Proc. Natl. Acad. Sci. USA
Vol. 75, No. 12, pp. 6290–6294, December 1978
Neurobiology

Histamine H_1 receptors identified in mammalian brain membranes with [^{3}H]mepyramine

(tricyclic antidepressants/antihistamine/neurotransmitter/amitriptyline)

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Communicated by Julius Axelrod, August 30, 1978

ABSTRACT **The antihistamine [^{3}H]mepyramine binds to H_1 histamine receptors in mammalian brain membranes. Potencies of H_1 antihistamines at the binding sites correlate with their pharmacological antihistamine effects in the guinea pig ileum. Specific [^{3}H]mepyramine binding is saturable with a dissociation constant of about 4 nM in both equilibrium and kinetic experiments and a density of 10 pmol per gram of whole brain. Some tricyclic antidepressants are potent inhibitors of specific [^{3}H]mepyramine binding. Regional variations of [^{3}H]mepyramine binding do not correlate with variations in endogeneous histamine and histidine decarboxylase activity.**

Histamine is a neurotransmitter candidate in mammalian brain (1–3). Effects of histamine on cyclic AMP and cyclic GMP formation may reflect H_2 and H_1 receptor influences, respectively (4–8). Preliminary reports described the labeling of histamine H_1 receptors in rat brain (9) and guinea pig ileum (10) with [^{3}H]mepyramine. We now present detailed properties of histamine H_1 receptors in mammalian brain labeled with [^{3}H]mepyramine.

MATERIALS AND METHODS

[^{3}H]Mepyramine (30 Ci/mmol) was prepared by tritium reduction of dibrominated mepyramine at New England Nuclear. The tritiated material migrated as a single band in thin layer chromatography on silica gel and cellulose in 1-butanol/glacial acetic acid/water, 2:4:1 (vol/vol). α-Chymotrypsin (EC 3.4.21.1; 60 units/mg), phospholipase C (EC 3.1.4.3; from *Clostridium perfringens*, 70 units/mg protein), and trypsin (EC 3.4.21.4; 195 units/mg) were obtained from Worthington. Phospholipase A (EC 3.1.1.4; from bee venom, 1230 units/mg protein), was purchased from Sigma. Compounds were obtained from the following sources: pyrathiazine (Upjohn); promethazine (Wyeth Laboratories, Philadelphia, PA); trimeprazine, diphenylpyraline, chlorpromazine, metiamide, cimetidine, trifluoperazine (Smith, Kline and French Laboratories); methdilazine (Mead Johnson, Evansville, IN); methapyraline, nortriptyline (Eli Lilly); mepyramine (Robinson Laboratory, San Francisco, CA); chlorcyclizine, triprolidine (Burroughs Wellcome, Research Triangle Park, NC); antistine, tripelennamine, dimethindene (CIBA, Summit, NJ); meclizine, doxepin (Pfizer); carbinoxamine (McNeil Laboratory, Fort Washington, PA); phenindamine (Hoffman–LaRoche); orphenadrine (Riker Laboratories, Northridge, CA); brompheniramine (Robins Research Laboratory, Richmond, VA); *d*- and *l*-chlorpheniramine (Schering, Bloomfield, NJ); fluphenazine, triflupromazine (Squibb); methysergide (Sandoz, Hanover, NJ); 1,4-methylhistamine, *N*-acetylhistamine (Calbiochem). Dimaprit and 4-methylhistamine were generous gifts from Marshall Plaut, Johns Hopkins University.

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Male Sprague–Dawley rats (150–200 g) were killed by cervical dislocation, their brains were rapidly removed and homogenized with a Polytron for 30 min (setting 5) in 30 vol of ice-cold Na/K phosphate buffer (50 mM, pH 7.5), and the suspension was centrifuged (50,000 × *g* for 10 min). The pellet was resuspended in the same volume of fresh buffer and centrifuged, and the final pellet was resuspended in the original volume of ice-cold buffer by Polytron homogenization. Calf brains were obtained from a local abattoir within 2 hr after the death of the animals and transferred to the laboratory in ice-cold saline. The brain was quickly dissected into individual regions on ice and stored frozen in plastic vials at −70°C until use. Male Hartley guinea pigs (300–400 g) were used.

