Naunyn-Schmiedeberg's Arch. Pharmacol. 301, 187–194 (1978)

Naunyn-Schmiedeberg's
Archives of
Pharmacology

Opiate Agonist Action of Antidiarrheal Agents in vitro and in vivo – Findings in Support for Selective Action

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Summary. Synthetic antidiarrheal agents like diphenoxylate and loperamide are characterized by strong opiate-like constipating activity with an almost complete lack of central morphinomimetic effects. The present investigation examines the pharmacological mechanisms underlying the action of such compounds.

1. In the radioreceptorassay, antidiarrheal agents competitively inhibited the stereospecific binding of opiates, having affinities comparable to those of strong narcotic analgesics. Binding was increased in the absence of sodium. No differences in the affinities of these compounds to central and peripheral opiate receptors, respectively, were found.

2. Antidiarrheal agents inhibited the electrically induced contractions of the isolated longitudinal muscle-myenteric plexus preparation of the guinea-pig ileum. This effect was completely antagonized by the narcotic antagonist naloxone; however, it was impossible to reverse drug action by washing the preparation. To obtain pharmacological effects on the isolated organ, up to 70-fold lower concentrations were necessary in case of the antidiarrheals than was calculated from their affinities in the radioreceptorassay.

3. The pharmacokinetic behaviour of ^{3}H-loperamide was tested in mice after i.v. injection. Compared to morphine, loperamide was rapidly eliminated from the general circulation and only small concentrations reached the CNS. This phenomenon provides an explanation for the fact, that opiate-like antinociception could only be obtained with antidiarrheal agents after application of subtoxic or toxic dose levels.

4. Up to 2 h after i.v. administration of ^{3}H-loperamide, an increase in radioactivity in the wall of the small intestine was found. Accumulation in the gastrointestinal tract might be related to distinct physicochemical properties of the compound. The surface tension lowering effect is a common characteristic by which all antidiarrheals differed from narcotic analgesics and could be related to the peculiar behaviour of these substances in vitro and in vivo.

Key words: Antidiarrheal drugs – Loperamide – Opiate receptor – Surface activity.

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INTRODUCTION

Opiates display pronounced constipating effects and narcotic analgesics like morphine have long been used in the symptomatic treatment of diarrhea. However, these drugs are known for high dependence liability, and research efforts have been directed towards the development of compounds in which antidiarrheal activity is separated from other morphine-like effects. Derived from the morphine congener pethidine, a group of chemically related substances has been synthesized; the first of these antidiarrheal drugs introduced into therapy were diphenoxylate (Janssen et al., 1959) and its active metabolite difenoxin (Van Wijngaarden and Soudijn, 1972). These drugs display selective constipating activity when administered orally in low doses; however, at higher doses central morphinomimetic effects become apparent (Jaffe and Martin, 1975). Recently, compounds with increased antidiarrheal activity and selectivity were synthesized (Stokbroekx et al., 1973) and loperamide, as one of the most specific drugs, is claimed to be devoid of central opiate-like effects (Niemegeers et al., 1976).

It has been shown before that loperamide reveals high affinity for opiate receptor sites in rat brain- and guinea-pig ileum homogenates (Terenius, 1975; Wüster et al., 1976; Wüster and Herz, 1976; Mackerer

0028-1298/78/0301/0187/$ 01.60

et al., 1976). Moreover, opiate-like inhibitory effects have been demonstrated on the longitudinal muscle-myenteric plexus preparation of the guinea-pig ileum (Wüster and Herz, 1976; Mackerer et al., 1976) or on the intact guinea-pig ileum segment (Van Nueten et al., 1976). However, these experiments failed to provide an explanation for the selective action of antidiarrheal agents on the intestinal tract. It is the aim of the present investigation to examine the opiate-like activity of a series of antidiarrheals in vitro and in vivo in order to get insight in the mechanism underlying their specific constipating activity.

Some reports assume that the site of antidiarrheal action of opiates is the CNS, which controls intestinal motility (Margolin and Plekks, 1965; Weinstock, 1971). However, it seems more likely that the constipating effect of opiates is achieved locally via receptors in the intestinal tract (Rinaldo et al., 1971; Dajani et al., 1975; Burks, 1976) and there are no indications for a different mechanism in the case of the antidiarrheal agents tested here.

