[^{3}H]Mianserin: Differential Labeling of Serotonin$_2$ and Histamine$_1$ Receptors in Rat Brain[1]

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Accepted for publication October 14, 1980

ABSTRACT

Peroutka, Stephen J. and Solomon H. Snyder: *[^{3}H]Mianserin: Differential labeling of serotonin$_2$ and histamine$_1$ receptors in rat brain.* J. Pharmacol. Exp. Ther. **216:** 142–148, 1981.

Mianserin, a tetracyclic antidepressant, is a potent serotonin (5-HT) and histamine H_1 antagonist in peripheral smooth muscle systems. Mianserin was found to possess high affinity for 5-HT_2 and histamine H_1 receptor binding sites in brain membranes. By using [^{3}H]mianserin, both 5-HT_2 and histamine H_1 receptors can be specifically labeled in rat cerebral cortex membranes. Simultaneous incubation of brain membranes with 300 nM triprolidine or 30 nM spiroperidol enables the selective labeling of 5-HT_2 or histamine H_1 receptors, respectively. In the guinea-pig cerebellum, [^{3}H]mianserin exclusively labels histamine H_1 receptors, since 5-HT_2 sites are virtually absent in this area.

Mianserin is a tetracyclic piperazine derivative that was developed as an antiallergic compound (van der Burg *et al.*, 1970). In peripheral smooth muscle systems, it displays potent antiserotoninergic and antihistaminic activity as well as some α-adrenolytic effects (Vargaftig *et al.*, 1971). Mianserin is also a clinically effective antidepressant (Itil *et al.*, 1972; Fell *et al.*, 1973) despite relatively weak inhibition of norepinephrine and serotonin reuptake (Kofoe and Leonard, 1973; Zis and Goodwin, 1979), failure to reverse reserpine-induced hypothermia (van Riezen, 1972) and inability to block monoamine oxidase (Fell *et al.*, 1973). Although central physiological effects of mianserin have been reported (Maj *et al.*, 1978), mianserin's interactions with neurotransmitter receptor sites have not been thoroughly evaluated.

Two distinct serotonin (5-HT) receptor sites can be labeled in brain membranes (Peroutka and Snyder, 1979). Receptors referred to as 5-HT_1 are labeled by [^{3}H]-5-HT, regulated by guanine nucleotides and appear to be linked to the 5-HT sensitive adenylate cyclase in brain membranes (Peroutka *et al.*, 1979, 1980). 5-HT_2 receptors, labeled by [^{3}H]spiroperidol, mediate the behavioral syndrome associated with central serotoninergic stimulation (Jacobs, 1976) since the potencies of numerous drugs in preventing the behavioral effects correlate closely with their affinities for 5-HT_2, but not 5-HT_1 receptors (Peroutka *et al.*, 1980).

There are some serious limitations in the use of [^{3}H]spiroperidol for labeling 5-HT_2 receptors. In the corpus striatum, only a small portion of [^{3}H]spiroperidol binding labels 5-HT_2 receptors as most of the binding is associated with dopamine receptors (Leysen *et al.*, 1978; Creese and Snyder, 1978; Peroutka and Snyder, 1980). Moreover, in portions of the limbic system [^{3}H]spiroperidol binding is largely associated with an anomalous site with selectivity for the spirodecanone structure (Howlett *et al.*, 1979). Because of these problems, we explored [^{3}H]mianserin as an alternative ligand for 5-HT_2 receptors.

We now report that [^{3}H]mianserin does label 5-HT_2 receptors in rat brain. Additionally, [^{3}H]mianserin binds to histamine H_1 receptors with a selectivity similar to that of [^{3}H]mepyramine (Tran *et al.*, 1978; Hill *et al.*, 1978). The binding of [^{3}H]mianserin to 5-HT_2 and histamine H_1 receptors can be differentiated by pharmacologic or anatomic manipulations.

Materials and Methods

Animals. Adult male Sprague-Dawley rats were obtained from Sprague-Dawley Laboratories (Madison, WI) and were used at weights of 150 to 200 g. Rats were maintained under a 12-hr light-dark cycle and had free access to food and water. After decapitation, brains were removed and specific regions dissected over ice immediately before assay. Frozen guinea-pig brains were purchased from Pel-Freez Biologicals, Inc. (Rogers, AK) and were defrosted at room temperature in saline before use.

Binding assays. Rat frontal cerebral cortices or guinea-pig cerebella were homogenized in Tris-HCl buffer (pH 7.7 at 25°C) using a Brinkmann Polytron for 10 sec. The homogenate was centrifuged at 50,000 × *g* for 10 min, resuspended in Tris-HCl buffer and centrifuged again at 50,000 × *g* for 10 min. The pellet was suspended in the standard assay buffer (Tris-HCl buffer and 0.1% ascorbic acid) and stored on ice until used.

Incubation tubes received 0.1 ml of ^{3}H-ligand, 0.1 ml of various drugs and 0.8 ml of tissue suspension. All assays were performed in triplicate.

Received for publication April 1, 1980.

[1] This work was supported by U.S. Public Health Service Grants DA-00266, MH-18501 and a grant of the McKnight Foundation.

[2] Recipient of Medical Scientist Training Grant 5T32GM0309 from the National Institutes of Health.

The final tissue concentration was 10 mg of wet weight tissue per ml for all brain regions except caudate (3.5 mg of wet weight tissue per ml). After incubation at 37°C for 15 min, the samples were rapidly filtered under vacuum through Whatman GF/B filters with three 5-ml rinses of ice-cold Tris-HCl buffer (pH 7.7 at 25°C). The filters were counted by liquid scintillation spectrometry in 10 ml of Formula 947 (New England Nuclear; Boston, MA) after 18 hr extraction at 4°C.