To measure specific binding of [^{3}H]mepyramine, [^{3}H]-mepyramine and unlabeled substances were added to 0.45 ml of tissue homogenate with a final incubation volume of 0.5 ml. Incuation was carried out at 25°C for 20–30 min and terminated by addition of 4 ml of ice-cold buffer followed by rapid filtration onto glass fiber filters (GF/B) positioned over a vacuum and subsequently with three 4-ml washes of cold buffer. Radioactivity trapped on the filters was counted in 10 ml of Aquasol (New England Nuclear) at an efficiency of 38% after a 12-hr stoarge at 4°C. Specific binding was defined as radioactivity bound after subtraction of nonspecific binding determined in the presence of 2 μM triprolidine. Maximal reduction of binding by triprolidine to 40% of total binding levels is constant over the range 0.1–10 μM. Thin layer chromotography of membranes and medium showed no metabolism of [^{3}H]-mepyramine.

RESULTS

Specific [^{3}H]mepyramine binding, total minus nonspecific binding, is linear with tissue concentrations in the range 2–20 mg of wet weight and displays a sharp pH optimum at 7.5.

Specific [^{3}H]mepyramine binding becomes saturated with increasing concentrations (Fig. 1). With 0.3 nM receptor concentration, which is 10% of the dissociation constant (K_d) (11), Scatchard analysis reveals a single population of binding sites with a K_d of 4.5 nM and a maximal number of binding sites B_{max} of 10.7 pmol per gram of tissue (Fig. 1 *middle*), resembling densities of muscarinic, cholinergic, opiate, and α- and β-adrenergic receptors (12). Hill plots are linear with a Hill coefficient of 1.01 (Fig. 1 *bottom*).

Kinetics of Specific [^{3}H]Mepyramine Binding. Specific [^{3}H]mepyramine binding associates at 25°C with a bimolecular association rate constant (k_1) of 0.073 nM^{-1} min^{-1} (Fig. 2 *upper*). The dissociation of bound [^{3}H]mepyramine at 25°C on a semilog scale is linear with a half-life of about 2.8 min (Fig. 2 *lower*) and a dissociation rate constant (k_{-1}) of 0.26 min^{-1}. The K_d estimated from the ratio (k_{-1}/k_1) is 3.6 nM, closely similar to the equilibrium K_d.

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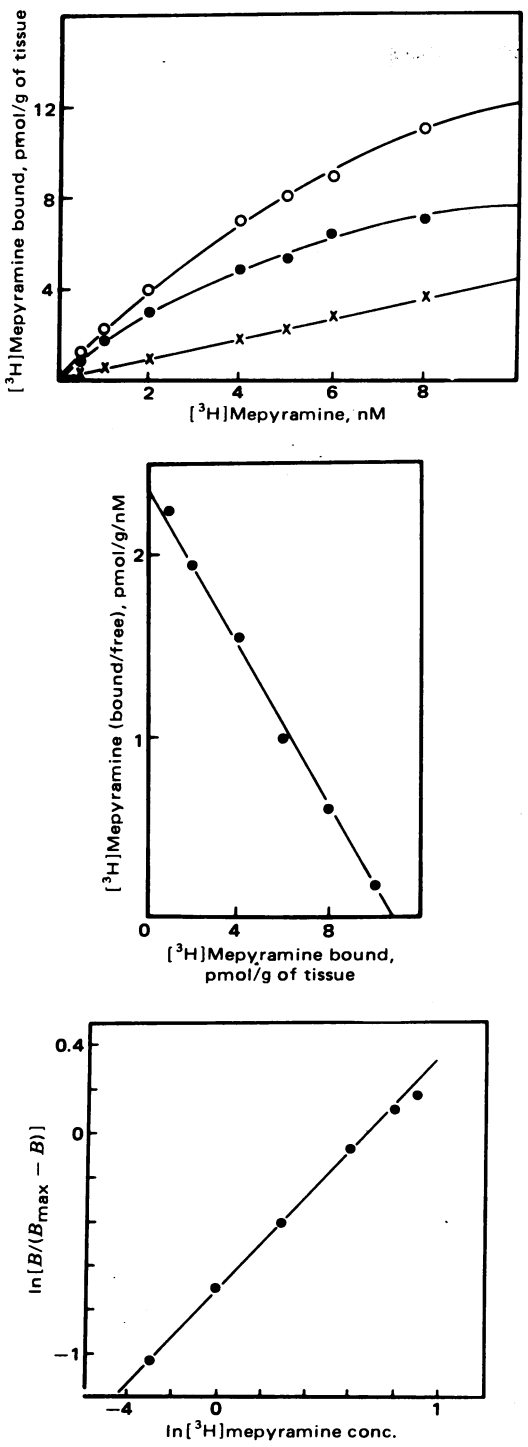