METHODS

Synthetic antidiarrheal agents generally displayed a poor solubility in most solvents, including water. Aqueous solutions of these drugs were therefore prepared by solubilization of the compounds in propylene glycol and dilution to the final volume with the appropriate solvent. The final concentration of propylene glycol never exceeded 10%, and control experiments revealed no influence of the vehicle except when the physico-chemical properties were determined. Experiments were not conducted for some compounds when there was no practicable method of solubilization available.

Radioreceptorassay

a) Opiate Receptor Binding in Rat Brain. Binding assays followed in general the method as described by Pert and Snyder (1973). Male Sprague Dawley rats (200 g) were decapitated and their brains quickly removed. Brains without cerebella were homogenized in the ice-cold buffers (Tris · HCl 50 mM or Tris · HCl 50 nM + NaCl 100 mM, pH 7.4) employing the Potter-Elvehjem technique. Homogenates were diluted with buffer to a final concentration of 1:100 (w/v). 2 ml aliquots were preincubated for 5 min with increasing amounts of unlabelled competitors at 25° C and subsequent incubation of 15 min was carried out after the addition of ^{3}H-naloxone (final concentration 2.5 nM). Each sample was then filtered under reduced pressure through glass-fibre filters (Whatman GF/B) and rinsed twice with 5 ml buffer. Filters were solubilized in Unisolve I (10 ml) and counted for radioactivity.

b) Opiate Receptor Binding in the Guinea-Pig Myenteric Plexus. Male albino guinea-pigs (500 g) were killed and the ileum removed. Longitudinal muscle strips with attached myenteric plexus were obtained by the method of Rang (1964) and placed in iced Krebs-Ringer-Tris buffer (pH 7.4) (Creese and Snyder, 1975). Strips from different animals were collected, minced with scissors and homogenized in the cold by means of an Ultra Turrax (1 min, max. speed). The homogenate was finally diluted to 1:100 (w/v). Binding studies were performed according to the method described above for rat brain homogenate with minor modifications: As experiments using ^{3}H-naloxone as a tracer revealed only low ratios of specific/unspecific binding, ^{3}H-etorphine (final concentration 0.5 nM) was used instead. To assay receptor affinities under conditions similar to those used when evaluating drug action on the isolated longitudinal muscle-myenteric plexus preparation, Krebs-Ringer-Tris buffer was used and incubation temperature was raised to 37° C.

Longitudinal Muscle-Myenteric Plexus Preparation of the Guinea-Pig Ileum (Strip)

Longitudinal muscle strips with attached myenteric plexus were mounted in a 5 ml organ bath containing Krebs-Ringer-bicarbonate solution (NaCl, 118 mM; KCl, 4.75; $CaCl_2$, 2.54; KH_2PO_4, 1.19; $MgSO_4$, 1.20; $NaHCO_3$, 25). Glucose (11 mM), choline chloride (20 µM) and mepyramine maleate (0.125 µM) was added and the bath maintained at 37° C, bubbled with 95% O_2 + 5% CO_2 (Kosterlitz et al., 1975). Electrical field stimulation was applied (80 V, 0.1 Hz, 0.5 ms) to the strip (resting tension 1 g) and isometric contractions were recorded via a transducer system (Hottinger Baldwin) on a Rikadenki recorder. Strips were allowed to equilibrate over a period of about 1 h during which electrical stimulation was applied, with washes at 5 min intervals, until maximal contraction was obtained. Drugs were tested for inhibition of electrically evoked twitch tension. ED_{50} was estimated from at least 6 experiments with strips of different animals.

Antinociceptive Activity

Drugs were tested for antinociceptive activity after i.v. injection in mice (20 g, NMRI-strain) and Sprague Dawley rats (200 g). Antinociceptive activity was tested employing a "vocalisation test" with vocalisation being elicited by electrical stimulation of the tail root (Bläsig et al., 1973).