Drugs. [^{3}H]Mianserin (16 Ci/mmol) was purchased from Amersham (Arlington Heights, IL). All other ^{3}H-ligands were obtained from New England Nuclear. ^{3}H-ligands were stored at −20°C and diluted in standard assay buffer immediately before assay. Unlabeled drugs were obtained from the following sources: mianserin (Organon, Amsterdam, Holland), cyproheptadine (Merck Sharp & Dohme; Rahway, NJ), *d*- and *l*-chlorpheniramine (Smith Kline and French Laboratories, Philadelphia, PA), triprolidine (Burroughs-Wellcome, Research Triangle Park, NC), iprindole (Wyeth Laboratories, Philadelphia, PA), *d*-lysergic acid diethylamide (LSD) (National Institute on Drug Abuse), 5-HT (Sigma Chemical Co., St. Louis, MO), spiroperidol (Janssen Pharmaceutica, Beerse, Belgium) and cinanserin (Squibb, Princeton, NJ).

Results

Influence of mianserin on various neurotransmitter receptor binding sites. Mianserin has been reported to possess blocking activity at 5-HT, histamine and *alpha* adrenergic receptors in peripheral smooth muscle systems (Vargaftig *et al.*, 1971). To evaluate the influence of mianserin on neurotransmitter receptors in the brain, we determined its potency in competing for the binding of various ^{3}H-ligands (table 1). Mianserin is most potent at 5-HT$_2$ receptors labeled by [^{3}H]spiroperidol and histamine H$_1$ receptors labeled by [^{3}H]mepyramine with K$_i$ values of 2.4 and 3.3 nM, respectively. In contrast to its high affinity for 5-HT$_2$ receptors, mianserin is about 400 times less potent at 5-HT$_1$ receptors labeled by [^{3}H]-5-HT (860 nM). Unlike spiroperidol, which has equally high affinity for both dopamine and 5-HT$_2$ receptors, mianserin is 1000 times less potent at dopamine receptors labeled by [^{3}H]spiroperidol in the caudate (2200 nM) than at 5-HT$_2$ receptors labeled by [^{3}H]-spiroperidol in the cerebral cortex. Mianserin also displays substantial affinity for *alpha*-2 receptors labeled by *p*-[^{3}H]aminoclonidine (19 nM) and *alpha*-1 sites labeled by [^{3}H]-WB-4101 (63 nM), although these effects are about an order of magnitude less than its affinity for 5-HT$_2$ and histamine H$_1$ sites. Mianserin is quite weak at muscarinic cholinergic, *beta* adrenergic, opiate and benzodiazepine receptor binding sites.

TABLE 1

Apparent affinity (K$_i$) of mianserin for neurotransmitter receptor binding sites

Standard assay conditions were used in all experiments. The ^{3}H-ligand concentrations were 0.25 nM [^{3}H]spiroperidol, 2.0 nM [^{3}H]-5-HT, 2.0 nM [^{3}H]mepyramine, 0.6 nM *p*-[^{3}H]aminoclonidine, 0.9 nM [^{3}H]-WB-4101, 0.2 nM [^{3}H]quinuclidinyl benzilate (QNB), 0.8 nM [^{3}H]dihydroalprenolol, 1.9 nM [^{3}H]naloxone, 1.4 nM *d*-[^{3}H]-ala-leu-enkephalin and 0.7 nM [^{3}H]flunitrazepam. Specific binding was defined as the excess over blanks taken in the presence of 1 μM *d*-LSD for 5-HT receptors, 1 μM triprolidine for histamine receptors,0.1 mM oxotremorine for muscarinic cholinergic receptors, 1 μM (±)-propranolol for *beta* adrenergic receptors, 1 μM levallorphan for opiate receptors, 1 μM diazepam for benzodiazepine receptors and 10 μM (−)-norepinephrine for *alpha*-1 and *alpha*-2 receptors. IC$_{50}$ values were determined from log-probit analysis of drug competition data using 4 to 5 concentrations of mianserin. K$_i$ values were calculated from the equation $K_i = IC_{50}/(1 + [^3H\ \text{ligand}]/K_D)$. The K$_D$ values were obtained from previous reports (Bennett, 1978; Peroutka and Snyder, 1979). Values given are the means ± S.E. of three or more experiments, each performed in triplicate.

Receptor	^{3}H-Ligand	Apparent K$_i$
		nM
5-HT$_2$	[^{3}H]Spiroperidol (cortex)	2.4 ± 0.51
5-HT$_1$	[^{3}H]-5-HT	860 ± 110
Histamine H$_1$	[^{3}H]Mepyramine	3.3 ± 0.63
Alpha-2 adrenergic	*p*-[^{3}H]Aminoclonidine	19 ± 5.0
Alpha-1 adrenergic	[^{3}H]-WB 4101	63 ± 7.4
Muscarinic cholinergic	[^{3}H]-QNB	2,100 ± 380
Dopamine	[^{3}H]Spiroperidol (caudate)	2,200 ± 610
Beta adrenergic	[^{3}H]Dihydroalprenolol	4,400 ± 910
Opiate	[^{3}H]Naloxone	10,000 ± 1,700
Opiate	*d*-[^{3}H]-Ala-leu-enkephalin	12,000 ± 2,000
Benzodiazepine	[^{3}H]Flunitrazepam	>100,000