FIG. 1. Specific [^{3}H]mepyramine binding to rat brain membranes. (*Top*) Membrane preparations (10–15 mg) were incubated with various concentrations of [^{3}H]mepyramine at 25°C for 20 min in 0.5 ml of 50 mM Na/K phosphate buffer (pH 7.5) in the presence or absence of 2 μM triprolidine. The experiment was replicated three times. O, Total; ●, specific; ×, nonspecific. (*Middle*) Scatchard analysis of data shown in *Top*. K_d = 4.5 nM and B_{max} = 10.7 pmol/g of tissue. (*Bottom*) Hill plot of the same data. Slope = 1.01.

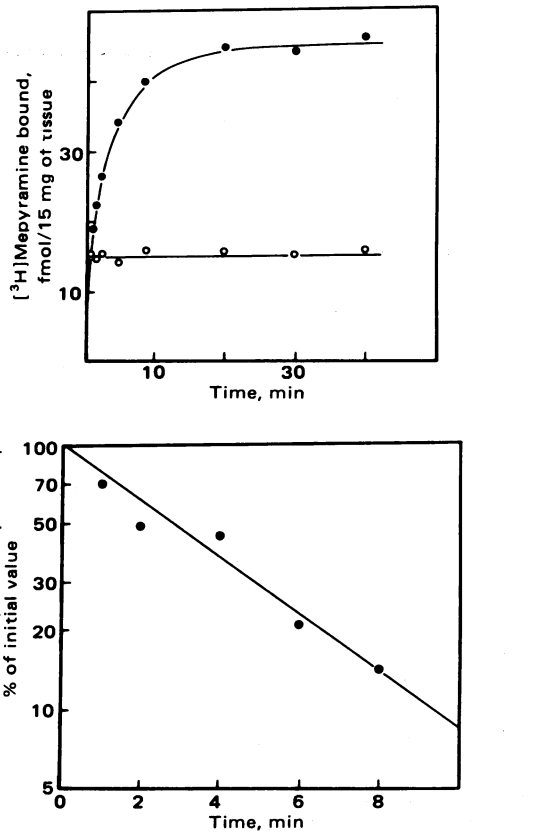

FIG. 2. Kinetics of [^{3}H]mepyramine specific binding. (*Upper*) Association. Specific [^{3}H]mepyramine binding to rat brain membranes (15 mg of wet weight) at 25°C was measured at various time intervals after the addition of [^{3}H]mepyramine (2 nM). Specific binding was defined as the difference between the absence and presence of triprolidine (2 μM). Points shown are those from a single experiment performed in triplicate which was replicated twice. The bimolecular rate constant is calculated according to the equation $k_1 = [2.203/t(a-b)]/\log[b(a-x)/a(b-x)]$, where a is the initial concentration of ^{3}H-mepyramine (2.0 nM) and b is the initial receptor concentration (0.3 nM). ●, Specific binding; O, nonspecific binding. (*Lower*) Dissociation. Specifically bound [^{3}H]mepyramine was assayed at 25°C at various intervals after incubation with [^{3}H]mepyramine to equilibrium under standard assay conditions. At time zero, triprolidine was added to the incubation mixture at a final concentration of 2 μM, and the reaction was terminated by filtration at various intervals. Points shown are those from a single experiment, performed in triplicate, which was replicated twice.

H_1 antihistamines are potent competitors for the specific binding of [^{3}H]mepyramine Table 1, Fig. 3). The phenothiazine antihistamines, pyrathiazine and trimeprazine, are most potent with K_i values of about 1 nM, 10–100 times more potent in reducing [^{3}H]mepyramine binding than are antipsychotic phenothiazines. Among the ethanolamine antihistamines, carbinoxamine and diphenylpyraline are the most potent with K_i values of 2–3 nM, about 5–8 times more potent than bromodiphenhydramine and diphenhydramine. Among the ethylenediamines, mepyramine, methapyrilene, and triprolidine all have K_i values of about 5 nM, whereas antazoline is only 1% as potent. *d*-Chlorpheniramine is about 100 times more potent that its *l* isomer indicating stereospecificity. The H_2 antihistamines, burimimide and metiamide, are extremely weak competitors for [^{3}H]mepyramine binding.