Pharmacokinetic Studies

Male NMRI-mice (20 g) were injected with 5 mg/kg corresponding to 10 µCi of either ^{3}H-loperamide or ^{3}H-morphine. After various time intervals, animals were decapitated and total radioactivity was estimated in blood, and various organs. All samples were prepared for scintillation counting by means of a 306 oxidizer (Packard Instruments). In a second set of experiments the amount of unchanged ^{3}H-loperamide was determined in plasma and brain using a modified procedure of Heykants et al. (1974). Tissues were homogenized by means of an Ultra Turrax (1 min, max. speed) in 10 ml of a mixture of methanol/water/acetic acid (30:20:1, v/v/v) with 10 mg of unlabelled loperamide added. After alkalisation with ammonia to pH 9.0, samples were extracted twice with chloroform (30 ml). The organic layer was evaporated to dryness and the residue spotted on thin layer chromatography (Merck 60 HF 254). After development of the plates in ethyl acetate/ethanol/ammonia 25% (90:10:1, v/v/v) spots were made visible by treatment with iodine vapour. Unchanged loperamide, displaying a single spot at R_f = 0.42, was scraped off and radioactivity determined by liquid scintillation counting.

Physico-Chemical Properties

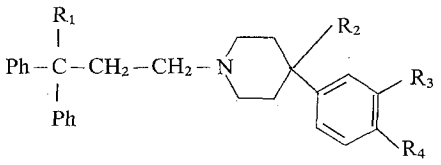
a) Partition coefficients of compounds between n-heptane and 0.2 M phosphate buffer (pH 7.4) were determined according to Herz and Teschemacher (1971). Partitioning of ^{3}H-loperamide between the two phases was estimated by liquid scintillation counting.

b) Plasma protein binding of the labelled drugs was estimated employing human plasma obtained from the blood bank. Protein binding of the drugs was measured by means of equilibrium-dialysis as described by Höllt and Teschemacher (1975).

c) The ability of drugs to lower surface tension of a solvent was estimated, employing an interfacial tensiometer (Le Comte de Noüy-tensiometer). Drugs were dissolved in Krebs-Ringer-Tris buffer (pH 7.4) and aliquots (20 ml) measured at 20° C ± 1 until the final value remained constant for at least 3 estimations.

Drugs

Antidiarrheal agents (Janssen Pharmaceutica, Beerse)



	R_1	R_2	R_3	R_4
Diphenoxylate	CN	$CO_2C_2H_5$	H	H
Difenoxin	CN	COOH	H	H
Loperamide	$CON(CH_3)_2$	OH	H	Cl
Fluperamide	$CON(CH_3)_2$	OH	CF_3	Cl
R 21345	$CONH_2$	OH	H	Cl
R 20905	$CONH(CH_3)$	OH	H	Cl
R 18936	$CON(CH_3)_2$	OH	H	H
R 18861	CON⟨⟩	OH	H	H

Morphine HCl (Merck AG, Darmstadt); etorphine (Reckitt & Colman, Kingston upon Hull); naloxone HCl (Endo Laboratories, Brussels); ^{3}H-loperamide (0.1 Ci/mmole, Janssen Pharmaceutica); ^{3}H-naloxone (23.6 Ci/mmole, New England Nuclear, Boston); ^{3}H-etorphine (41 Ci/mmole, The Radiochemical Centre, Amersham).

RESULTS

Effects on Stereospecific ^{3}H-Naloxone Binding in Rat Brain Homogenate

The antidiarrheal agents competitively inhibited stereospecific ^{3}H-naloxone binding. Log-probit analysis of antidiarrheals as well as narcotic analgesics gave linear plots with similar slopes. Receptor affinities of loperamide and its derivatives were in the range of those of potent narcotic analgesics (Table 1). The concentration necessary to inhibit ^{3}H-naloxone receptor binding by 50 % (IC_{50}) for loperamide and morphine in Tris · HCl buffer was 2.1 nM and 14 nM, respectively. The two main metabolites of loperamide in rats correspond to the compounds R21345 and R20905 (Karim and Heykants, 1976), the latter displaying a similar high affinity as the original drug. The most potent drug studied in the binding assay was R 18936. However, diphenoxylate and difenoxin, compounds with a chemical structure not closely related to loperamide, revealed receptor affinities lower than morphine. Because of the hypothesis that the ratio of binding affinities estimated in the presence and absence of sodium reflects the agonistic or antagonistic character of an opiate (Pert et al., 1973; Pert and Snyder, 1974), bindings assays were repeated with addition of NaCl (100 mM) to the buffer. All drugs under investigation displayed a reduced receptor affinity in the presence of sodium, with a quotient for loperamide resembling that of pure narcotic agonists.