Properties of [^{3}H]mianserin binding to rat cerebral cortex membranes. The binding of [^{3}H]mianserin to brain membranes is saturable (fig. 1). Specific binding accounts for approximately 50% of total binding at a concentration of 0.7 nM, the amount of [^{3}H]mianserin used in routine drug competition studies. At ligand concentrations above 1.0 nM, specific binding represents a decreasing percentage of total binding and is only 25% of total binding at 10 nM [^{3}H]mianserin. Specific [^{3}H]mianserin binding reaches plateau levels at about 6 nM and attains half maximal binding at approximately 3 nM. By contrast, nonspecific binding increases linearly with increasing [^{3}H]mianserin concentrations. This nonspecific binding is not altered by 30 nM spiroperidol or 300 nM triprolidine, which are employed (below) to differentiate [^{3}H]mianserin interactions with histamine H$_1$ and 5-HT$_2$ receptors. Scatchard analysis indicates the presence of a single population of binding sites with an apparent dissociation constant (K$_D$) of 3.7 nM.

Specific [^{3}H]mianserin binding is linear between 2 and 20 mg/ml of brain membrane, with all experiments performed using 10 mg wet weight tissue per ml. The specific binding of [^{3}H]mianserin displays a relatively broad pH optimum with about the same levels of binding between pH 7 and 8. The amount of [^{3}H]mianserin binding obtained in standard Tris-HCl assay buffer is not significantly altered by the addition of 4 mM calcium chloride, 100 mM sodium ions or 10 mM potassium ions.

Kinetics of [^{3}H]mianserin binding. Specific [^{3}H]mianserin binding is time-dependent (fig. 2A). At 37°C, binding plateaus between 7 and 30 min and attains half-maximal binding at approximately 1 min. The rate constant for association (k$_1$) is 0.10 nM^{-1}min^{-1} at 37°C determined from the equation

$$k_1 = \frac{k_{obs} - k_{-1}}{[L]}$$

where k_{-1} equals the first order dissociation rate constant, [L] equals the steady-state free ligand concentration and k_{obs} is the observed rate of association.

To determine the dissociation rate of specifically bound [^{3}H]-mianserin, we conducted incubations to equilibrium, at which time 1 μM cyproheptadine was added and residual binding was examined at various intervals. The dissociation appears to be a first-order process, as represented by a straight line when plotted on a semi-logarithmic scale (fig. 2B). The half-time for dissociation is about 2.5 min and the rate constant for dissociation (k_{-1}) is 0.31 min^{-1}. The apparent dissociation constant (K$_D$) determined from the ratio k_{-1}/k_1 is 3.1 nM, which agrees well with the K$_D$ value determined in saturation experiments.

Displacement of [^{3}H]mianserin binding by 5-HT and histamine-related agents. Displacement of specifically bound [^{3}H]mianserin by the antihistamines *d*-chlorpheniramine and triprolidine and by the 5-HT-related agents *d*-LSD and spiroperidol yield extremely shallow displacement curves (fig. 3A). At least five orders of magnitude are required to displace 90% of the bound [^{3}H]mianserin. Clearcut biphasic displacement is apparent for triprolidine, whose displacement

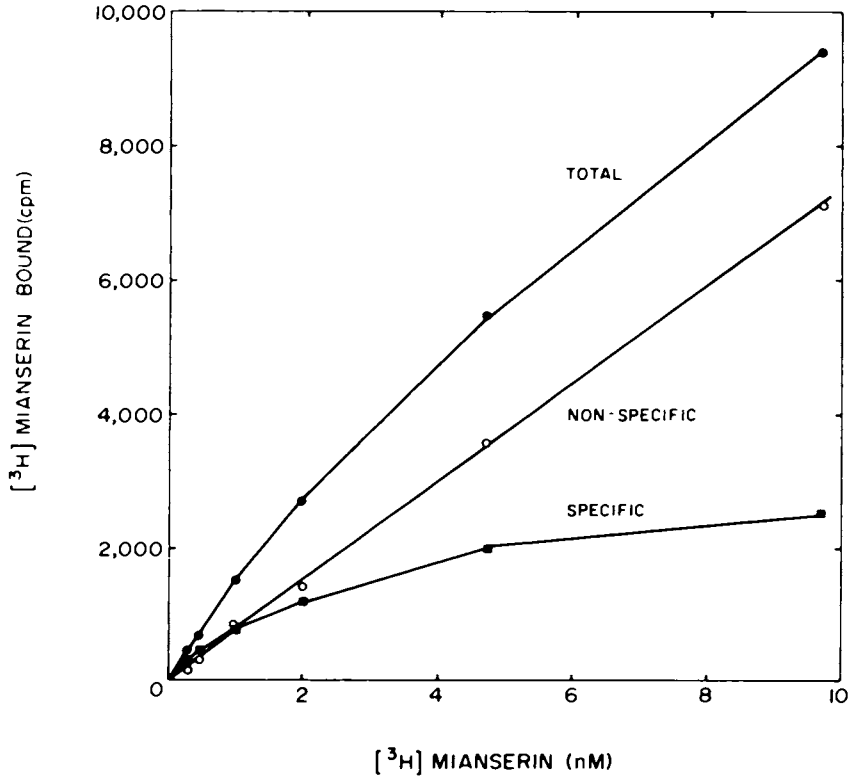

Fig. 1. [^{3}H]Mianserin binding as a function of increasing concentrations of [^{3}H]mianserin. Rat frontal cerebral cortex membranes were incubated under standard binding assay conditions with increasing concentrations of [^{3}H]mianserin. Nonspecific binding was defined as the amount of [^{3}H]mianserin bound in the presence of 1 μM cyproheptadine. Specific binding represents the difference between total and nonspecific binding. The experiment was replicated three times.

curve is essentially flat between 0.1 and 1.0 μM concentrations. Hill coefficients for *d*-chlorpheniramine, triprolidine, *d*-LSd and spiroperidol are 0.33, 0.23, 0.52 and 0.32, respectively.

