Table 1. Mean values of pA_2 and slope for atenolol and ICI 118,551 in guinea-pig ileum.

	(−)-Isoprenaline		Fenoterol	
	pA_2	Slope	pA_2	Slope
Atenolol	6·56 ± 0·08 (8)	0·87 ± 0·06 (4)	6·42 ± 0·04 (8)	0·91 ± 0·04 (4)
ICI 118,551	6·55 ± 0·05 (6)	0·97 ± 0·02 (3)	6·35 ± 0·09 (8)	1·01 ± 0·03 (4)

Values are mean ± s.e.m. from (n) experiments.

As a check on these findings, further experiments were undertaken in which the atenolol/(−)-isoprenaline interaction was studied in the continuous presence of 0·1 μM ICI 118,551. The mean value of slope (0·91 ± 0·05, n = 4) was not significantly different from unity and the mean pA_2 value (6·42 ± 0·01, n = 8) was not significantly different from the value obtained in the absence of ICI 118,551 (P = 0·617, 6 d.f., unpaired t-test).

The results of the present study indicate that at a functional level, an homogeneous population of β_1-adrenoceptors subserve inhibitory responses in the guinea-pig ileum. Thus this preparation may be of use in determining the actions of drugs on β_1-adrenoceptors in smooth as opposed to cardiac muscle preparations.

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REFERENCES

Arunlakshana, O., Schild, H. O. (1959) Br. J. Pharmacol. 14: 48–58

Daly, M. J., Levy, G. P. (1979) in: Kalsner, S. (ed.) Trends in Autonomic Pharmacology. Volume 1, Urban & Schwarzenberg, Baltimore-Munich, pp 347–385

Iakovidis, D., Malta, E., McPherson, G. A., Raper, C. (1980) Br. J. Pharmacol. 68: 677–685

O'Donnell, S. R., Wanstall, J. C. (1975) Clin. Exp. Pharmacol. Physiol. 2: 541–547

O'Donnell, S. R., Wanstall, J. C. (1983) Br. J. Pharmacol. 78: 417–424

Wikberg, J. (1977) Acta Physiol. Scand. 99: 190–207

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Central and peripheral inhibition of gastrointestinal transit in rats: narcotics differ substantially by acting at either or both levels

FRANCESCA PERACCHIA, GIANCARLO BIANCHI, ROBERTO FIOCCHI, PAOLA PETRILLO, ALESSANDRA TAVANI, LUCIANO MANARA*, *Istituto di Ricerche Farmacologiche Mario Negri, Via Eritrea 62, 20157, Milan, Italy*

The roles of local intestinal and centrally mediated opiate-specific mechanisms underlying gastrointestinal transit inhibiton by five typical narcotic analgesics were assessed by the rat charcoal meal test. The doses (mg kg^{-1} s.c.) reducing the progression of charcoal to 50% of drug-free controls in 5 min (ID50) were approximately 1 for morphine and methadone, 0·5 and 40 for diamorphine and pethidine (all given 25 min before charcoal) and 0·001 for etorphine (10 min before charcoal). The delay in test meal travel caused by these ID50 doses was completely prevented by i.p. naloxone. Intracerebroventricular (i.c.v.) naloxone fully antagonized pethidine and etorphine but had no effect on morphine. Morphine, but either pethidine nor etorphine, was antagonized by i.p. *N*-methyl naloxone (a peripheral antagonist). Diamorphine and methadone were partially antagonized by i.c.v. naloxone or i.p. *N*-methyl naloxone.

The recognized loci of the therapeutic pain-relieving action of narcotic analgesics are confined to the cns whereas their constipating side effect is currently attributed to both central and local intestinal opiate-specific mechanisms (Jaffe & Martin 1980). Recently we have demonstrated that the direct action of systemically administered morphine on the rat gastrointestinal tract virtually accounts for the drug's inhibition of transit in the gut which in fact can be prevented by quaternary narcotic antagonists without impairment of analgesia (Bianchi et al 1983). On these grounds we have in animals successfully approached a possible, clinically applicable dissociation of morphine analgesia from its intestinal side effect (Tavani et al 1979b; Ferretti et al 1981; Bianchi et al 1982). It remains to be established whether narcotics other than morphine have a similar mechanism of action on the intestine. In the present study we compared the roles of the local and cns mediated components of inhibition of transit in the gut by morphine and four typical narcotic analgesics.