Effects on ^{3}H-Etorphine Binding in the Myenteric Plexus

In preliminary experiments, the inhibition of ^{3}H-etorphine binding was assayed both in rat brain and myenteric plexus homogenates. For all drugs under investigation, no significant differences in receptor affinities to central and peripheral opiate receptors, respectively, were found. However, results derived from experiments with brain homogenate proved of lower variability. Moreover, despite some differences in experimental conditions, there is a fair correlation between receptor affinities estimated using ^{3}H-etorphine and ^{3}H-naloxone as tracer compounds (Table 1).

Inhibition of Electrically Induced Contractions of the Strip Preparation

The inhibitory action of antidiarrheal drugs on the strip preparation was exclusively opiate-like, as indicated by the unaltered response of the muscle to acetylcholine after drug treatment, and the complete reversal of the inhibition by naloxone (100 nM). However, on- and offset of action of the antidiarrheals clearly differed from that of narcotic analgesics (Fig. 1). Loperamide was found to exhibit an extremely slow onset of action compared to morphine; at least 30 min were required to establish a half-maximal inhibition (ED_{50}). Washing of the preparation failed to reverse the inhibitory action of loperamide and even continued renewal of the suspension medium at 10 min-intervals for more than 3 h did not affect inhibition caused by the drug. At any time, however, drug action was completely antagonized by naloxone; subsequent washing of the preparation reestablished the inhibition.

The potency of drugs to inhibit electrically induced twitches (ED_{50}) of the strip are correlated with results obtained from radioreceptorassay (Fig. 2). The ED_{50} of loperamide was 0.85 nM, which is about 30 times lower than receptor affinity (IC_{50}) in strip homogenate. In general, antidiarrheals displayed up to 70-fold increased pharmacological activity when compared to their IC_{50}-values, whereas morphine was equieffective in both preparations (100 nM). It has

Table 1. Inhibition of stereospecific binding by antidiarrheal agents and morphine

Drug	Rat brain			Myenteric plexus Krebs-Ringer-Tris buffer
	Tris HCl buffer IC_{50} (nM)	Tris HCl buffer + 100 nM NaCl IC_{50} (nM)	Quotient $\frac{\text{Tris HCl + NaCl}}{\text{Tris HCl}}$	IC_{50} (nM)
Loperamide	2.1	31	14.8	25
Fluperamide	2.4	10	4.2	17
R 21345	38	280	7.4	370
R 20905	2.8	42	15.0	35
R 18936	0.18	2.3	12.8	0.6
R 18861	3.8	46	12.1	50
Diphenoxylate	68	1180	17.3	1050
Difenoxin	29	350	12.1	400
Morphine	14	160	11.4	100

