orally administered loperamide did not produce analgesia as measured in the TWR test. This result does not exclude the possibility that subtle central morphine-like effects could be disclosed by other methods. Potentiation of methohexital hypnosis in rats and of ethanol hypnosis in mice are rather sensitive effects of the administration of narcotic analgesics. Also, diphenoxylate produced potentiation at doses of 5 to 10 mg/kg, smaller than the lowest analgesic dose in the TWR test. Loperamide, however, remained inactive up to near lethal or lethal doses (71).

From a study of many narcotic analgesics in a drug discrimination procedure, it was concluded that the narcotic cue in rats is produced at virtually the same dose that produces analgesia (21). For many reasons, such as the fact that the narcotic cue is not subject to tolerance, analgesia and narcotic discrimination are considered to be independent expressions of the activity of narcotic analgesics. Loperamide was found to be devoid of narcotic discriminative stimulus properties (22, 39). It was therefore predicted that loperamide would have little or no abuse potential (23). In adults with and without extensive opioid experience a very high dose of loperamide (60 mg) was compared to a threshold dose of oral codeine (96 mg base) and to placebo. Only codeine induced pupillary constriction. Loperamide induced a detectable subjective effect in somewhat over half the subjects, was "liked" little or not at all, and was identified as "dope" at a frequency less than that for the threshold dose of codeine (52). After treatment of 10 days with 4 mg loperamide twice daily an intravenous naloxone challenge resulted in no evidence of physical dependence (61).

In two studies (86, 130) the pharmacology of intravenously injected loperamide has been reported. In both, central opiate-like effects were reported for intravenous loperamide at doses equal to or only slightly lower than the lethal dose. The analgesic effects were associated with high plasma levels ($\geqslant$ 1,000 ng/ml), which could be reached after intravenous injection but not upon oral administration of loperamide (86). In man a normal therapeutic dose of 4 mg of loperamide produced a plasma level of about 1 ng/ml. Twenty-seven capsules of 2 mg loperamide taken at once produced peak plasma levels up to 12.4 ng/ml (59, 76, 128, 129). It is not known whether very high plasma levels of loperamide can induce analgesia in man, but deliberate large overdoses do not induce CNS-effects; they produce plasma levels far below those associated with central effects in the rat, and

also far below the plasma level of 100 ng/ml, which can produce opioid physical dependence in monkeys (133). Oral loperamide may therefore be considered devoid of central narcotic effects largely because of very distinctive pharmacokinetics (see below).

Side effects of any type have been rare in several years of intensive use of loperamide for acute and chronic diarrhea (86). Probably this will remain so as long as inappropriate use of the drug is avoided. Diarrheal disease with high fever or blood and mucus in the stools are examples of contraindication (30).

RECEPTOR BINDING OF ANTIDIARRHEALS

It is now accepted that morphine-like drugs and endogenous opioids, as well as their antagonists, act by binding to opiate receptors (104). Furthermore the saturable stereospecific binding sites for opiates can be demonstrated in the two target tissues of greatest interest for antidiarrheals, the ileum and the brain (112).

Binding studies have demonstrated that loperamide inhibits binding of ^{3}H-naloxone or of other suitable ligands to the opiate receptors in much the same way as do classical opiate agonists (20, 67, 68, 109, 112, 131). Furthermore, there is little difference in the affinity of loperamide for receptor preparations from brain and from ileum. So, classical binding experiments alone suggest that loperamide behaves as an opiate and offer no basis to elucidate the preferential intestinal action of the drug.

When labelled loperamide was used as a ligand it was extremely difficult to obtain a valid measure of binding specific for opiate receptors (66). Aspecific binding, which is usually low for morphine-like compounds, even in crude receptor preparations, was found to be extremely important for loperamide. Both the total capacity of the loperamide uptake by nonspecific membrane binding and the affinity of this nonspecific binding were unusually high. It is difficult at the present time to appreciate fully the pharmacological implications of non-opiate binding of high affinity and capacity. The minimum conclusion is that this nonspecific binding accounts for a large reservoir of drug close to its sites of action, of which two have been identified: the intestinal opiate receptors (109) and the calmodulin binding sites (134).

PHARMACOKINETICS AND TARGET TISSUES

The missing link between the restriction of loperamide's effects to the gastrointestinal tract and the high affinity for opiate receptors in in vitro studies was clarified by pharmacokinetic studies. One hour after oral administration to rats loperamide has a characteristic tissue distribution. Most of

the drug (85%) is recovered from the gastrointestinal tract, about 5% is found in the liver, and less than 0.04% in the brain (45). Upon intravenous injection the distribution in other peripheral tissues is more prominent, but even by this route loperamide rapidly concentrates in the small intestine (130). Pharmacokinetic studies in rat, mouse, and monkey point to the same conclusions: oral loperamide is well absorbed from the gut lumen (about 70%), but less than 1% enters the systemic circulation; uptake, metabolism, and redistribution occur within an efficient enterohepatic shunt (B. S. Butterworth, unpublished).

Within the intestinal wall the drug binds preferentially to the myenteric plexus-longitudinal muscle preparation rather than to either the circular muscle or the mucosa (121). The tendency to concentrate in the vicinity of the nervous structures that regulate the contractile activity of the gut is therefore the most likely basis of the extremely selective action of loperamide.

