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Stereoselective blockade of central [^{3}H]5-hydroxytryptamine binding to multiple sites (5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C}) by mianserin and propranolol

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The interaction of the enantiomers of mianserin and propranolol with the binding of [^{3}H]5-hydroxytryptamine ([^{3}H]5-HT) to the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} sites, and with the binding of [^{3}H]ketanserin to the 5-HT_2 site, has been evaluated in rat brain membranes. A stereoselective interaction at the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} sites was demonstrated for both compounds, with (+)-mianserin being a more potent displacer than (−)-mianserin and (−)-propranolol being more potent than (+)-propranolol. Only mianserin interacted in a stereoselective manner with the 5-HT_2 site, (+)-mianserin being the more potent isomer. The stereoselective association of mianserin and propranolol with the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} sites may prove useful in the characterization of these sites.

The heterogeneity of brain 5-hydroxytryptamine (5-HT) receptors has been supported by many studies. Peroutka & Snyder (1979) classified 5-HT receptor sites into 5-HT_1 (labelled by [^{3}H]5-HT) and 5-HT_2 (labelled by [^{3}H]spiperone). More recently, by means of radioligand binding techniques, distinct subtypes of the 5-HT_1 receptor have been demonstrated and termed the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} sites (Hoyer et al 1985). These sites have been shown to satisfy some of the criteria for neurotransmitter receptor binding sites: they are saturable, possess a high affinity for the neurotransmitter 5-HT, have differential pharmacological specificities, and are unevenly distributed throughout the brain (Hoyer et al 1985; Alexander et al 1986; Blurton & Wood 1986). The 5-HT_{1A} site has high affinity for 8-hydroxy-2-(di-*N*-propylamino)tetralin (8-OH-DPAT) and spiperone; the 5-HT_{1B} site displays high affinity for methoxy-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole (RU 24969) and propranolol; and the 5-HT_{1C} site shows high affinity for mianserin and mesulergine. The hippocampus is rich in 5-HT_{1A} sites, whereas the striatum is rich in 5-HT_{1B} and 5-HT_{1C} sites. Another important consideration in the identification of neurotransmitter receptor binding sites is that of stereospecificity. Both propranolol and mianserin interact with differing potencies at the three subsites, and both possess an optically active chiral centre. We have therefore examined the interaction of the enantiomers of mianserin and propranolol with the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} subsites, and also with the 5-HT_2 site, in rat brain membranes.

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Materials and methods

[^{3}H]5-HT binding. The binding of [^{3}H]5-HT was as described by Blurton & Wood (1986). The hippocampus and striatum were dissected from 12 male Sprague-Dawley rats (200–250 g) and homogenized in 50 vol Tris-HCl buffer (50 mM, pH 7·4 at 37 °C) using a Polytron (setting 5 for 30 s). The homogenates were centrifuged (40 000*g* for 10 min) and the pellets washed twice by centrifugation and resuspension. After the second wash, the pellet was resuspended in 100 vol Tris-HCl buffer and incubated at 37 °C for 15 min before centrifugation to remove endogenous 5-HT. The final pellet was resuspended in 35 vol Tris-HCl buffer containing 10 μM pargyline, 4 mM $CaCl_2$ and 0·1% ascorbic acid. Throughout the procedure the tissue was kept at 0–4 °C unless otherwise stated.

Tissue homogenate (350 μL), [^{3}H]5-HT (2 nM final concentration, 50 μL), buffer or specific binding displacer (50 μL) and the appropriate concentrations of drug (50 μL) were incubated at 37 °C for 12 min. Incubations were terminated by rapid filtration through Whatman GF/B filters under reduced pressure using a 24 place cell harvester (Brandel, USA). The filters were washed twice with 7·5 mL ice-cold Tris-HCl buffer. Retained radioactivity was measured after extraction into NE266 (Nuclear Enterprise) liquid scintillator at an efficiency of 40–45%. Binding to the 1A site was studied in hippocampal membranes. Drug potencies at the 1B and 1C sites were derived by computer analysis from displacement data in striatal tissue, which were resolved into 1B and 1C components based on the relative proportion of 1B : 1C sites (60 : 40) in this tissue (Blurton & Wood 1986). Specific binding was defined as that displaced by 10^{-5} M 5-HT.

[^{3}H]Ketanserin binding. The binding of [^{3}H]ketanserin was studied in membranes from the rat frontal cortex

prepared as described above. The final pellet was resuspended in 100 vols Tris-HCl buffer.

Tissue homogenate (0·9 mL), [^{3}H]ketanserin (1 nM, 0·05 mL) and the appropriate concentration of drug (0·05 mL) were incubated for 20 min at 37 °C and the reaction terminated as above. Specific binding was that displaced by 10^{-5} M methysergide.