Histamine itself, with a K_i of 40 μM, is substantially weaker than the H_1 antihistamines. However, its effects are selective, because 1,4-methylhistamine, virtually inactive at H_1 receptors in smooth muscle, is much weaker in competing for [^{3}H]-mepyramine binding. Moreover, dimaprit, 4-methylhistamine, and 2-aminoethylpyridine, selective H_2 receptor agonists, are substantially weaker than histamine itself.

The most potent inhibitors of binding are certain tricyclic antidepressants. Doxepin (K_i = 0.7 nM) is more potent than any of the H_1 antihistamines evaluated. Amitriptyline (K_i = 4 nM) is comparable in potency to the most active H_1 antihistamines,

Table 1. Inhibition of [^{3}H]mepyramine binding in rat brain by various drugs

Drug	K_i, nM	Drug	K_i, nM	Drug	K_i, nM	Drug	K_i, nm
Antihistamines (H_1 antagonists)				*Tricyclic antidepressants*			
Ethanolamines		Phenothiazines		Doxepin	0.7 ± 0.1	Protriptyline	60 ± 21
Carbinoxamine	2.3 ± 0.4	Pyrathiazine	1.5 ± 0.7	Amitriptyline	4.1 ± 0.9	Desipramine	250 ± 59
Diphenylpyraline	3.2 ± 1.2	Trimeprazine	1.3 ± 0.1	Imipramine	26 ± 2.8	Iprindole	100 ± 10
Bromodiphenhydramine	13.0 ± 2.1	Methdialzine	2.3 ± 0.2	Nortriptyline	46 ± 2.8		
Diphenhydramine	17.0 ± 2.3	Promethazine	2.9 ± 0.8	*Neuroleptics*			
Ethylenediamines		Piperazines		*cis*-Thiothixene	27	Trifluoperazine	182
Mepyramine	4.5 ± 1.5	Chlorcylcizine	9.0 ± 0.7	Chlorpromazine	36	Spiroperidol	670
Methapyrilene	4.5 ± 0.1	Meclizine	250 ± 12	Trifluopromazine	52	(+)-Butaclamol	1,300
Triprolidine	5.6 ± 1.6			Fluphenazine	67	Haloperidol	3,300
Antazoline	610			Promazine	30	Thioridazine	20 ± 6
Alkylamines		Miscellaneous		*Muscarinic antagonists*			
d,l-Bromopheniramine	4.7 ± 0.5	Cyproheptadine	3.1	Atropine	2,000	Scopolamine	17,000
d-Chlorpheniramine	8.0 ± 2.7	Phenindamine	20	*Adrenergic agonists and antagonists*			
l-Chlorpheniramine	700 ± 135			WB-4101	13,000	Clonidine	NE
Dimethindene	8.0			Phentolamine	40,000	(−)-Isoproterenol	NE
Tripelennamine	35 ± 5.7			Tolazoline	NE	(−)-Alprenolol	NE
Antihistamines (H_2 antagonists)				*Serotonergic agonists and antagonists*			
Burimamide	50,000	Metiamide	100,000	*d*-lysergic acid diethylamide	1,300	Psilocybin	67,000
Histamine and related substances				Methysergide	33,000	5-Hydroxytryptamine	NE
Histamine	40,000	Dimaprit	270,000	*Miscellaneous drugs*			
N-Acetylhistamine	NE	Histidine	NE	*d*-Tubocurarine	NE	Cocaine	NE
1,4-Methylhistamine	NE	Imidazole	NE	Carbachol	NE	Muscimol	NE
2-Aminoethylpyridine	220,000	Imidazoleacetic acid	NE	Flunitrazepam	17,000		
				α-Bungarotoxin	NE		

The inhibition of specific binding of [^{3}H]mepyramine (2 nM) was determined with five or six concentrations of competing drugs assayed in triplicate. The mean inhibitory concentration (IC_{50}) values were determined from the log-probit analysis and K_i values were calculated from the equation $K_i = IC_{50}/1 + ([^3H]\text{mepyramine}/K_d)$. Valid K_i determinations by this procedure require bound/free rations <0.15. In these experiments this ratio in all cases was between 0.06 and 0.10.

