Stereoselective Interaction of Mianserin with 5-HT3 Receptors

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Abstract—The interaction of the enantiomers of mianserin with the 5-HT$_3$ receptor was determined. Using [^{3}H]granisetron binding, (−)-mianserin was more potent than (+)-mianserin (pK$_i$ 8·46 and 6·95, respectively). The enantiomers competitively antagonized the depolarizing effect of 5-hydroxytryptamine in the rat vagus nerve preparation (pK$_{app}$: (−)-mianserin 8·13, (+)-mianserin 6·58). This stereoselectivity was maintained in-vivo as determined using ex-vivo inhibition of [^{3}H]granisetron binding. Therefore, in contrast to its enantiomeric selectivity for the 5-HT$_{1C}$ and 5-HT$_2$ receptors, where the (+)-isomer is more potent, the enantiomeric selectivity of mianserin for the 5-HT$_3$ receptor was reversed. This differential selectivity of the enantiomers of mianserin may be useful in elucidating its utility in anxiety states.

The antidepressant compound mianserin has long been known to interact with 5-hydroxytryptamine (5-HT) receptors. It was first shown to interact with the 5-HT$_2$ receptor (Peroutka & Snyder 1979) and was subsequently used in the identification of the 5-HT$_{1C}$ recognition site (Pazos et al 1984; Blurton & Wood 1986). Recently, mianserin has also been shown to interact at low concentrations with central 5-HT$_3$ receptors labelled with [^{3}H]granisetron (Nelson & Thomas 1989) and [^{3}H]ICS 205-930 (Hoyer & Neijt 1988). Mianserin has also been shown to antagonize 5-HT-induced inward currents in N1E-115 cells, believed to be mediated by 5-HT$_3$ receptors (Guharay & Usherwood 1981; Zwart et al 1990).
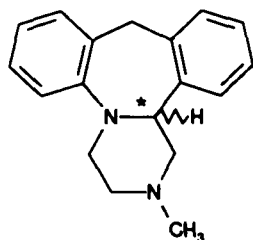

FIG. 1. A representation of the structure of mianserin showing the chiral centre.

Mianserin (Fig. 1) possesses an optically active chiral centre and its enantiomers interact stereoselectively with multiple 5-HT binding sites (Alexander & Wood 1987). It was, therefore, of interest to determine whether the enantiomers of mianserin interact in a stereoselective manner with the 5-HT$_3$ receptor. This was investigated in radioligand binding assays using [^{3}H]granisetron both in-vitro and ex-vivo. In addition, the mode of antagonism of mianserin was studied in a functional 5-HT$_3$ receptor model, the rat isolated vagus nerve (Ireland & Tyers 1987). Preliminary findings have been reported elsewhere (Wood et al 1991).

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Materials and Methods

[^{3}H]Granisetron binding in-vitro

The binding of [^{3}H]granisetron was studied in membranes prepared from the caudal cortex plus hippocampus from male Sprague-Dawley rats (Interfauna, 200–300 g). Briefly, the tissue was excised over ice and homogenized (Polytron) in 50 vol ice-cold buffer (Tris-HCl, 50 mM, pH 7·4 at 37°C). The homogenate was washed three times by centrifugation (40 000 g for 10 min) with a 15-min incubation at 37°C before the final wash and the pellets frozen at −20°C.

On the day of the experiment, the membranes were thawed and resuspended in 100 vol Tris buffer. Portions (0·9 mL) were incubated with [^{3}H]granisetron (50 µL, 0·3 nM final concentration) and either 50 µL Tris buffer (for determining total binding), or drug solution, and incubated at ambient temperature for 40 min. The reaction was terminated by rapid filtration through Whatman GF/B filters (presoaked in 0·01% polyethyleneimine) using a Brandel cell harvester, and the filters washed twice with 7·5 mL ice-cold Tris buffer. Incubations were performed in triplicate with 10 concentrations of the inhibitor. Specific binding was defined in the presence of 10 µM ondansetron.

[^{3}H]Granisetron binding ex-vivo

The ability of compounds administered in-vivo to inhibit the binding of [^{3}H]granisetron in-vitro was determined as an index of CNS penetration and receptor occupancy (Wood & Piper 1990). Male CFY rats (6 per group) were pretreated intraperitoneally with varying doses of the enantiomers of mianserin or standard 5-HT$_3$ antagonists. Thirty minutes later the rats were killed and the caudal cortex plus hippocampus removed. Tissue was homogenized in 25 vol Tris-HCl buffer incubated for 15 min at 37°C to reduce endogenous 5-HT content, and kept on ice. Portions (0·4 mL) were then incubated with [^{3}H]granisetron (0·3 nM final) in a final volume of 0·5 mL for 40 min at 37°C. The reaction was terminated by rapid filtration through filters (Whatman GF/B presoaked in 0·01% polyethyleneimine) using a Millipore manifold and the filters washed twice with 7·5 mL ice-cold Tris buffer.