Values are the means of 4 experiments, each run in triplicate

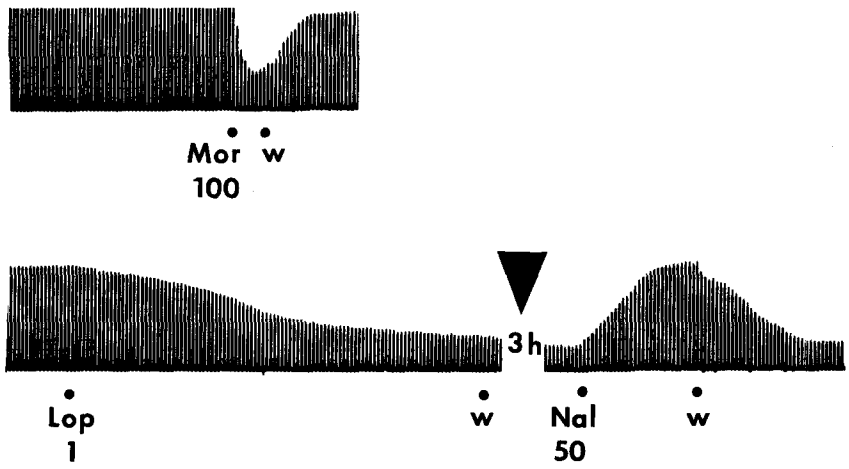


Fig. 1
Inhibition of electrically induced contractions of the longitudinal muscle-myenteric plexus preparation of the guinea-pig ileum by morphine (*Mor*) and loperamide (*Lop*), and reversal of the effect by naloxone (*Nal*). Numbers give the final drug concentrations in the organ-bath in nM. A 3 h-washing period with renewal of the suspension medium in 10 min intervals is indicated by the arrow (w = washing)

been demonstrated for a series of opiate agonists and antagonists that a half-maximal effect in both in vitro tests is established by equimolar concentrations, as is indicated by the straight line in Figure 2 (Creese and Snyder, 1975). However, due to the differ--ent physicochemical properties of opiates, this correlation is not always displayed; e.g. fentanyl or methadone have been shown to behave similarly to loperamide, in that they also possess greater pharmacological activities than was expected from their receptor affinities (Wüster and Herz, 1976).

Analgesic Activity

Opiate-like effects of loperamide, diphenoxylate and R 18936 in the central nervous system were observed after i.v. injection in mice and rats (Table 2). However, with regard to the LD_{50}, antiociception only occurred at subtoxic or toxic doses. Compared to morphine, in the case of the antidiarrheals the safety margin for central effects is only very small. These compounds also displayed morphinomimetic effects in mice (Straub-tail, increased locomotor activity) at doses causing antinociception. Manifestation of the opiate-like symptoms alternated with intervals when animals appeared to be sedated. Antinociception as well as the other opiate-like effects were completely abolished by subsequent application of naloxone (1 mg/kg i.p.).

Pharmacokinetics of ^{3}H-Loperamide

Distribution of ^{3}H-loperamide was studied in mice after i.v. injection. Figure 3 gives the total amount of radioactivity found in several organs, tissues and blood up to 12 h after application of a single dose (5 mg/kg).

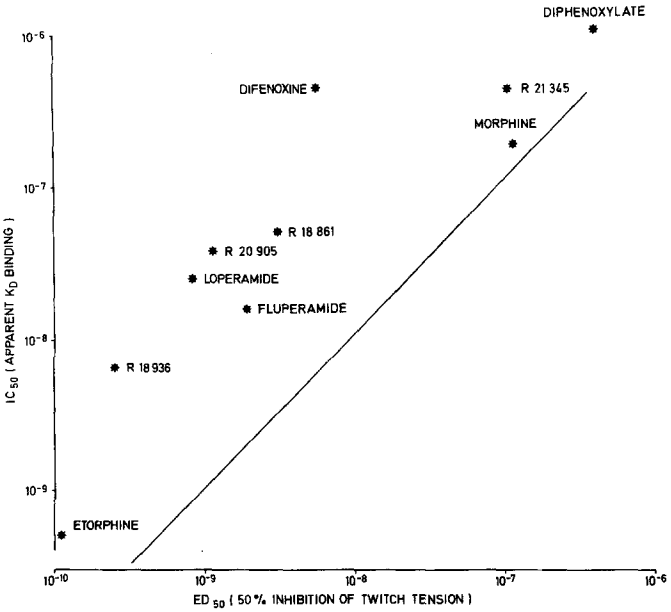


Fig. 2. Correlation between receptor binding and pharmacological activity of antidiarrheal agents and narcotic analgesics on the strip. The IC_{50} values (determined by inhibition of stereospecifically bound ^{3}H-etorphine in the longitudinal muscle-myenteric plexus homogenate) are plotted against the ED_{50}'s (concentration necessary to inhibit electrically induced twitches of the strip by 50%). Equal concentrations needed for a half maximal effect in both in vitro tests is indicated by the straight line