The shallow displacement curves and similar potencies of 5-HT and histamine antagonists could indicate that [^{3}H]mianserin binds to a unique receptor site displaying considerable cooperativity. Alternatively, it is possible that [^{3}H]mianserin labels both 5-HT$_2$ and histamine H$_1$ receptors. The low Hill coefficients may reflect drug competition for the binding of [^{3}H]-mianserin to the two receptor sites with markedly different affinities. If this were the case, it might be possible to obtain [^{3}H]mianserin binding selectively to 5-HT$_2$ receptors if the binding to histamine H$_1$ sites were pharmacologically blocked. Accordingly, we examined the displacement of [^{3}H]mianserin binding in the presence of 300 nM triprolidine, a concentration of unlabeled antagonist which saturates all H$_1$ receptors (Tran *et al.*, 1978). Under these conditions, *d*-LSD and spiroperidol become substantially more potent with about 50% inhibition of binding at 10 and 3 nM, respectively (fig. 3B). Moreover, *d*-chlorpheniramine and triprolidine became much weaker in inhibiting [^{3}H]mianserin binding with half-maximal displacement at approximately 10 μM. For all four drugs, the Hill coefficients are significantly increased in the presence of 300 nM triprolidine to about 1.0.

Conversely, possible labeling of histamine H$_1$ receptors by [^{3}H]mianserin should be apparent in the presence of 30 nM spiroperidol, a concentration sufficient to fully occupy 5-HT$_2$ receptors (Peroutka and Snyder, 1979). Under these conditions, *d*-chlopheniramine and triprolidine become substantially more potent with 50% displacement at 5 to 8 nM for both drugs (fig. 3C). Moreover, displacement curves for the antihistamines are significantly steeper in the presence of 30 nM spiroperidol with Hill coefficients of about 1.0. At the same time, the potencies of *d*-LSD and spiroperidol are decreased while their Hill coefficients are raised to 1.0.

The 50% decrease in [^{3}H]mianserin binding in the presence of 300 nM triprolidine or 30 nM spiroperidol derives from 50% site occupancy and therefore should alter the apparent B_{max} with no change in apparent K_D. To test this prediction, we conducted saturation studies of [^{3}H]mianserin in the presence of 300 nM triprolidine or 30 nM spiroperidol (fig. 4). Addition of triprolidine or spiroperidol elicits no change in K_D of specific [^{3}H]mianserin binding. However, both drugs reduce the maximal number of binding sites (B_{max}) by approximately 50%. Thus, total specific [^{3}H]mianserin binding in the rat cerebral cortex appears to be a composite of the labeling of two distinct sites, half of which involve 5-HT$_2$ receptors and the other half histamine H$_1$ receptors. The B_{max} values for apparent 5-HT$_2$ and histamine H$_1$ sites (13.7 and 13.3 pmol/g of wet weight tissue) determined in this way agree well with parallel determinations using [^{3}H]mepyramine for histamine H$_1$ sites (12.8 pmol/g) and [^{3}H]spiroperidol for 5-HT$_2$ receptors (14 pmol/g).

Differential effects of drugs on [^{3}H]mianserin binding in the presence or absence of 300 nM triprolidine or 30 nM spiroperidol. To further determine whether the binding sites labeled by [^{3}H]mianserin represent both 5-HT$_2$ and histamine H$_1$ receptors, we evaluated a series of 5-HT- and histamine-related agents (table 2). We compared drug potencies in inhibiting [^{3}H]mianserin binding in the absence and presence of 300 nM triprolidine or 30 nM spiroperidol. These values were also compared to the drug potencies at [^{3}H]spiroperidol labeled 5-HT$_2$ receptors and [^{3}H]mepyramine labeled H$_1$ receptors in the rat cerebral cortex.

