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The selectivity of the opioid antagonist, naltrindole, for δ-opioid receptors

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Abstract—In the mouse vas deferens, naltrindole gave pK_B values of 9·7, 8·3, and 7·5 at the δ-, μ-, and κ-sites and in binding assays, pIC50 values of 9·6, 7·8 and 7·2 at the same sites. The affinity of naltrindole for the δ binding site was increased in the presence of sodium ions and 5′-guanylylimidophosphate. Naltrindole is, thus, a potent opioid antagonist with marked selectivity for the δ-opioid receptor.

Until recently the only selective antagonists available for the δ-opioid receptor were peptides, that is ICI 154129 (Shaw et al 1982) and ICI 174864 (Cotton et al 1984). Those compounds, although they are highly selective for the δ-opioid receptor type over the μ- and κ-types, have the disadvantage that they do not readily cross the blood-brain barrier. Portoghese et al (1988a, b) reported recently that naltrindole is a non-peptide, δ-selective, antagonist. The experiments described below characterize more fully the receptor selectivity of naltrindole in an in-vitro preparation possessing μ-, δ-, and κ-receptors: the mouse vas deferens; and in individual binding assays for the μ-, δ-, and κ-opioid binding sites.

Materials and methods

Vasa deferentia were removed from CRH mice (25–40 g) and each one was suspended between a pair of platinum electrodes in a 5 mL organ bath. The vasa were bathed with a magnesium-free Krebs solution of the following composition (mM): NaCl 118, $NaHCO_3$ 25, glucose 11, KCl 4·7, $CaCl_2$ 2·5, and KH_2PO_4 1·2, gassed with 95% O_2/5% CO_2 at 37°C. Vasa were stimulated with three square-wave pulses of 0·5 ms duration at 200 μs intervals. Trains of three pulses at supramaximal voltage were delivered at a frequency of 0·1 Hz. The preparation was maintained under 0·2 g tension and contractions of the tissue were recorded isometrically. Concentration-response curves were constructed by cumulative addition of agonists to the bathing fluid. Naltrindole was applied for 30 min before repetition of an agonist concentration-response curve. The ratios of the IC50 values for the agonist in the presence and absence of naltrindole were used to calculate the pK_B value, and the Schild slope was calculated from a plot of log (concentration ratio-1) vs log [naltrindole].

For the binding assays, homogenates were prepared from brains (without cerebellum) of male Wistar rats (250 g) or cerebella of male Dunkin-Hartley guinea-pigs as outlined by Gillan et al (1980). Membranes were incubated at 25°C for 40 min in Tris HCl buffer (50 mM, pH 7·4) in the presence or absence of sodium chloride (100 mM) and the non-hydrolysable analogue of GTP, 5′-guanylylimidophosphate (GppNHp; 50 μM). κ-Sites were labelled using [^{3}H]diprenorphine (0·2 nM) with 20 nM 16-methylcyprenorphine (M8008; Smith 1987) in guinea-pig cerebellum, μ-sites using [^{3}H]naloxone (0·2 nM) in rat brain and δ-sites using [^{3}H]diprenorphine (0·5 nM) with cyprodime (1·26 μM; Schmidhammer et al 1989) and norbinaltorphimine (20 nM) to suppress μ- and κ-sites, respectively.

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Drugs. The drugs used were: [D-Pen^2, D-Pen^5]enkephalin (DPDPE; Cambridge Research Biochemicals), [DAla^2, MePhe^4, Gly^5-ol]enkephalin (DAGO; Cambridge Research Biochemicals), U69593X((5′,7′,8a)-(−)-*N*-methyl-*N*-(7-(1-pyrrolidinyl)-1-oxa-spiro-(4,5)-dec-8-yl)-benzeneacetamide; Upjohn), 5′-guanylylimidophosphate (GppNHp; Sigma) [^{3}H]diprenorphine (Amersham International), [^{3}H]naloxone (Amersham International), cyprodime and 16-methylcyprenorphine (Colin Smith; Reckitt & Colman). Naltrindole and norbinaltorphimine were synthesized by A. Naylor, Chemistry Department, Glaxo.

Results

Field-stimulated mouse vas deferens. In the mouse vas deferens, following 30 min equilibration, naltridole produced concentration-dependent, parallel shifts of the concentration-response curve for the δ-agonist, [D-Pen^2,D-Pen^5]enkephalin (DPDPE; Fig. 1). Naltrindole alone had no effect on the twitch height nor did it depress the maximum response to DPDPE. The mean pK_B value calculated from the IC50 ratios was 9·74 ± 0·04 and the Schild slope was not significantly different from unity (see Table 1).

Parallel shifts of the concentration-response curves for the μ-agonist, [D-Ala^2,MePhe^4,Gly^5-ol]enkephalin (DAGO) and the κ-agonist, U69593X were also obtained in the presence of naltrindole. However, higher concentrations of naltrindole were required to shift the curves for DAGO and U69593X than for DPDPE. The pK_B values for naltrindole against DAGO and U69593X were 8·28 ± 0·06 and 7·49 ± 0·05, respectively (Table 1).