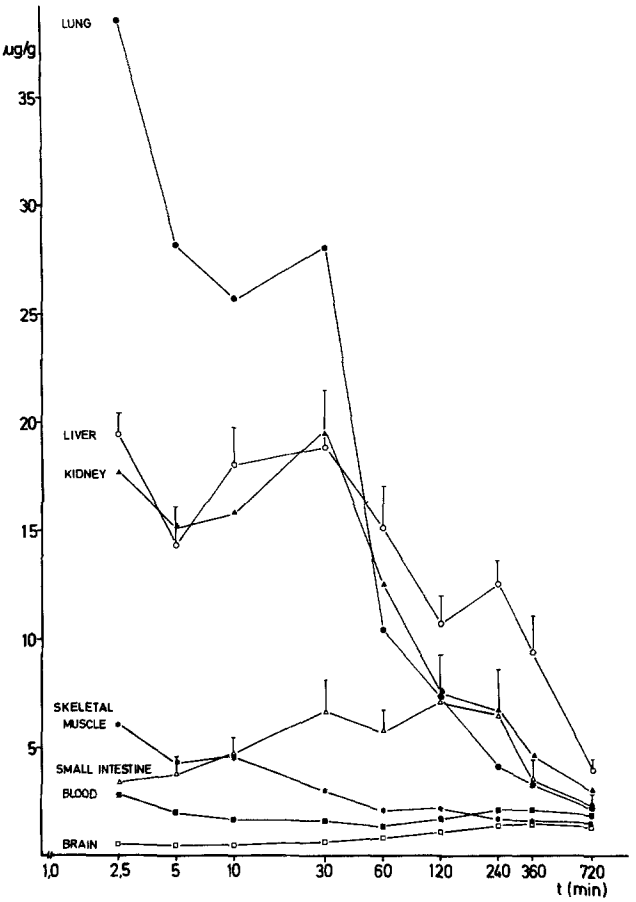


Fig. 3. Time-course of radioactivity in organs, tissues, and blood at increasing times after i.v. injection of ^{3}H-loperamide (5 mg/kg) in mice. Each symbol represents the mean of at least 6 experiments. Standard deviations are omitted from most of the curves in order not to complicate the graphs. Those which are given are representative also for the other curves

Table 2. Antinociceptive activity of antidiarrheal agents in rodents after i.v. injection

	Mice		Rats	
	ED_{50}	LD_{50}	ED_{50}	LD_{50}
	mg/kg			
Morphine	2.5	200.0	3.5	150.0
Diphenoxylate	2.3	7.0	–	–
Loperamide	1.9	10.0	3.7	5.0
R 18936	0.2	2.5	0.75	2.0

$n = 10$; LD_{50} approximately estimated

The highest concentration of radioactivity was found in the lung immediately after injection, declining to 19% of its value after 2 h. Similar kinetics were observed in the liver and the kidney. In contrast, the concentration in the wall of the small intestine increased over a 2-h period. There is a similar decrease of radioactivity in all organs thereafter. Most striking, in view of the lipophilic character of the drug, are the small concentrations in both blood and brain; moreover, radioactivity in the brain being 3–4 times lower than the corresponding blood levels. The concentrations of unchanged loperamide in plasma and brain are given in Figure 4. Loperamide appears to be metabolized very rapidly, as indicated by the low portion of unchanged drug in the blood even 5 min after injection. The low ability of the drug to penetrate the blood-brain-barrier becomes obvious again when the amounts of unchanged loperamide are regarded. Then the concentration in the blood is about 6 times higher than in the brain.

Physico-Chemical Properties

The lipid solubility and plasma protein binding capacity of ^{3}H-loperamide was found to be comparable to that of etorphine (Table 3), a potent narcotic analgesic of considerable lipophilicity. Drugs could be divided into two groups on the basis of surface activity: Antidiarrheals, which decreased surface ten-