Mianserin and cyproheptadine are potent blockers of both 5-

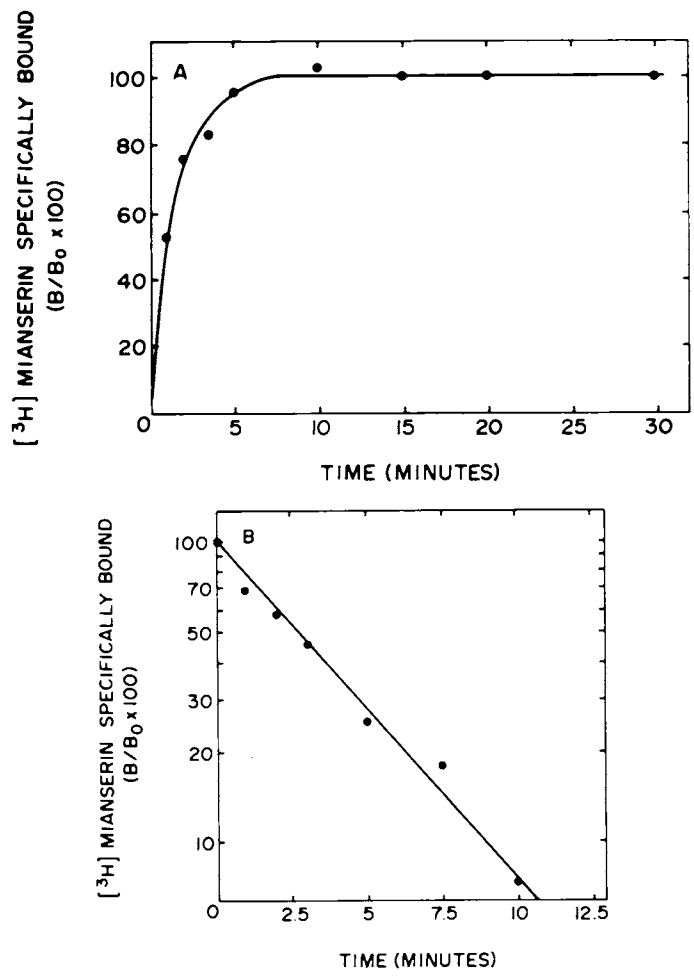

Fig. 2. Kinetics of [^{3}H]mianserin binding. A, association of [^{3}H]mianserin and its receptor at 37°C as a function of time. Specific binding was defined as the excess over blanks taken in the presence of 1 μM cyproheptadine during standard binding assay conditions. The experiment was replicated three times with values that varied less than 10%. B, dissociation of [^{3}H]mianserin from its receptor at 37°C. The sample volumes were incubated for 15 min at 37°C under standard binding assay conditions at which time 1 μM cyproheptadine was added (time zero) to initiate dissociation. The samples were then filtered at various time intervals. The experiment was replicated three times.

HT_2 and histamine H_1 receptors as shown by their high affinities for [^{3}H]spiroperidol and [^{3}H]mepyramine binding, respectively. These two drugs also have nanomolar affinity for [^{3}H]-mianserin binding whether assayed alone or in the presence of triprolidine or spiroperidol. By contrast, *d*-chlorpheniramine has 1000 times greater affinity for histamine receptors labeled by [^{3}H]mepyramine (8.0 nM) than for 5-HT_2 sites labeled by [^{3}H]spiroperidol (8200 nM). Its potency in reducing [^{3}H]mianserin binding (260 nM) is approximately the logarithmic mean between its affinity for 5-HT_2 and histamine H_1 receptor binding sites. However, in the presence of 300 nM triprolidine, *d*-chlorpheniramine has about the same affinity (4200 nM) for [^{3}H]-mianserin as for [^{3}H]spiroperidol binding while, in the presence of 30 nM spiroperidol, the affinity of *d*-chlorpheniramine (7.8 nM) approximates its affinity for [^{3}H]mepyramine binding. An even greater differentiation of 5-HT_2 and histamine H_1 sites is obtained with triprolidine which has over 2000 times greater affinity for [^{3}H]mepyramine (4.7 nM) than for [^{3}H]spiroperidol (11,000 nM) binding sites. Similarly, the affinity of triprolidine varies more than 2000-fold between its affinity for [^{3}H]mianserin sites in the presence of 300 nM triprolidine (11,000 nM) and in the presence of 30 nM spiroperidol (5.0 nM). The same pattern of potency differences also occurs with *d*-chlorpheniramine and the antidepressant iprindole.

A reciprocal pattern occurs with drugs active at 5-HT receptors. *d*-LSD is only one-fortieth as potent at [^{3}H]mepyramine (390 nM) as at [^{3}H]spiroperidol (10 nM) sites. Its potency at [^{3}H]mianserin sites in the presence of 300 nM triprolidine (12 nM) resembles its effects at 5-HT_2 receptors while it competes for [^{3}H]mianserin binding in the presence of 30 nM spiroperidol (180 nM) with a potency similar to its affinity for histamine H_1 receptors. This pattern of differential drug potencies also occurs with 5-HT itself and the 5-HT antagonist cinanserin. Of the