* Correspondence and present address: Groupe SANOFI, Research Center MIDY S.p.A., Via Piranesi 38, 20137 Milan, Italy.

Methods

Overnight-fasted male CD-COBS rats (Charles River, Italy) 180–220 g, housed in standard conditions (60% relative humidity, 22 °C), were given a charcoal meal (2 ml/rat) consisting of 10% vegetable charcoal plus 10% gum arabic (F.U., Farmitalia-Carlo Erba, Milan, Italy) in water. Five min later (Tavani et al 1980) animals were decapitated and their small intestine was

removed, its length measured from the pyloric sphincter to the ileocecal junction and the distance travelled by the test meal was recorded as a percentage of the total length (% intestine traversed). In these conditions intestinal transit does not appear to depend significantly on the effect of opioids on gastric emptying (Fiocchi et al 1982).

Drugs were administered in 0·9% NaCl water solution, 2 ml kg^{-1} subcutaneously (s.c.) or intraperitoneally (i.p.) (Tavani et al 1980) or 10 µl/rat intracerebroventricularly (i.c.v.). Rats injected i.c.v. were chronically implanted in the left lateral cerebral ventricle with a polyethylene tube as previously described (Tavani et al 1980) and the correct positioning of the implanted cannula was checked after death. To ensure accurate delivery of the drug, i.c.v. administration consisted of injecting 5 µl saline containing the drug followed by 5 µl saline. Drug doses were calculated for the following salts, gifts of which are gratefully acknowledged: etorphine HCl (Reckitt & Colman, UK); naloxone HCl (Endo, USA); *N*-methyl naloxone bromide (MRZ 2593, Dr H. Merz, Boehringer, W. Germany); pethidine HCl (Hoechst, W. Germany). Morphine HCl was purchased from Farmitalia-Carlo Erba, Italy, diamorphine (heroin HCl) from Hubert Lando International, USA, and methadone HCl from Franco Tosi, Italy.

Results in Fig. 1 were obtained the same day and were analysed by linear regression. The two experiments in Table 1 were run on two separate days and were analysed separately by analysis of variance and Duncan's (1955) test.

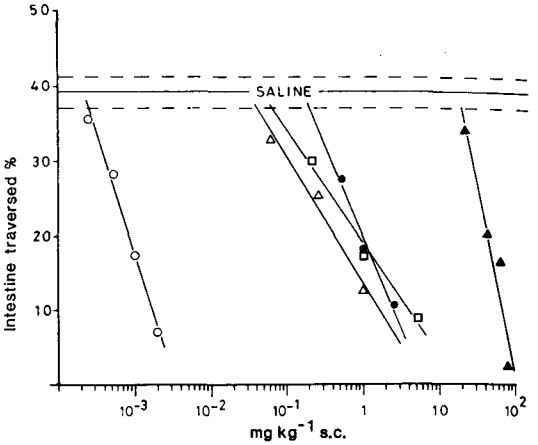

FIG. 1. Narcotic dose-related inhibition of gastrointestinal transit in rats. Animals (4 each point) were injected with saline (mean ± s.e. — —), diamorphine (△—△), morphine (□—□), methadone (●—●), or pethidine (▲—▲) 25 min before a charcoal meal or with etorphine (○—○) 10 min before the meal. % intestine traversed was measured 5 min after the meal. The slopes of the curves were −16·6, −14·9, −24·0, −48·2 and −31·9 respectively for diamorphine, morphine, methadone, pethidine and etorphine and differed significantly for the following drugs: etorphine from diamorphine and methadone from pethidine ($P < 0·05$); morphine from etorphine and pethidine, and pethidine from diamorphine ($P < 0·01$).