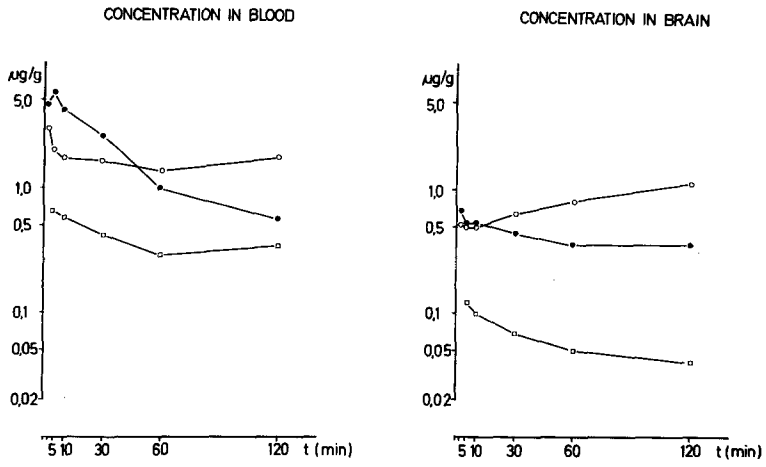


Fig. 4
Time-course of ^{3}H-loperamide amounts [total radioactivity (○——○), unchanged drug (□——□)] in blood and brain after i.v. injection (5 mg/kg) in mice. For comparison, ^{3}H-morphine concentrations [total radioactivity (●——●)] after i.v. injection (5 mg/kg) are given

Table 3. Physico-chemical properties

	Partition coefficient heptane/buffer pH 7.4	Plasma protein binding at 10^{-7} M	Surface tension lowering effect at 10^{-3} M dyn/cm
Morphine	<0.00001	25 ± 0.2%	<0.1
Etorphine	1.4 ± 0.25	73.5 ± 0.7%	<0.1
Loperamide	2.4 ± 0.42	78 ± 2.1%	9.8 ± 0.2
R 18936	not estimated	not estimated	18.3 ± 0.1

Mean values of 3 experiments ± S.E.M.

sion of the buffer, and a series of narcotic agonists and antagonists, 2 of which are shown here, which did not exhibit measurable activity at 10 mM. It has to be mentioned, however, that fentanyl, which is chemically related to loperamide, is surface active, too.

DISCUSSION

The present study demonstrates high opiate receptor affinity of the antidiarrheal drugs in the binding assay (Table 1) and on the isolated guinea-pig ileum (Fig. 2). Moreover, an almost "pure" agonistic character of the compounds is indicated by the high sodium quotient.

In order to explain the selective action on the gut, we considered the possibility that receptor interaction of the antidiarrheals with peripheral and central binding sites may differ. However, all compounds tested, narcotic analgesics as well as antidiarrheals, displayed identical affinities when tested in parallel for opiate receptor binding on the gut and the central nervous system. This finding provides evidence for the assumption that the molecular structure of central and peripheral receptors must be very similar.

It was shown, that there is a close correlation between the receptor affinities of opiates and their pharmacological activities on the electrically stimulated strip (Kosterlitz and Waterfield, 1975). Subsequently, it was reported that equimolar concentrations of an opiate are necessary for a half-maximal effect in both in vitro tests (Creese and Snyder, 1975). In contrast, a large discrepancy was observed in the case of the antidiarrheals and some narcotic analgesics (Wüster and Herz, 1976; Mackerer et al., 1976). As shown in Figure 2, some compounds are up to 70 times more effective on the strip than could be expected from their receptor affinities. These differences do not necessarily contradict the concept of Creese and Snyder (1975); their findings that the concentrations of opiate agonists required to occupy 50% of the receptors were the same as those, necessary to induce 50% of the maximal pharmacological response, suggest that receptor affinity alone might be sufficient to account for the pharmacological potency, and omits the necessity for invoking the assumption of "intrinsic activity". The unexpected high pharmacological activity of the antidiarrheal agents on the isolated organ could not be taken as an expression of a specific action profile, e.g. antidiarrheal activity, since this phenomenon is shared by some narcotic analgesics, too. Instead, it has been explained as a consequence of certain physico-chemical properties of some drugs, indicating an accumulation of these compounds in the tissue (Wüster and Herz, 1976).

Loperamide and some derivatives were introduced into therapy as specific antidiarrheal agents, with little if any central effects after oral administration (Niemegeers et al., 1974). The observed opiate-like antinociception in rodents after i.v. injection occurred at doses comparable to that of morphine; however, in the case of the antidiarrheals, analgesia was only observed at almost lethal doses.