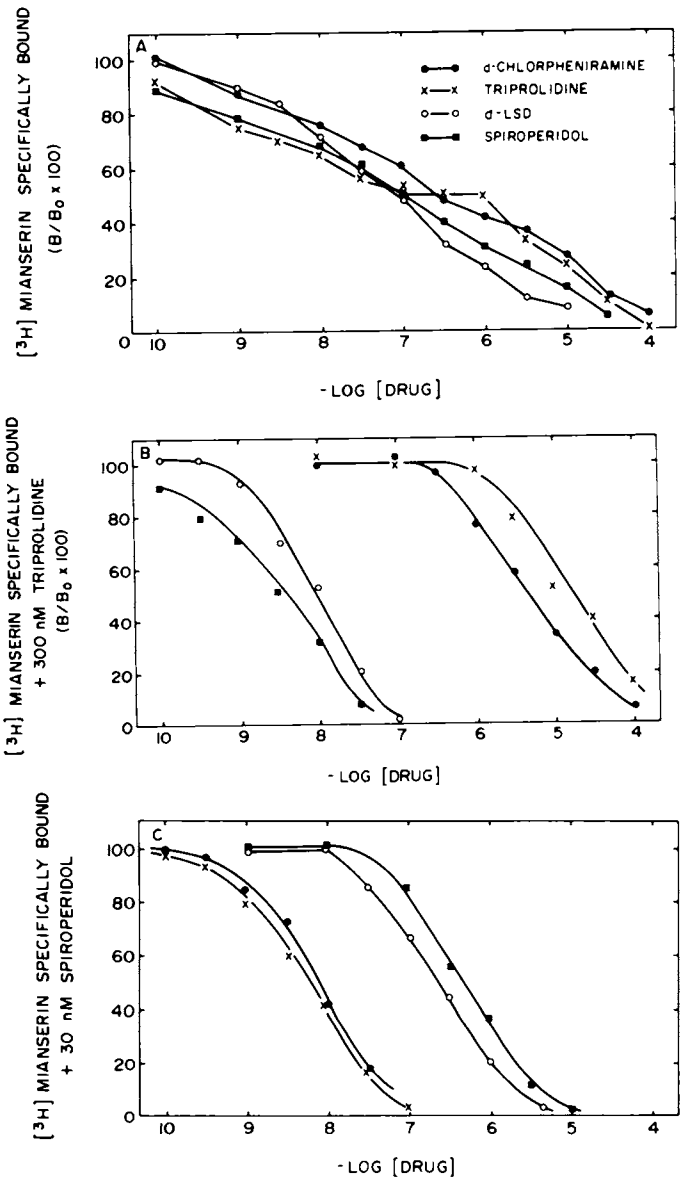

Fig. 3. Inhibition of [^{3}H]mianserin binding in rat cerebral cortex membranes. Increasing concentrations of drugs were incubated under standard assay conditions with [^{3}H]mianserin. Data shown are the means of triplicate assays performed in a single experiment. The experiment was replicated 3 to 5 times. A, inhibition of [^{3}H]mianserin binding. B, inhibition of [^{3}H]mianserin binding in the presence of 300 nM triprolidine. C, inhibition of [^{3}H]mianserin binding in the presence of 30 nM spiroperidol.

drugs tested, spiroperidol is best able to differentiate 5-HT_2 and histamine H_1 receptors. Its affinities for [^{3}H]spiroperidol (1.0 nM) and [^{3}H]mepyramine (670 nM) are approximated by spiroperidol's affinity for [^{3}H]mianserin sites in the presence of triprolidine (3.7 nM) or spiroperidol (570 nM).

The differential effects of the 5-HT_2 and histamine H_1 selective drugs indicate that the binding sites labeled by [^{3}H]mianserin represent both 5-HT_2 and histamine H_1 receptors in rat frontal cerebral cortex.

Specific [^{3}H]mianserin binding in the guinea-pig cerebellum. It would be desirable to evaluate [^{3}H]mianserin binding to 5-HT or histamine receptors in isolation. Histamine H_1 receptors are relatively ubiquitous in the brains of various species, including the guinea pig (Chang *et al.*, 1979). By contrast, preliminary studies have shown that [^{3}H]spiroperidol-labeled 5-HT_2 receptors display marked regional variations with little, if any, binding in the guinea-pig cerebellum (S. J. Peroutka and S. H. Snyder, manuscript in preparation). Accordingly, we evaluated the saturation of [^{3}H]mianserin binding to membranes prepared from guinea-pig cerebellum (fig. 5). Specific [^{3}H]mianserin binding to guinea-pig cerebellum is triple nonspecific binding and is saturable with a K_D value of 2.4 nM for a single population of binding sites. The B_{max} for [^{3}H]-mianserin (23.1 pmol/g of wet weight tissue) is the same as the B_{max} in the same tissue determined in parallel for [^{3}H]mepyramine labeling of histamine H_1 sites (21 pmol/g). In marked contrast to the results obtained in rat cerebral cortex, [^{3}H]-mianserin binding in guinea-pig cerebellum is totally abolished by 300 nM triprolidine, while 30 nM spiroperidol has no effect on either K_D or B_{max} values. These results suggest that [^{3}H]-mianserin exclusively labels histamine H_1 receptors in this brain region.

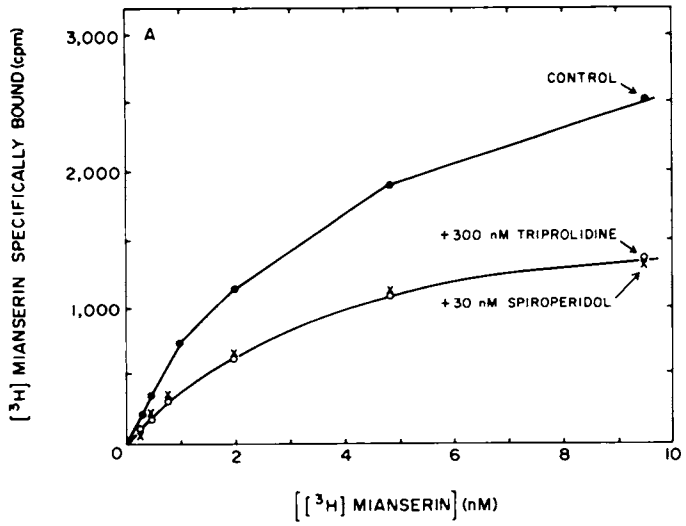

Fig. 4. Saturation of [^{3}H]mianserin binding in rat frontal cerebral cortex membranes. At each [^{3}H]mianserin concentration, total binding was measured in the absence of inhibitor (●), in the presence of 300 nM triprolidine (○) or in the presence of 30 nM spiroperidol (×). Specific binding was defined as the excess over blanks taken in the presence of 1 μM cyproheptadine. Points shown are the means of triplicate assays performed in a single experiment. The experiment was performed three times with values which varied less than 15%.