Inhibition of [³H]granisetron binding was compared with specific binding (that displaced by 10 μM ondansetron added in-vitro) in vehicle-treated controls. The ID50 (mg kg⁻¹) was determined and expressed after correction for the dilution factor in the resuspension and incubation stages. No correction was made for the possibility that these compounds may have different rates of dissociation from the 5-HT₃ receptor resulting in differential dilution; therefore the calculated ID50 only represents a relative potency.

Vagus nerve

Male Sprague–Dawley rats (125–270 g, Harlan-Olac) were killed by a heavy blow to the back of the head followed by exsanguination. Two cervical vagus nerve trunks (1·0–1·5 cm in length) were excised from each animal and desheathed and each vagus was set up in a three-compartment bath (Newberry et al 1991a). About 0·5 cm of the vagus was suspended in the central compartment with each end of the nerve trunk protruding through a greased gap in one of the barriers dividing up the bath. The central compartment (volume ca. 0·5 mL) was perfused (at 2·0–2·5 mL min⁻¹) with an aqueous medium at 25°C. The same medium in the outer two chambers was static. The d.c. potential between the central compartment and one of the outer compartments was continuously monitored on a chart recorder using Ag/AgCl electrodes. The aqueous medium was equilibrated with 95% O_2–5% CO_2 and contained (in mM): NaCl 125, NaHCO₃ 25, D-glucose 5, CaCl₂ 2·5, KCl 2, KH₂PO₄ 1 and MgSO₄ 1.

5-HT was applied to the vagus for 1-min periods via the medium flowing through the central chamber. After setting up the preparation and when the d.c. potential had stabilized, 10 μM 5-HT was applied at 30-min intervals until the evoked depolarizing response was stable (varying by less than 10%), this usually took 3–4 applications. The peak amplitude of the last of these responses was given the arbitrary unit of 1·0. Superfusion of an antagonist (1 h) began 15–20 min after the last response to 5-HT. In the presence of the antagonist, concentration-response relationships were constructed by increasing the applied concentration of 5-HT in half log₁₀ (molar concentration) units (30, 10, 100 nM etc.) at intervals of 10–20 min. The response to 5-HT were expressed as a ratio of the last response to 10 μM 5-HT. Controls were obtained by using the same procedure on the second vagus from the same rat, but without adding an antagonist to the superfusing medium.

Where appropriate, the apparent affinity of an antagonist was determined from the equation $pK_{app} = \log_{10} (CR - 1) - \log_{10}$ (molar concentration of antagonist). The concentration-ratio (CR) was the ratio of the geometric mean concentrations of 5-HT necessary to produce a response level of 0·5 in the presence and absence of the antagonist. The pEC50 is the negative logarithm (base 10) of the concentration which evokes half of the maximum response. Unless otherwise indicated, all values are either the arithmetic mean ± s.e.m. or the geometric mean (+s.e.m., −s.e.m.).

Data analysis

Inhibition curves from both in-vitro and ex-vivo binding studies were analysed using the 4-parameter logistic program ALLFIT (DeLean et al 1978) to obtain potency estimate

(IC50/ID50) and slope factor. K_i values were derived according to Cheng & Prusoff (1975). The relative response unit in the vagus nerve studies is a ratio, consequently the data were expressed as their log₁₀ values before calculating the mean and s.e.m.

Materials

[³H]Granisetron (61·0 Ci mmol⁻¹) was obtained from NEN DuPont UK Ltd. The enantiomers of mianserin were prepared at SmithKline Beecham Medicinal Research Centre, Harlow and as a gift from Organon International. ICS 205-930 and ondansetron were prepared at the SmithKline Beecham Medicinal Research Centre, Harlow. 5-HT hydrochloride, Tris-HCl and polyethyleneimine were obtained from Sigma.

Results

In-vitro binding

(−)-Mianserin was a more potent inhibitor of [³H]granisetron binding (pK_i 8·46±0·14, slope 0·86±0·08; mean± s.e.m., n=4) than (+)-mianserin (pK_i 6·95±0·06, slope 1·12±0·17; n=4). This is in contrast to the stereoselectivity in favour of (+)-mianserin for other 5-HT binding sites (Table 1).

Table 1. Interaction of (+)- and (−)-mianserin with 5-HT binding sites.

	IC50 (nM)	
	(+)-Mianserin	(−)-Mianserin
5-HT₃	276	9·1
*5-HT₁ₐ	654	3790
*5-HT₁ᵦ	2930	13 900
*5-HT₁c	13·9	95·2
*5-HT₂	2·6	70·0

Relative potencies (IC50 values) for (+)- and (−)-mianserin at various 5-HT receptor binding subtypes in-vitro. *Alexander & Wood (1987).