Values are the means ± SEM for experiments employing six independent determinations. For experiments containing three to five determinations, results varied by less than 20% and are presented as mean values.

NE = less than 15% displacement observed at 10 mM.

and other tricyclic antidepressants are simliar in activity to clinically employed H_1 antihistamines. Curves of displacement by several H_1 antihistamines, tricyclic antidepressants, and phenothiazines of [^{3}H]mepyramine binding are parallel, suggesting a single population of binding sites with Hill coefficients of about 1.0.

To establish specificity of [^{3}H]mepyramine binding, we compared the relative potencies of drugs in inhibiting specific [^{3}H]mepyramine binding and in blocking histamine-induced contractions of the guinea pig ileum (Fig. 3). The correlation between the molar potencies of drugs in inhibiting ileal contractions and specific [^{3}H]mepyramine binding ($r = 0.89$) is extremely close and highly significant ($P < 0.001$), indicating that [^{3}H]mepyramine labels H_1 histamine receptors that are quite similar in brain and guinea pig ileum.

In rat brain, the highest level of [^{3}H]mepyramine binding occurs in the hypothalamus with values in the thalamus, cerebral cortex, midbrain, and brainstem about 70% of hypothalamic levels and binding levels in the hippocampus, corpus striatum, and cerebellum about half that of the hypothalamus (9).

In calf brain (Table 2), the highest binding level is in the cerebral cortex, in which subdivisions vary considerably with highest values in pareital and occipital cortex and similar binding is observed in parts of the limbic system, including olfactory cerebral cortex, hippocampus, parahippocampus, and amygdala, as well as in all areas of the thalamus evaluated. Levels in the limbic system and thalamus are about one-third those of the parietal cerebral cortex. Whereas the hypothalamic binding level is highest in the rat, hypothalamic calf binding is only 20–35% of that of the parietal cerebral cortex. Whereas in the rat, the brainstem and midbrain have high levels of binding, in the calf, midbrain, pons, and medulla oblongata are among the lowest in the brain. The cerebellum displays the lowest levels of binding in the rat, but in the calf all portions of the cerebellum except for the deep nuclei have substantial binding levels.

Regional variations in guinea pig brain differ somewhat from both rat and calf. Guinea pig cerebellum displays highest levels, almost 3 times those in the cerebral cortex and 5 times the lowest levels detected in the corpus striatum.

Specific [^{3}H]mepyramine binding is readily degraded by the proteolytic enzymes trypsin and α-chymotrypsin, which at 5 units/ml reduce binding 52% and 62%, respectively. Phospholipids appear to play a role in binding sites, because binding is extensively degraded by phospholipase A (96% reduction at 1.1 unit/ml) with lesser effects by phospholipase C (61% fall at 2 units/ml). The calcium dependence of the phospholipase effects and the blockage of the trypsin effects by soybean trypsin inhibitor favors selectivily. Triton X-100 at 0.01%, less

Table 2. Regional distribution of specific [^{3}H]mepyramine binding in calf and guinea pig brain