OTHER DRUG CLASSES WITH ANTIDIARRHEAL ACTIVITY

Table 6 lists compounds with known gastrointestinal constipating activity. They were studied in the castor oil test and in another test, which varies according to the pharmacological class but measures an "undesirable" systemic activity of a compound with potential therapeutic value in diarrhea. The list includes two anticholinergics, atropine and isopropamide,

Table 6 Specificity and safety of the antidiarrheal activity of drugs of various pharmacological classes (doses in mg/kg p.o.)

Compound	Castor oil test ED_{50}, 2 h A	Restrictive activity ED_{50} B	Antidiarrheal specificity B/A	LD_{50} C	Safety margin C/A
Atropine	9.30	0.39[a]	0.042	—	—
Isopropamide	74.6	21.4[a]	0.29	—	—
Indomethacin	8.68	6.2[b]	0.71	23.9	2.75
Acetylsalicylic acid	95.3	38.0[b]	0.40	⩾1280	⩾13.4
Suprofen	14.1	2.70[b]	0.19	≅640	≅45.4
Clonidine	0.028	0.085[c]	3.0	—	—
Lidamidine	1.67	24.9[c]	14.9	184	110
Nufenoxole	1.72	85.9[d]	50	105	61
Loperamide	0.29	>160[d]	>552	185	638

[a] Mydriasis.
[b] Nystatin paw oedema test.
[c] Autonomic side-effects.
[d] Tail withdrawal reaction test.

which belong to a class of pharmacological agents with antisecretory and antimotility properties (127); three inhibitors of prostaglandin biosynthesis, indomethacin, suprofen, and acetylsalicylic acid [clinically useful antidiarrheal activity of aspirin in patients under cancer radiotherapy has been reported (73)]; two substituted amidinoureas, clonidine, and lidamidine (77), and the diphenoxylate-like agent nufenoxole (26).

The antimuscarinic agents atropine and isopropamide induced mydriasis at a dose much lower than the dose that protected from diarrhea for only two hours. These results agree with the known side effects of therapeutically active doses of the compounds in gastrointestinal disease (127). Inhibitors of prostaglandin synthesis in general have been found to produce a significant delay of castor oil diarrhea (5). For a two hour protection the required doses were already higher than the effective doses for anti-inflammatory activity in the nystatin paw oedema test (81): for indomethacin the required dose was only slightly below the lethal dose. Clonidine was particularly potent in the castor oil test. At three times the dose for two hour protection from diarrhea autonomic side effects were induced. The synthetic analogue, lidamidine, was much less potent than clonidine, but more specific. Lidamidine was, however, still less specific and much less safe than diphenoxylate. The lack of specificity of clonidine and lidamidine has also been confirmed by comparing their antidiarrheal and diuretic action (64). Nufenoxole was less potent in the castor oil test than diphenoxylate and loperamide; when compared to its activity in the tail withdrawal reaction test, the antidiarrheal specificity of nufenoxole was about twice that of diphenoxylate. Comparison with the lethal dose, however, indicates that nufenoxole was less safe than diphenoxylate.

It appears, therefore, that even within pharmacological classes with well-known gastrointestinal actions on either motility, hypersecretion, or both, none of the available compounds reaches the antidiarrheal specificity and safety of diphenoxylate, and even the preferential intestinal action and safety of diphenoxylate is low in comparison to that of loperamide. Future pharmacological developments, as illustrated in the progress with the amidinoureas, may, however, lead to specific antidiarrheals with a different mechanism of action.

GENERAL CONCLUSIONS

The pharmacological study of potential antidiarrheal drugs is based on the measurement of inhibition of diarrhea in laboratory animals. Stimuli inducing diarrhea have previously been reviewed, and it was concluded that the castor oil test in rats is an adequate method of detecting and comparing the antidiarrheal activity of compounds (83). In this test morphine-like antidiarrheals, but also drugs of various other pharmacological classes, clearly

change the excretory pattern induced by castor oil. Quantitative and qualitative differences between the drugs appear at this level: e.g. in contrast to morphine-like drugs that inhibit diarrhea, the aspirin-like drugs produce only a short delay of watery excretion, which is still as copious as in control rats.

Most drugs are active in the castor oil test at a dose that exceeds the effective dose for their characteristic pharmacological action. For anticholinergics, aspirin-like drugs, and clonidine-like drugs, which are all known to possess pronounced intestinal actions, antidiarrheal specificity is still absent or very low. For loperamide only, the antidiarrheal specificity and safety are remarkably high. These properties are linked to a particular distribution in the organism, in which loperamide and its metabolites are practically confined to an enterohepatic circuit.

The local action of loperamide appears to be at least partially mediated by opiate receptors and results in inhibition of both excessive propulsive and secretory activity of the intestine. Smooth muscle and mucosal involvement in the action of loperamide is not surprising as the known mediators of diarrhea activate contraction as well as secretion. The therapeutic implications of this action have perhaps not been fully investigated. Severe secretory diarrhea, particularly when caused by bacterial toxins (29, 31), is frequently considered to require the development of new drugs with high "antisecretory" activity. Various preparations have been set up for accurate measurement of fluid accumulation and electrolyte fluxes in response to secretory stimuli. However, drugs shown recently to counteract such stimuli offer no hope for a therapeutically acceptable treatment, with the exception of loperamide, for which the specificity and safety in the organism should allow adaptation of the dose to the intensity of the intestinal challenge.

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