Results

As shown in Fig. 1, systemic administration of any of the 5 narcotics tested caused dose-related inhibition of the transit of charcoal along the small intestine of the rat. The doses reducing the progression of the test meal to 50% that of drug free controls (ID50) were approximately (mg kg^{-1} s.c.): 0·001 for etorphine, 0·5 for diamorphine, 1 for morphine and methadone and 40 for pethidine. These doses were selected for additional experiments summarized in Table 1.

The first experiment (see Table 1) consisted in challenging gastrointestinal transit inhibition by these narcotics with an i.p. injection of either naloxone or *N*-methyl naloxone. Naloxone restored charcoal transit to drug-free control values in all cases. Conversely *N*-methyl naloxone affected the delay in test meal travel differently, depending on the narcotic producing it: it failed to antagonize etorphine and pethidine, fully

Table 1. Antagonism of narcotic induced inhibition of gastrointestinal transit in rats. Etorphine was injected 10 min and all the other narcotics 25 min before a charcoal meal; naloxone and *N*-methyl naloxone 15 min before the meal.

Treatment mg kg^{-1} s.c.	Saline —	Morphine 1	Diamorphine 0·5	Methadone 1	Pethidine 40	Etorphine 0·001
	% Intestine traversed in 5 min					
Experiment I						
Saline	38·2 ± 3·6	21·6 ± 4·1[2]	16·8 ± 2·4[3]	18·0 ± 1·5[3]	13·9 ± 4·1[3]	14·0 ± 4·1[3]
Naloxone 1 mg kg^{-1} i.p.	36·4 ± 5·1	31·2 ± 2·1	36·3 ± 3·7[5]	32·8 ± 2·1[4]	34·4 ± 4·1[5]	35·4 ± 5·7[5]
N-methyl naloxone 4 mg kg^{-1} i.p.	32·2 ± 3·2	35·9 ± 3·5[5]	29·0 ± 2·7[5]	25·2 ± 5·3[2]	8·5 ± 2·8[3,5]	15·1 ± 3·3[3,5]
Experiment II						
Saline	38·8 ± 2·0	19·2 ± 2·6[3]	14·8 ± 1·5[3]	17·9 ± 2·1[3]	9·2 ± 2·7[3]	25·6 ± 1·6[3]
Naloxone 2 µg rat^{-1} i.c.v.	34·2 ± 3·7	18·6 ± 1·3[3,5]	27·9 ± 3·8[3,5]	25·1 ± 1·5[3,4]	35·3 ± 3·6[5]	37·8 ± 2·0[5]

[1] Data are means ± s.e., n = 4 in experiment I and n = 5 in experiment II.
[2] $P < 0·05$ from saline – saline. [3] $P < 0·01$ from saline – saline. [4] $P < 0·05$ from saline as appropriate. [5] $P < 0·01$ from saline as appropriate.

antagonized morphine, partially antagonized diamorphine and possibly methadone.

In the second experiment (see Table 1) the intestinal action of the narcotics was evaluated in rats given naloxone i.c.v.; this fully antagonized etorphine and pethidine, failed to antagonize morphine, partially antagonized diamorphine and methadone. Neither naloxone nor *N*-methyl naloxone when given alone significantly changed gastrointestinal transit compared to drug-free controls under any of the conditions specified in Table 1.

Discussion

Our results disclose substantial differences between narcotic analgesics as regards their sites of inhibition of gastrointestinal transit in rats. The variability in control responses is an unavoidable drawback entailed in our choice to study central and peripheral antagonism at the ID50 of each narcotic. This choice in principle provides an appropriate comparison of the results with the different narcotics and—more important—permits the use of low doses of antagonists; higher doses of i.c.v. naloxone and systemic *N*-methyl naloxone, as we showed previously (Bianchi et al 1982, 1984, in the press), may in fact no longer be selective respectively centrally and peripherally.

The delay in the travel of a test meal along the small intestine caused by any of five typical narcotic analgesics at approximately equi-effective doses was antagonized by intraperitoneal naloxone. This attests to the opiate-specific nature of the intestinal action monitored, but does not clarify the relative roles of local versus cns-mediated components since i.p. naloxone is similarly effective centrally and peripherally (Ferretti et al 1981).