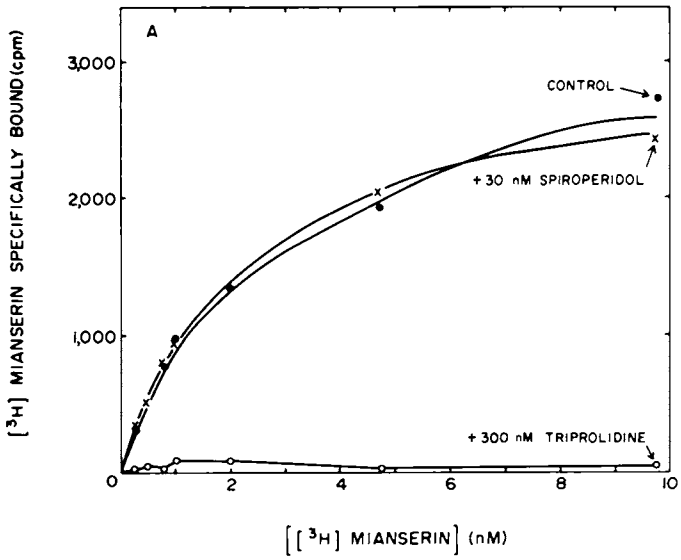

Fig. 5. Saturation of [^{3}H]mianserin binding in guinea-pig cerebellar membranes. At each [^{3}H]mianserin concentration, total binding was measured in the absence of inhibitor (●), in the presence of 300 nM triprolidine (○) and in the presence of 30 nM spiroperidol (×). Specific binding was defined as the excess over blanks taken in the presence of 1 μM cyproheptadine. Points shown are the means of triplicate assays performed in a single experiment. The experiment was replicated three times with values which varied less than 20%.

TABLE 2

Drug affinities for ^{3}H-ligand binding sites in rat frontal cerebral cortex

Binding was conducted as described in "Materials and Methods." Values are means ± S.E.M. for three to six independent IC_{50} determinations. Each IC_{50} value was obtained from log-probit analysis of the effects of four to six drug concentrations.

Drug	IC_{50}				
	[^{3}H]Spiroperidol	[^{3}H]Mianserin + 300 nM Triprolidine	[^{3}H]Mianserin	[^{3}H]Mianserin + 30 nM Spiroperidol	[^{3}H]Mepyramine
	nM	*nM*	*nM*	*nM*	*nM*
5-HT_2 and H_1 active					
Mianserin	2.5 ± 0.51	7.9 ± 1.1	2.4 ± 0.53	5.8 ± 0.14	3.3 ± 0.63
Cyproheptadine	3.0 ± 0.27	4.2 ± 1.6	3.7 ± 0.25	5.8 ± 1.5	4.1 ± 0.81
H_1 selective					
d-Chlorpheniramine	8,200 ± 2,400	4,200 ± 1,300	260 ± 70	7.8 ± 1.5	8.0 ± 2.7
l-Chlorpheniramine	14,000 ± 2,000	12,000 ± 1,400	3,500 ± 700	790 ± 250	700 ± 400
Triprolidine	11,000 ± 1,500	11,000 ± 820	190 ± 85	5.0 ± 1.6	4.7 ± 1.2
Iprindole	2,900 ± 640	2,000 ± 500	580 ± 49	370 ± 200	200 ± 51
5-HT_2 selective					
d-LSD	10 ± 3.6	12 ± 3.0	70 ± 7.9	180 ± 16	390 ± 23
5-HT	3,200 ± 610	1,000 ± 360	22,000 ± 5,000	35,000 ± 6,600	90,000 ± 25,000
Cinanserin	36 ± 9.2	75 ± 2.6	300 ± 89	1,100 ± 190	1,000 ± 210
Spiroperidol	1.0 ± 0.12	3.7 ± 0.22	44 ± 19	570 ± 76	670 ± 120

To further examine this possibility, we analyzed displacement curves of *d*-chlorpheniramine, triprolidine, *d*-LSD and spiroperidol in the guinea-pig cerebellum (fig. 6). *d*-Chlorpheniramine and triprolidine potently displace [^{3}H]mianserin binding with IC_{50} values between 1 and 5 nM. By contrast, *d*-LSD and spiroperidol are much weaker displacing agents, requiring about 1 μM concentrations to displace 50% of [^{3}H]mianserin binding. Additionally, the displacement curves for each of these drugs are monophasic with Hill coefficients of approximately 1.0.

Relative affinities of various drugs for guinea-pig cerebellum binding sites of [^{3}H]mianserin also confirm the notion that [^{3}H]mianserin selectively labels histamine H_1 receptors in this brain region (table 3). For all classes of drugs tested, potencies in reducing [^{3}H]mianserin binding in guinea-pig cerebellum closely resemble potencies at sites in rat cerebral cortex labeled by [^{3}H]mepyramine or by [^{3}H]mianserin in the presence of 30 nM spiroperidol. Interestingly, mianserin, cyproheptadine, *d*- and *l*-chlorpheniramine and triprolidine are somewhat more potent in guinea-pig cerebellum than in rat cortex, a characteristic noted in earlier studies (Chang *et al.*, 1979).