Pharmacokinetic studies in rats revealed that, after oral administration of radioactive loperamide, the bulk of the drug remains in the gastro-intestinal tract, and is excreted as unchanged drug or as active metabolites (R 21345, R 20905) nearly exclusively in the feces (Karim and Heykants, 1976). In the present investigation it was shown in Figure 3 that even after i.v. injection of ^{3}H-loperamide there is a time-dependent increase in radioactivity in the intestinal tissue; a finding which is in accordance with the observed accumulation of the compound in the isolated longitudinal muscle-myenteric plexus preparation of the guinea-pig (Wüster and Herz, 1976). The accumulation in the gut after i.v. injection might be due to an intensive enterohepatic circulation (Heykants et al., 1974). As this phenomenon was demonstrated for morphine, too (Walsh and Levine, 1975), the main difference between narcotic analgesics and antidiarrheal agents may be due to differences in the degree of fixation to the intestinal tissue. It seems highly probable that following oral administration, which is recommended for obtaining the most selective action, the drug adheres to the gut where it accomplishes its action locally on opiate receptors of the intestinal nervous tissue, while only small concentrations are reached in the plasma. In addition, loperamide-action outside the gut is lowered by a rapid metabolism of the drug.

On the evidence of these results, the lack of central effects of antidiarrheals cannot be accounted for by the specificity of receptor interaction. If high opiate receptor affinity of loperamide is balanced against weak antinociception and low brain concentration, there is no evidence that the drug behaves differently from narcotic analgesics at central receptor sites. However, antidiarrheal agents are characterized by a low access of the compound to the central nervous system.

The reason for the unusual pharmacokinetic behaviour was evaluated in terms of the physico-chemical correlates. Investigations of the partition coefficient or plasma protein binding of ^{3}H-loperamide, which revealed properties similar to that of ^{3}H-etorphine, did not provide enlightment about a possible mechanism of selective action. On the contrary, drugs with considerable lipid solubility like etorphine are known to pass lipid membranes without difficulties. However, narcotic analgesics and antidiarrheal agents clearly differ in their ability to reduce surface tension (Table 3). This difference is considered to be in some way correlated to the selective action of the antidiarrheals. The restricted absorption from the intestinal tract has been shown for other surfactants like chlorpromazine or Δ^9-tetrahydrocannabinol (Sundaresan and Rivera-Calimlim, 1975; Garrett and Hunt, 1974). Therefore, it is highly suggestive that antidiarrheal agents accumulate in the wall of the small intestine after oral administration; their particular properties cause them to be adsorbed on membrane-surfaces from which they are released extremely slowly.

The suggestion that surface activity is a determining factor is also indicated by the characteristic behaviour of the antidiarrheals on the strip. While it was reported for narcotic analgesics, that on- and offset of action is determined by the lipid solubility of the drug (Kosterlitz et al., 1975), the particular behaviour of the antidiarrheals must be established by additional factors. The long lasting inhibition of the drugs is not due to a covalent binding to opiate receptor sites, as indicated by the ability to antagonize drug effects with low amounts of naloxone. However, that the drug is still present in the tissue near to the receptor becomes obvious, when, after washing of the preparation and removal of the antagonist, the action of the agonist returns unaltered. These effects and additional phenomena, like accumulation in the isolated organ (Wüster and Herz, 1976) or restricted ability to cross biological membranes (unpublished observation) are best explained by the surface active properties of the compound. Further evidence for the importance of surface activity for drug action were obtained from binding studies employing highly labelled ^{3}H-loperamide. These experiments, which are the subject of another report (in preparation) revealed an adsorption to membranes, which is characterized by unspecific binding of low affinity and high capacity.

It may concluded from the results reported here that antidiarrheal compounds display agonist activity on opiate receptor sites in vivo and in vitro. However, a nearly complete separation of central and peripheral opiate effects is achieved by their unique pharmacokinetics. The most likely explanation for this phenomenon is that the tenside-like character of the compounds is responsible for their accumulation in the vicinity of peripheral opiate receptors.

This investigation was supported by Deutsche Forschungsgemeinschaft.

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Received July 27 / Accepted November 2, 1977