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An homogeneous population of β_1-adrenoceptors subserves inhibitory responses in guinea-pig ileal preparations

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In transmurally stimulated guinea-pig ileal preparations, the pA_2 values obtained with the selective β_1- and β_2-adrenoceptor antagonists atenolol and ICI 118,551 were independent of the β-adrenoceptor agonist used ((−)-isoprenaline or fenoterol) and were similar to values reported in isolated cardiac tissue. On the basis of these results, the relaxant responses to β-adrenoceptor agonists in guinea-pig ileal preparations appear to be mediated by an homogeneous population of β_1-adrenoceptors.

It is now well accepted that β-adrenoceptors mediate inhibitory effects in the longitudinal smooth muscle of the guinea-pig ileum (see Wikberg 1977 for references). For both β_1- and β_2-adrenoceptor selective agonists, potency ratios with respect to isoprenaline in ileal preparations are similar to those observed in cardiac, as opposed to bronchial and uterine tissue (O'Donnell & Wanstall 1975; Daly & Levy 1979; Iakovidis et al 1980). It would therefore appear that the predominant β-adrenoceptor present is of the β_1-subtype. The similarities in the EC50 values and relative potencies of (−)-isoprenaline and RO363 (1-(3,4-dihydroxyphenoxy)-3[2-(3,4-demethoxyphenyl)ethylamino]-propan-2-ol) in ileal preparations stimulated transmurally or by exogenous acetylcholine (Iakovidis et al 1980) suggest that these β_1-adrenoceptors are located postjunctionally.

In order to determine whether the inhibitory responses are produced via the activation of an homogeneous population of β_1-adrenoceptors, or whether β_2-receptor activation might also be involved, the effects of the selective β-receptor antagonists atenolol and ICI 118,551 were tested against responses to the non-selective agonist (−)-isoprenaline and the selective β_2-receptor agonist fenoterol.

Method

Segments of guinea-pig ileum, obtained from animals pretreated with reserpine (1 mg kg^{-1} i.p. 18 h) were mounted on a platinum electrode assembly and bathed in Krebs solution (NaCl 120, KCl 7·3, $MgSO_4$ 0·6, dextrose 11·1, $NaHCO_3$ 25, NaH_2PO_4 1·0 and $CaCl_2$ 2·6 mM) maintained at 37 °C and aerated with 5% CO_2 in O_2. A resting tension of 0·5 g was applied and changes in isometric tension in response to transmural stimulation (0·1 Hz, 2·5 ms pulses at supramaximal voltage) were recorded with a Grass FTO3c transducer coupled to a Grass 79D polygraph. The bathing solution contained cocaine (30 μM), hydrocortisone (50 μM) and phentolamine (10 μM) to reduce interference from uptake systems and α-adrenoceptor effects, and ascorbic acid (0·1 mM) to reduce oxidation of the compounds.

Cumulative concentration-effect curves to (−)-isoprenaline or fenoterol were first established at 20–30 min intervals. After constant responses had been obtained, the tissues were incubated with increasing concentrations of either atenolol or ICI 118,551 (erythro-DL-1(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol) and agonist curves re-established. A 30 min equilibration time was allowed with each concentration of antagonist used.

Responses to the agonists were expressed as a percentage of the maximal reduction in twitch height elicited in the control period. Dose-ratios were determined using the EC50 value for each curve and the values of slope of the Arunlakshana & Schild (1959) plot [log (dose-ratio-1) vs log (molar antagonist concentration)] calculated. Once the presence of competitive antagonism had been established (value of slope not significantly different from unity), pA_2 values were calculated using the equation:

$$pA_2 = \log (DR - 1) - \log (B)$$

The range of dose-ratios used to assess antagonism was 3–55 with atenolol and 3–31 with ICI 118,551.

Results and discussion

(−)-Isoprenaline (1–300 nM) and fenoterol (0·1–100 μM) produced a concentration-dependent inhibition of the ileal contractions. The maximum inhibitory effects of (−)-isoprenaline and fenoterol, expressed as a percentage reduction of total twitch height, were 82 ± 3% (n = 7) and 87 ± 3% (n = 8) respectively. The mean pD_2 values for (−)-isoprenaline and fenoterol were 8·11 ± 0·06 (n = 7) and 6·13 ± 0·04 (n = 8) respectively.

Atenolol (2–10 μM) and ICI 118,551 (2–10 μM) produced parallel rightward shifts of the (−)-isoprenaline and fenoterol concentration-effect curves without affecting their maximal inhibitory responses. The calculated pA_2 values and values of slope (indicative of competitive antagonism) together with their respective standard errors of the mean are shown in Table 1. The values obtained for atenolol and ICI 118,551 were independent of the agonist used and were similar to the pA_2 values obtained for the actions of these antagonists on β_1-adrenoceptor mediated responses (atenolol 6·8, ICI 118,551 6·7; see O'Donnell & Wanstall, 1983 for more detail and references).

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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

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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.

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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