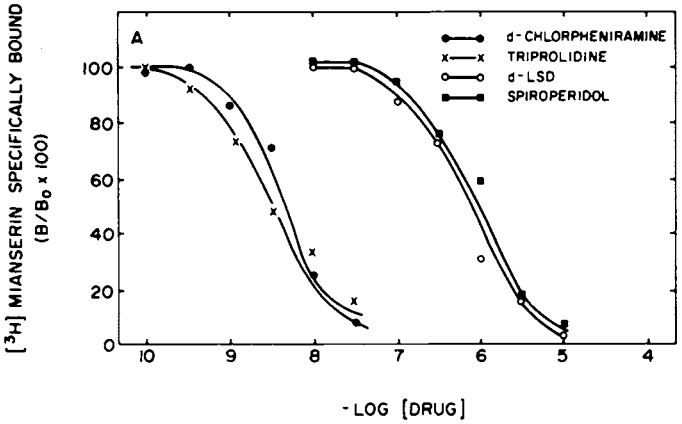

Fig. 6. Displacement of [^{3}H]mianserin binding to guinea-pig cerebellar membranes by *d*-chlorpheniramine (●), triprolidine (×), *d*-LSD (○) and spiroperidol (■). The experiment was performed three times with results varying less than 20%.

TABLE 3

Drug affinities for ^{3}H-ligand binding in guinea-pig cerebellum

Binding was assayed as described in "Materials and Methods." Values are means ± S.E.M. for three to six independent IC_{50} determinations. Each IC_{50} value was obtained from log-probit analysis using four to seven drug concentrations.

Drug	IC_{50}	
	[^{3}H]Mepyramine	[^{3}H]Mianserin
	nM	
5HT$_2$ and H$_1$ active		
Mianserin	2.1 ± 0.72	2.8 ± 0.66
Cyproheptadine	1.2 ± 0.24	1.8 ± 0.24
H$_1$ selective		
d-Chlorpheniramine	2.4 ± 0.8	2.7 ± 0.26
l-Chlorpheniramine	230 ± 50	230 ± 48
Triprolidine	1.2 ± 0.3	2.5 ± 0.86
Iprindole	530 ± 76	430 ± 27
5-HT$_2$ selective		
d-LSD	480 ± 110	570 ± 130
5-HT	6000 ± 950	5500 ± 200
Cinanserin	4200 ± 640	35100 ± 110
Spiroperidol	3200 ± 120	1700 ± 27

Discussion

The present study demonstrates that [^{3}H]mianserin has high affinity for both 5-HT$_2$ and histamine H_1 receptors in brain membranes. Central anti-5-HT effects of mianserin have been reported on the basis of its blockade of 5-hydroxytryptophan-induced head twitches (van Riezen, 1972; Maj *et al.*, 1978) and inhibition of 5-HT-induced hyperthermia (Maj *et al.*, 1978). Mianserin also causes a uniquely complex set of electroencephalographic changes that may relate to central neurotransmitter interactions (Itil *et al.*, 1972). Clinically, the sedative side-effects of mianserin (Fell *et al.*, 1973) may be consistent with its potency at central α-noradrenergic receptors (U'Prichard *et al.*, 1978). The present study provides the first direct demonstration of the interactions of mianserin with central nervous system receptors.

As a radioligand, [^{3}H]mianserin may prove useful in 5-HT$_2$ and histamine H_1 receptor binding studies. All the binding of [^{3}H]mianserin observed here can be accounted for by histamine H_1 and 5-HT$_2$ receptors. No selective reduction of binding by *alpha*-1 or *alpha*-2 drugs is demonstratable (S. J. Peroutka, unpublished observations). Until the present time, [^{3}H]spiroperidol was the only ligand available for the study of 5-HT$_2$, but not 5-HT$_1$ receptors. However, in brain regions such as the corpus striatum, nucleus accumbens and olfactory tubercle, the extremely high density of dopamine receptors make it technically difficult to study selectively [^{3}H]spiroperidol-labeled 5-HT$_2$ receptors. In addition, [^{3}H]spiroperidol may bind to a third, as yet undefined, receptor site that has a selective high affinity for the spirodecanone class of drugs (Howlett *et al.*, 1979). By contrast, when assayed in the presence of 300 nM triprolidine, [^{3}H]mianserin appears to label 5-HT$_2$ binding sites exclusively. In the present study, only about 50% of total [^{3}H]mianserin binding is specific. This limitation is related to the low specific activity (16 Ci/mmol) of the ^{3}H-ligand used in this study. With new preparations of [^{3}H]mianserin with higher specific activity, up to 75% of total binding is associated with 5-HT and histamine receptors (S. J. Peroutka, unpublished observations).

[^{3}H]Mianserin has proved valuable in assessing relative amounts of 5-HT$_2$ receptor binding in different brain regions, especially those in which [^{3}H]spiroperidol binding largely involves sites other than 5-HT$_2$ receptors (Peroutka and Snyder, 1980). Numbers of 5-HT$_2$ receptors quantified with [^{3}H]mianserin are similar to levels obtained using [^{3}H]LSD with appropriate displacers.

[^{3}H]Mianserin represents the first labeled antidepressant that has been demonstrated to bind to previously characterized neurotransmitter receptor sites. Raisman *et al.* (1979) obtained specific high affinity binding of [^{3}H]imipramine that displays a unique pharmacological profile which does not fit with any known receptor site nor with the equipotent clinical effects of antidepressants. [^{3}H]Desipramine binding displays biphasic saturation and a unique pharmacological profile, suggesting that multiple neurotransmitter sites may be labeled (Biegon and Samuel, 1979). Similarly, the multiphasic saturation of [^{3}H]amitriptyline also suggests the labeling of multiple receptor sites (Rehavi and Sokolovsky, 1978). It remains unclear whether any of the binding of the above antidepressants involves 5-HT$_2$ or histamine H_1 receptors.

Acknowledgments

The authors wish to thank Richard Lebovitz for technical assistance and Dawn Hanks and Nancy Bruce for excellent manuscript preparation.

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