Ex-vivo binding

The 5-HT₃ receptor antagonists ICS 205-930 and ondansetron were potent inhibitors of ex-vivo [³H]granisetron binding (Table 2) indicating that the radioligand was binding to

Table 2. Ex-vivo inhibition of [³H]granisetron binding to central 5-HT₃ receptors.

Compound	Relative potency ID50 (mg kg⁻¹, i.p.)
ICS 205-930	0·013±0·001
Ondansetron	0·089±0·061
(−)-Mianserin	0·58±0·07
(+)-Mianserin	2·08±0·22

Relative potency (ID50) for the ex-vivo inhibition of [³H]granisetron binding relative to vehicle controls. The ID50 values and approximate standard errors (not true standard errors as they were derived from nonlinear curve-fitting) were generated from inhibition curves using 3–5 doses of the antagonist with six animals per group.
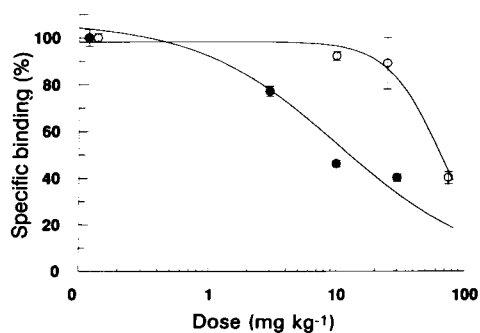
FIG. 2. Ex-vivo inhibition of [³H]granisetron binding by (+)-mianserin (O) and by (−)-mianserin (●). Each point represents the mean ± s.e.m. from six animals using different doses (shown not corrected for dilution factor).

central 5-HT₃ recognition sites. In agreement with the in-vitro studies, (−)-mianserin was a more potent inhibitor of ex-vivo [³H]granisetron binding than (+)-mianserin (Table 2, Fig. 2).

Vagus

5-HT (10 μM) reproducibly depolarized the rat isolated vagus nerve by 0.61 ± 0.03 mV (n = 36). Its effect was concentration-dependent with a pEC50 of 6.15 ± 0.05 (n = 16) (cf. Ireland & Tyers 1987) and was blocked by 0.1 μM (±)-mianserin (Fig. 3). This antagonism was reversible and
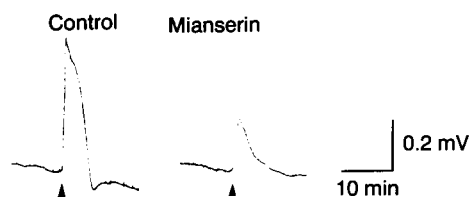

FIG. 3. Antagonism of the 5-HT₃ receptor mediated response of the rat vagus nerve by mianserin. In this experiment showing the depolarization induced by 5-HT (10 μM), the rat vagus nerve was superfused for 45 min with 0.1 μM (±)-mianserin and then washed with antagonist-free medium for a further 60 min. The application of 5-HT (1 min) is indicated by the arrow.

shifted the concentration-response curve to 5-HT to the right with a pK_{app} of 7.83 (n = 5, not shown). The individual enantiomers of mianserin also induced rightward surmountable shifts of the concentration-response curve to 5-HT. (−)-Mianserin was clearly the more potent of the two antagonists (Fig. 4). For the (−)-isomer the pK_{app} was 8.26 at 0.03 μM and 8.00 at 0.3 μM, whereas for the (+)-isomer it was 6.71 at 1 μM and 6.46 at 10 μM. When averaged these correspond to pK_{app} values of 8.13 and 6.58, respectively. There was some flattening of the lower part of the concentration-response curve to 5-HT seen with high concentrations of the enantiomers. This was evident in Fig. 4 as the responses evoked by low concentrations of 5-HT (up to 1 μM) were little affected by the enantiomers.

