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^{3}H-MIANSERIN BINDING IN CALF CAUDATE: POSSIBLE INVOLVEMENT OF SEROTONIN RECEPTORS IN ANTI-DEPRESSANT DRUG ACTION

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Mianserin has been shown to be a serotonin receptor antagonist both in peripheral [1,2] and central [3,4] receptor systems. It has also been well established as a clinically effective tetracyclic anti-depressant [5,6].

Recently, a tritiated form of mianserin has become available with a specific activity high enough for use in direct binding assays of central neurotransmitter receptors. These assays would be of interest for two reasons: (i) they could introduce a new ligand for central serotonin receptors in addition to ^{3}H-d-LSD [7] and ^{3}H-serotonin, [4], both of which have limited usefulness, and (ii) the results would be interesting to compare with recent findings in ^{3}H-tricyclic antidepressant direct binding assays [8,9,10] in order to establish a possible common site of action shared by the tricyclics and a novel antidepressant.

The caudate nucleus was dissected from frozen calf brains, homogenized in 10 volumes of 0.32 M sucrose and centrifuged at 1,000 g. for 10 min. The resulting supernatant was diluted to 100 volumes with 50 mM Tris /HCl buffer (pH 7.4) and centrifuged at 50,000 g. for 20 min. The pellet was resuspended in 40 volumes of buffer and frozen. Immediately prior to use the pellet was thawed and homogenized using a polytron homogenizer at a setting of 5 for 10 seconds. Final protein concentration was 1.5 mg/ml.

Direct binding assays were performed by incubating at room temperature the following aliquots: 0.2 ml. of buffer alone or buffer containing mianserin hydrochloride (final concentration 1,000 nM) or buffer containing varying concentrations of drug competitor; 0.2 ml. of ^{3}H-mianserin HCl (final concentration 5 nM); 0.2 ml. of calf caudate homogenate (0.3 mg of protein). After 30 min., 3.5 ml of buffer was added to each tube and the entire contents filtered under vacuum through a Whatman GF/B filter and washed by a further 3.5 ml of buffer. Filters were then monitored for tritium using a toluene/Triton scintillation mixture. Specific binding of ^{3}H-mianserin was defined to be the total amount of ^{3}H-mianserin bound in the presence of buffer minus the ^{3}H-mianserin bound in the presence of 1,000 nM non-radioactive mianserin.

The K_i values of tested drugs were determined by linear regression analysis of log-probit plots of binding inhibition using at least 8 different drug concentrations, each one

measured in quadruplicate. These data are given in the following tables.

Table 1 : K_i Values of Neurotransmitters on Specific Binding of ^{3}H-Mianserin

Neurotransmitter	K_i (nM)
Serotonin	390
Noradrenaline	>1,300
Histamine	No effect at 15,000
Dopamine	No effect at 15,000
Acetylcholine	No effect at 15,000
GABA	No effect at 15,000

Table 2 : K_i Values of Drugs Active in Serotonin Systems on Specific Binding of ^{3}H-Mianserin

Tryptamines	K_i (nM)
Tryptamine	780
N,N Dimethyltryptamine	620
5, methoxy DMT	1,030
5,6 dihydroxytryptamine	4,000
Ergots	
LSD	1.7
Metergoline	1.7
Dihydroergocryptine	30.0
Dihydroergotamine	52.0

Table 3 : K_i Values of Antidepressants on Specific Binding of ^{3}H-Mianserin

Antidepressant	K_i (nM)
Mianserin	1.2
Imipramine	5.0
Chlorimipramine	7.2
Trazodone	16.7
Amitriptyline	26.0
Nortriptyline	28.0
Maprotiline	38.6
Protriptyline	71.0
Nomifensine	200.0

Saturation analysis revealed the number of binding sites (B_{max}) to be 139 fmol/mg protein and the K_D to be 0.73 nM. A single class of binding sites was observed.

The most interesting finding is the high potency of a wide range of antidepressants at competing for bound ^{3}H-mianserin. This includes the tricyclics as well as novel antidepressants such as trazodone, nomifensine and maprotiline.

Although a number of studies on tritiated anti-depressant binding have been done in the past [8,9,10] , this is the first study to show potent binding inhibition by a variety of anti-depressant structures including the atypical antidepressants. A possible explanation is that previously studied anti-depressants were all tricyclics, which are known to label more than one site including a muscarinic cholinergic site. This is evident in their clinically observed anticholinergic side effects. Mianserin, on the other hand, has no anticholinergic side effects in clinical use [6,12] and based on our results is binding to only one site, although saturation analysis would not distinguish between sites with similar K_D's. Based on our low K_i values, this site labelled by ^{3}H-mianserin is shared by all the antidepressants tested.

It is possible that this is a specific antidepressant binding site, similar to the specific site for benzodiazepines [13] however it is also possible that the site or sites being labelled is specific to a neurotransmitter or to a group of neurotransmitters. From Table 1 the neurotransmitter receptor most likely involved would be one for serotonin. The K_i values for serotonergic agents given in Table 2 further support this suggestion in that both tryptamines and ergots are effective at competing for the bound ^{3}H-mianserin. The apparent K_D of this site for serotonin would be 390 nM, which differs from previously reported values in ^{3}H-serotonin [4] or ^{3}H-d-LSD [7] binding. Although we found spiroperidol to have a K_i in ^{3}H-mianserin binding of 1.6 nM, the K_i of serotonin is much lower in ^{3}H-mianserin binding than in ^{3}H-spiroperidol binding in cortex [14] (390 nM us 2700 nM). This suggests that the site labelled by mianserin is not identical to that labelled by other serotonergic ligands.

There are other neurotransmitter receptors which must also be considered as potential sites of ^{3}H-mianserin binding. From data in Table 1, it is possible that adrenergic sites could be involved. This would agree with data showing that tricyclic antidepressants compete for α-adrenergic receptors labelled by ^{3}H-WB-4101 [15] . Mianserin itself has been shown to block pre-synaptic α-adrenergic receptors although this effect was not shared by other antidepressants [16] .

The other site possibly being labelled is a histamine receptor. There have been reports showing that tricyclic antidepressants inhibit histamine-stimulated adenylate cyclase [17,18] , compete for ^{3}H-mepyramine binding to H_1 sites in mammalian brain, [19] and block histamine effects in guinea pig ileum [20] . Although we have found histamine itself to be ineffective at competing for ^{3}H-mianserin binding, we have found at least one antihistamine, promethazine, to have a potent effect (K_i = 32 nM).

In summary, if ^{3}H mianserin is labelling one specific type of neurotransmitter receptor

it would appear that the receptor is mostly serotonergic in binding characteristics. The possibility that a common site of action of antidepressants is on serotonergic receptors is not new and has support from a variety of other experimental procedures [21,22,23]. It remains possible, however, that ^{3}H-mianserin is labelling a collection of receptor types and that the other neurotransmitter receptors involved are histaminergic and α-adrenergic.

We are currently characterizing further the sites labelled by ^{3}H-mianserin to determine their distribution throughout various brain regions and in subcellular fractions. We are also establishing a more precise pharmacological profile which may correlate with a known physiological function of serotonin and thus establish with some certainty a site of action of antidepressants.

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