Other procedures. All incubations were performed in triplicate generally using 10 different drug concentrations which were prepared and dispensed using a diluter/dispenser (Hamilton Microlab M). Displacement data were analysed using the ALLFIT program (De Lean et al 1978) to obtain inhibitory potency (IC50) and slope factor. Displacements with shallow slopes were analysed further to 1- or 2-site binding models using the non-linear least squares program PATTERNSEARCH (Green et al 1982) and the best fit determined. [^{3}H]Ketanserin (80 Ci mmol^{-1}) was purchased from NEN, and [^{3}H]5-HT (30 Ci mmol^{-1}) from Amersham International. (+)- and (−)-Mianserin were kindly donated by Dr R. Pinder, Organon, and (+)- and (−)-propranolol were kindly donated by ICI Ltd.

Results

[^{3}H]5-HT binding studies. Both mianserin and propranolol displaced [^{3}H]5-HT binding from the hippocampal 5-HT_{1A} site in a stereospecific manner (Table 1), with (+)-mianserin being more potent than (−)-mianserin and (−)-propranolol being markedly more potent than (+)-propranolol. Both the active isomers ((+)-mianserin and (−)-propranolol) inhibited [^{3}H]5-HT binding with shallow slopes and were best described by a two-site fit with the high affinity site representing 65–70% of specific binding and corresponding to an interaction with the 5-HT_{1A} subsite.

In the striatum, (+)-mianserin was more potent that (−)-mianserin (Fig. 1) and (−)-propranolol was more potent than the (+)-isomer in displacing [^{3}H]5-HT (Table 2). The stereoisomers of both compounds displaced [^{3}H]5-HT binding with shallow slopes, and were best described by a two binding site model. The high affinity site recognized by (+)- and (−)-propranolol represented 60–70% of specific [^{3}H]5-HT binding, corresponding to an interaction with the 5-HT_{1B} site. The site recognized with high affinity by (+)- and (−)-mianserin represented 35–40% of specific striatal [^{3}H]5-HT binding, corresponding to a 5-HT_{1C} site. The isomers of propranolol also interacted in a stereoselective manner with the 5-HT_{1C} site, although this was difficult to quantify due to the low potency of (+)-propranolol. Separate experiments in striatal membranes using 250 nM mianserin to occlude the binding of [^{3}H]5-HT to the 5-HT_{1C} site gave IC50 values of 13 500 nM ± 5100 and 24 600 nM ± 2700 (results mean ± s.e.m., n = 3) for the (−)- and (+)-isomers of propranolol, respectively.

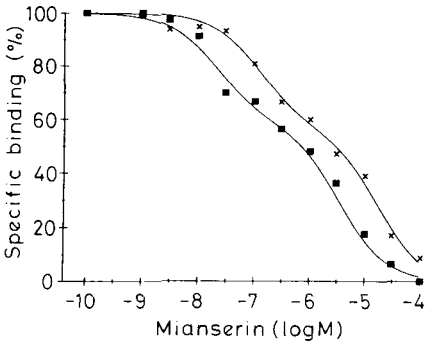

FIG. 1. Stereoselective inhibition of striatal [^{3}H]5-HT binding by the enantiomers of mianserin. Varying concentrations of (+)- and (−)-mianserin were incubated in triplicate with 2 nM [^{3}H]5-HT as described in the Methods. Inhibition of specific [^{3}H]5-HT binding is shown with the solid lines representing the computer-determined best-fit to a two-site model. The site displaced by nanomolar concentrations of mianserin corresponds to the 5-HT_{1C}, and the site displaced by micromolar concentrations corresponds to the 5-HT_{1B} site. The results are from one experiment, which was repeated 3 times with similar results. Key: × —— × (−)-mianserin, ■ —— ■ (+)-mianserin.

Table 1. Inhibition of [^{3}H]5-HT binding to rat hippocampal membranes. Results are computer-determined parameters for the inhibition of [^{3}H]5-HT binding. Displacements were analysed to a 1-site binding model to obtain IC50 and slope factor. Those with shallow slopes were analysed further using a 2-site model: the IC50 and relative % of [^{3}H]5-HT binding to the high affinity site (IC50 high and % high) are shown.

	1-site analysis		2-site analysis	
Compound	IC50 (nM)	Slope	IC50 high	% high
(−)-Propranolol	97·1 ± 11[a]	0·61 ± 0·04[b]	54·2 ± 11	65·7 ± 3·0
(+)-Propranolol	4220 ± 400	0·97 ± 0·13	N/A	N/A
(−)-Mianserin	3790 ± 560	0·91 ± 0·17	N/A	N/A
(+)-Mianserin	1190 ± 280[c]	0·81 ± 0·03[b]	654 ± 160	69·0 ± 9·5

Data are the means ± s.e.m. of 3–5 experiments.
N/A = not applicable.
[a] $P < 0·01$ compared with (+)-propranolol, Student's t-test.
[b] $P < 0·05$ slope significantly less than unity, F-test.
[c] $P < 0·05$ compared with (−)-mianserin, Student's t-test.