Discussion

The present results show that mianserin interacts in a stereoselective manner with 5-HT₃ receptors in the rat and
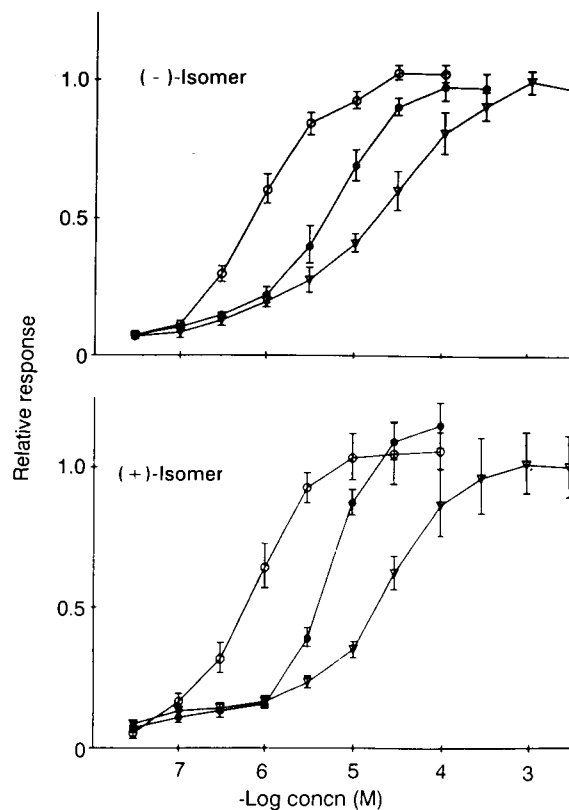

FIG. 4. Stereoselective antagonism of the 5-HT-induced depolarization of the rat vagus nerve by the enantiomers of mianserin. The relative responses have been pooled (see Materials and Methods) from 4 to 7 tissues, each from a different rat. The geometric mean concentrations of 5-HT necessary to evoke response levels of 0.5 were as follows: 0.63 μM for controls, 4.07 and 19.5 μM in 0.03 and 0.3 μM (−)-mianserin, and 3.86 and 19.03 μM in 1 and 10 μM (+)-mianserin, respectively.

that it appears to be a competitive antagonist at those receptors. The stereoselective interaction of (−)-mianserin with [³H]granisetron binding is an important criterion of neurotransmitter receptor binding. It is also unusual in that for the other pharmacological actions of mianserin, either there is little stereoselectivity or the biological activity resides mainly in the (+)-isomer (Nickolson & Wieringa 1981; Johnson et al 1983). That this selectivity is maintained in-vivo was shown in ex-vivo binding experiments. It should be noted that the apparent stereoselectivity observed in-vivo was less than that seen in-vitro. This may reflect differences in the dilution correction as the enantiomers may have different dissociation rates from 5-HT₃ receptor or from the tissue. Confirmation that the ex-vivo binding method does reflect occupancy of central 5-HT₃ receptors was shown by the marked inhibition seen with the selective 5-HT₃ receptor antagonists ICS 205-930 (Richardson et al 1985) and ondansetron (Butler et al 1988).

In the vagus nerve, both (+)- and (−)-mianserin produced clear rightward shifts of the concentration-response curve to 5-HT. The surmountable antagonism and the good agreement in pK_{app} values indicate that both isomers act in a competitive manner. Relatively few of the 5-HT₃ receptor antagonists tested in this preparation produce surmountable effects (Ireland & Tyers 1987; Butler et al 1990; Newberry et

al 1991b, 1992). The slight flattening of the concentration-response curve to 5-HT seen with high concentrations of the isomers has been observed with other 5-HT₃ receptor antagonists and may be due to the presence of a 5-HT₃ receptor antagonist-resistant response, possibly mediated by 5-HT₄ receptors (Rhodes & Coleman 1991). Mianserin has previously been found to have a lower affinity for 5-HT₃ receptors labelled with [³H]quipazine (pIC50 7·18 (Schmidt & Peroutka 1989)) and [³H]ICS 205-930 (pK_D 7·19 in NIE-115 cell membranes) (Hoyer & Neijt 1988); pK_D 6·97 in NG 108-15 cell membranes (Neijt et al 1988)). The close correlation between affinity in a functional model of 5-HT₃ receptors and displacement of [³H]granisetron binding in the same species suggests that the use of quipazine and ICS 205-930 as radioligands may not be predictive of 5-HT₃ affinity or that the 5-HT₃ receptor in those cell lines may be pharmacologically different from that in the rat. It should be noted that a similar anomaly exists for methiothepin between affinity on binding in NIE-115 cells (Hoyer & Neijt 1988) and on function in rat vagus nerve (Ireland & Tyers 1987).

The high potency of mianserin for the 5-HT₃ receptor suggests that blockade of these receptors may occur at doses similar to those used to block 5-HT₁C and 5-HT₂ responses. The differential selectivity reported here of (−)-mianserin, for the 5-HT₃-receptor binding site and of (+)-mianserin for the 5-HT₁C and 5-HT₂ binding sites may provide a means of separating these effects. Mianserin has been reported to have clinical utility in the treatment of anxiety states (Murphy 1978). Since both 5-HT₃ receptors (Jones et al 1988) and 5-HT₁C receptors (Kennett 1992) have been suggested to mediate anxiolytic effects it may be of interest to examine the effects of the enantiomers of mianserin in the clinic to determine the anxiolytic mechanism.

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