The *N*-methyl quaternary analogue of naloxone which, under comparable conditions, fails to antagonize centrally elicited antinociception by morphine (Ferretti et al 1981), fully prevented its intestinal action. Quaternary naloxone, however, had no effect on inhibition of transit in the gut induced by pethidine or etorphine and only partially antagonized that caused by diamorphine or methadone. These findings are consistent with previously presented comprehensive evidence that a gut-located site plays a primary role in inhibition of gastrointestinal transit by systemically administered morphine in rats (Bianchi et al 1983), but suggest that under the test conditions pethidine and etorphine act primarily at central sites whereas diamorphine and methadone act centrally and peripherally to the same extent.

The results with i.c.v. naloxone, which can be reasonably assumed to reach opiate receptors only within the brain, further support the above view. Thus pethidine and etorphine (both refractory to quaternary naloxone) were completely antagonized by i.c.v. naloxone which in turn failed to antagonize morphine (fully antagonized by quaternary naloxone).

It remains to be established precisely why narcotic analgesics, as shown in this study, may differ substantially in the extent to which central or local intestinal mechanisms underly their slowing of transit in the gut. Opiate-specific sites for inhibition of gastrointestinal propulsion in rats are present in the intestine and brain (Manara et al 1980) and possibly in the spinal cord (Porreca et al 1983). Although opiate receptors in any of these regions may be different, present evidence points to the pharmacokinetic properties of individual compounds (i.e. their distribution in these regions after systemic administration) as the only factor currently investigated accounting at least in part for the reported differences between the narcotics tested. For example, predominantly central and local intestinal mechanisms of gastrointestinal transit inhibition respectively are to be expected for etorphine and morphine since the latter is much more readily available to the intestine than to the brain (Bianchi et al 1983), whereas the opposite is true for etorphine (Tavani et al 1979a).

The main practical implication of our present findings is whether narcotic analgesia can be dissociated from constipating side effects (Manara et al 1980; Tavani et al 1979b) depending on the compound given. Meaningful clinical extrapolations, however, should be based on effects of systemically administered narcotics like in the animal model that we investigated.

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REFERENCES

Bianchi, G., Fiocchi, R., Tavani, A., Manara, L. (1982) Life Sci. 30: 1875–1883

Bianchi, G., Ferretti, P., Recchia, M., Rocchetti, M., Tavani, A., Manara, L. (1983) Gastroenterology 85: 852–858

Bianchi, G., Fiocchi, R., Peracchia, F., Petrillo, P., Tavani, A., Manara, L. (1984) J. Pharm. Pharmacol. 36: 326–330

Duncan, D. B. (1955) Biometrics 11: 1–42

Ferretti, P., Bianchi, G., Tavani, A., Manara, L. (1981) Res. Commun. Substance Abuse 2: 1–12

Fiocchi, R., Bianchi, G., Petrillo, P., Tavani, A., Manara, L. (1982) Life Sci. 31: 2221–2223

Jaffe, J. H., Martin, W. R. (1980) in: Goodman Gilman, A., Goodman, L. S., Gilman, A. (eds) Goodman and Gilman's the Pharmacological Basis of Therapeutics. 6th edn, Macmillan, New York, pp 494–534

Manara, L., Bianchi, G., Ferretti, P., Monferini, E., Strada, D., Tavani, A. (1980) in: Way, E. L. (ed) Endogenous and Exogenous Opiate Agonists and Antagonists. Pergamon Press, New York, pp 143–146

Porreca, F., Filla, A., Burks, T. F. (1983) Eur. J. Pharmacol. 86: 135–136

Tavani, A., Luini, A., Manara, L. (1979a) J. Phar. Exp. Ther. 211: 140–144

Tavani, A., Bianchi, G., Manara, L. (1979b) Eur. J. Pharmacol. 59: 151–154

Tavani, A., Bianchi, G., Ferretti, P., Manara, L. (1980) Life Sci. 27: 2211–2217