[^{3}H]Ketanserin binding studies. Both (+)- and (−)-propranolol interacted with the 5-HT_2 site, as labelled by [^{3}H]ketanserin, only at micromolar concentrations. There was no stereoselectivity between the isomers with IC50s of 4670 nM ± 660 ($\bar{x}$ ± s.e.m., n = 5) and 3272 nM ± 334 ($\bar{x}$ ± s.e.m., n = 7) for (+)- and (−)-propranolol, respectively. In contrast, mianserin blocked [^{3}H]ketanserin binding stereoselectively with IC50s of 2·6 nM ± 0·3 ($\bar{x}$ ± s.e.m., n = 3) and 70·0 nM ± 19 (n = 3, $P < 0·5$ Student's paired t-test) for (+)- and (−)-mianserin, respectively.

Discussion

Although there have been a number of reports on the identification of distinct 1A, 1B and 1C subsites of 5-HT binding (Hoyer et al 1985; Blurton & Wood 1986; Heuring et al 1986), these binding sites have still to be

Table 2. Inhibition of [^{3}H]5-HT binding to rat striatal membranes. Results are computer-determined parameters for the inhibition of [^{3}H]5-HT binding. Displacements were analysed to a 1-site binding model to obtain IC50 and slope factor. Those with shallow slopes were analysed further using a 2-site model: the IC50 and relative % of [^{3}H]5-HT binding to the high affinity site (IC50 high and % high) are shown.

	1-site analysis		2-site analysis		
Compound	IC50 (nM)	Slope	IC50 high	IC50 low	% high
(−)-Propranolol	84 ± 20[a]	0·61 ± 0·04[b]	22·9 ± 4·9[a]	9080 ± 1800	63 ± 3·6
(+)-Propranolol	4880 ± 990	0·70 ± 0·07[b]	3550 ± 134	>20000	67 ± 6·6
(−)-Mianserin	2080 ± 510	0·50 ± 0·02[b]	95·2 ± 2·7	13900 ± 1650	37 ± 4·9
(+)-Mianserin	725 ± 88[c]	0·49 ± 0·02[b]	13·9 ± 4·8[c]	2930 ± 740[c]	35 ± 3·1

Data are the means ± s.e.m. of 3–5 experiments.
[a] $P < 0·01$ compared with (+)-propranolol, Student's t-test.
[b] $P < 0·01$ slope significantly less than unity, F-test.
[c] $P < 0·05$ compared with (−)-mianserin, Student's t-test.

shown to satisfy all of the criteria required for neurotransmitter receptor binding sites (see Burt 1978). The 1A, 1B and 1C sites have been shown to be saturable and have high affinity for the neurotransmitter 5-HT (Hoyer et al 1985; Blurton & Wood 1986), to have differential pharmacological specificity (Hoyer et al 1985; Alexander et al 1986), and to have an uneven regional distribution (Pazos & Palacios 1985; Blurton & Wood 1986). The present investigation has shown that the enantiomers of mianserin and propranolol interact stereoselectively with the 1A, 1B and 1C subsites. The (−)-isomer of propranolol was reported to be more potent than the (+)-isomer in displacing 5-HT_{1B} binding (Middlemiss 1984), and the (−)-isomer also showed higher affinity for the 1A and 1C sites than the (+)-isomer. (+)-Mianserin was more potent than (−)-mianserin as an inhibitor of noradrenaline release and reuptake (Nickolson & Wierring 1981), and a similar selectivity was found at the 1A, 1B and 1C binding sites. Stereoselectivity is an important consideration in the identification of neurotransmitter receptor binding (see Burt 1978). Thus the occurrence of stereoselectivity implies that an extremely close relationship exists between the binding site and the compound such that the topography of the recognition site can discriminate stereoisomers. It is envisaged that such a precise relationship does not occur by chance, but reflects the presence of a physiologically relevant recognition site. The high degree of stereoselectivity displayed by propranolol probably reflects the importance of the side chain amino group (where the chiral centre resides) in the 5-HT recognition site.

Although the demonstration of stereoselectivity suggests the presence of a specific recognition site, it does not constitute proof of receptor identification as shown by the stereospecific binding of opiates to glass fibre filters (Snyder et al 1975). Thus the demonstration of stereoselectivity at the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} sites has little meaning if activation of these sites does not produce a response and so satisfy the most important criterion in receptor identification. Recent studies suggest that functional correlates for these sites do exist: the 5-HT_{1A} site may be coupled to adenylate cyclase (Shenker et al 1985), the 5-HT_{1B} site appears to regulate 5-HT release from 5-HT terminals (i.e. autoreceptor function) (Hibert & Middlemiss 1986; Engel et al 1986) and the 5-HT_{1C} site may mediate inhibition of twitch in the rat fundus (Buchheit et al 1986). The stereoselective interaction of the enantiomers of mianserin and propranolol should be useful in the characterization of such functional correlates.

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