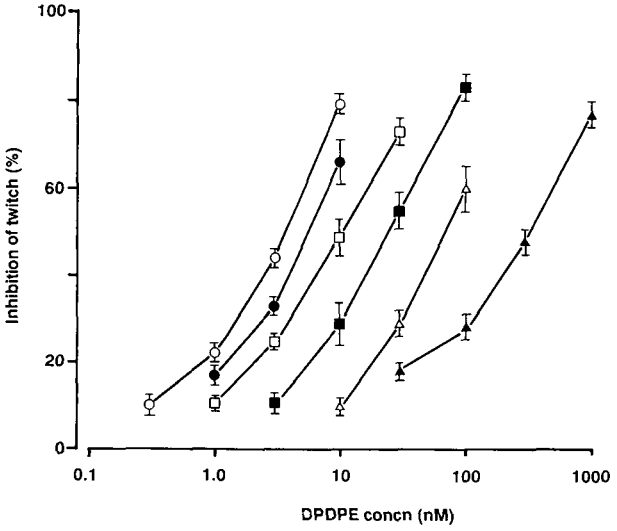

FIG. 1. Effect of naltrindole on concentration-response curves to DPDPE in the field-stimulated mouse vas deferens. Concentration-response curves are mean ± s.e.m. of four tissues. The complete range of concentrations of naltrindole was applied to each tissue. DPDPE concentration-response curve in the absence of naltrindole (○) or in the presence of 0·1 (●), 0·3 (□), 1·0 (■), 3·0 (△), and 10 nM (▲) naltrindole.

Table 1. Affinity of naltrindole for δ-, μ-, and κ-receptors. Agonist concentration-response curves were constructed for [D-Pen^2,D-Pen^5]enkephalin (δ), [D-Ala^2, MePhe^4,Gly^5-ol]enkephalin (μ), and U69593X (κ). Each tissue was then incubated for 30 min in the presence of naltrindole before repeating the agonist concentration-response curve. Naltrindole was applied at five, increasing, concentrations to each tissue and the ratio of the IC50 values for the agonist in the presence and absence of naltrindole calculated for each concentration. These concentration-ratios were then used to calculate the mean pK_B value ($\pm$s.e.m.) and to construct a Schild plot from which the Schild slope ($\pm$s.e.m.) was obtained. Binding assays were performed in the presence and absence of sodium ions and GppNHp. Binding sites were defined as follows: κ-sites were labelled using [^{3}H]diprenorphine (0·2 nM) in guinea-pig cerebellum in the presence of 20 nM 16-methylcyprenorphine, μ-sites using [^{3}H]naloxone (0·2 nM) in rat brain and δ-sites using [^{3}H]diprenorphine (0·5 nM) with cyprodime (1·26 μM) and norbinaltorphimine (20 nM) to suppress μ- and κ-sites, respectively. In the binding assays the standard errors were within 5% of the mean.

Receptor	Mouse vas deferens			Binding assay		
				Tris	Tris-NaCl-GppNHp	
	pK_B	slope	(n)	pIC50 (nM)	pIC50 (nM)	(n)
δ	9·74±0·04	1·12±0·05	(4)	9·59	11·85	(3)
μ	8·28±0·06	0·94±0·10	(4)	7·77	7·88	(3)
κ	7·49±0·05	0·94±0·07	(3)	7·20	7·62	(3)

The Schild slope in both cases was not significantly different from unity and naltrindole had no effect on the maximum response to either agonist. Thus, the relative selectivity of naltrindole in the mouse vas deferens for δ/μ-receptors is approximately 19- to 23-fold and for δ/κ-receptors 145- to 220-fold.

Binding assays. Naltrindole displaced the specific binding of opioids to μ-, δ-and κ-binding sites. The negative logarithms of the IC50 values (pIC50) obtained are given in Table 1. As has been prevously shown for δ-antagonists,the affinity of naltrindole for the δ-binding site was markedly increased in the presence of sodium ions and GppNHp. The presence of sodium ions and GppNHp did not significantly change the affinity of naltrindole for the μ- or κ-sites. The leftward shift of the displacement curve for naltrindole increased the selectivity ratios of naltrindole for δ/μ-receptors from 66-fold to 9330-fold and for δ/κ- from 250-fold to 17000-fold.

Discussion

The results show that, in both a standard in-vitro screen for opioid receptor interaction and in binding assays for the individual opioid receptor types, naltrindole is a potent, competitive, antagonist that shows a marked degree of selectivity for the δ-type of opioid receptor. The selectivity ratios obtained in the mouse vas deferens were not as large as those reported originally by Portoghese et al (1988a, b). In the study of Portoghese et al, the affinity of naltrindole for δ-receptors in the mouse vas deferens was compared with the affinity for μ- and κ-receptors in the guinea-pig ileum, which gave a δ/μ ratio of 81 and a δ/κ ratio of 370. However, the K_e values given were based upon shifts obtained using a single concentration of naltrindole, whereas in the present study a full Schild analysis was performed. In addition, in the present study, pK_B values have been obtained at all three receptor types in the same tissue.

In the binding assays, naltrindole exhibited a greater selectivity for the δ-receptor than in the mouse vas deferens, and the values agreed more closely with those of Portoghese et al (1988a, b). As with ICI 174864 (Appelmans et al 1986) the displacement curve for naltrindole at the δ-site shifted markedly to the left in the presence of sodium ions and GppNHp.

Thus, this study confirms that naltrindole shows a high degree of selectivity for the δ-opioid receptor in-vitro, and should its selectivity and potency be maintained in-vivo, this drug should prove a useful tool for the characterisation of δ-opioid receptor-mediated responses.

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