Regions	pmol/g tissue	Regions	pmol/g tissue
	Calf brain		
Cerebrum		Hypothalamus	
Parietal cortex	4.7 ± 0.4	Post. hypothal.	1.6 ± 0.3
Occipital cortex	3.9 ± 0.4	Ant. hypothal.	1.5 ± 0.2
		Mammillary	
Temporal cortex	2.0 ± 0.5	body	1.1 ± 0.1
Frontal cortex	1.9 ± 0.1		
Cingulate cortex	1.6 ± 0.2		
Basal ganglia		Mesencephalon	
Caudate, head	3.1 ± 0.4	Superior colliculi	1.1 ± 0.1
Caudate, body	2.3 ± 0.3	Substantia nigra	0.9 ± 0.1
Caudate, tail	1.8 ± 0.3	Inferior colliculi	0.8 ± 0.1
Putamen	1.8 ± 0.2	Red nucleus	0.7 ± 0.1
Limbic system		Pons	
Olfactory cortex	1.5 ± 0.3	Dorsal	0.5 ± 0.1
Hippocampus	1.4 ± 0.4	Ventral	0.5 ± 0.1
Parahippocampus	1.4 ± 0.3		
Amygdala	1.3 ± 0.3		
Thalamus		Medulla oblongata	
Anterior thalamus	2.1 ± 0.3	Obex	0.9 ± 0.2
Pulvinar	1.4 ± 0.2	Below obex	0.7 ± 0.2
Dorsal medial thal.	1.3 ± 0.3	Above obex	0.6 ± 0.1
Vent. tier of thal.	1.3 ± 0.4		
Cerebellum		Others	
Flocculonodular lobe	2.9 ± 0.5	Fornix	0.2 ± 0.08
Hemispheres	2.6 ± 0.4	Corpus callosum	0.2 ± 0.07
Vermis	2.2 ± 0.4	Pineal	0.2 ± 0.008
Deep nuclei	0.6 ± 0.1		
	Guinea pig brain		
Cerebellum	9.2 ± 0.5	Midbrain	3.8 ± 0.5
Thalamus	5 7 ± 0.4	Cerebral cortex	3.4 ± 0.3
Hippocampus	4.5 ± 0.4	Pons medulla	2.9 ± 0.3
Hypothalamus	3.8 ± 0.5	Corpus striatum	1.7 ± 0.4

Specific [^{3}H]mepyramine binding in each region was determined. Binding was assayed with 2 nM [^{3}H]mepyramine in triplicate and the experiment was repeated three times. Results shown are the mean values from three experiments ± SEM.

than levels that solubilize most membrane proteins, lowers specific [^{3}H]mepyramine binding by 60%, whereas 0.1% Triton X-100 reduces binding by 90%.

DISCUSSION

The substrate specificity of specific [^{3}H]mepyramine binding in the present study indicates an association with H_1 histamine receptors. The potencies of H_1 antihistamines in blocking histamine-induced contractions of the guinea pig ileum are closely similar to their potencies in competing for specific [^{3}H]mepyramine binding in brain membranes. However, histamine is about 100 times more potent in inducing contractions in the guinea pig ileum than in blocking [^{3}H]mepyramine binding; this might reflect existence of spare receptors in the guinea pig ileum.

Regional variations in the density of [^{3}H]mepyramine binding sites in rat, calf, and guinea pig do not parallel endogenous histamine and histidine decarboxylase. Failure of receptor density to parallel the endogenous neurotransmitter has been observed for several other transmitter receptors (12).

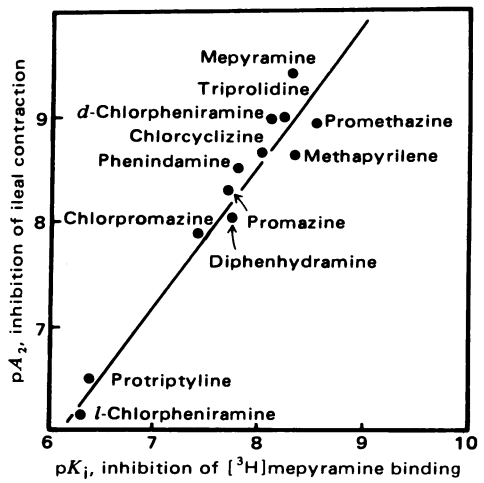

FIG. 3. Correlation of affinity of drugs at [^{3}H]mepyramine binding receptor sites with their potencies in blocking histamine-induced guinea pig ileal contractions. pA_2 values were derived from published data (13–16). Slope = 1.4.

Several tricyclic antidepressants are extremely potent inhibitors of specific [^{3}H]mepyramine binding and also block histamine H_1 stimulation of cyclic GMP accumulation in neuroblastoma clones (7). Tricyclic antidepressants inhibit neuronal uptake of catecholamines and serotonin but at much higher concentrations (about 0.1–1.0 μM) (17, 18) than are required to block specific [^{3}H]mepyramine binding. Moreover, iprindole, a tricyclic antidepressant that does not inhibit biogenic amine uptake (19), is as potent as some other tricyclic antidepressants in competing for mepyramine binding.

Could the antidepressant efficacy of these drugs be due in part to blockage of H_1 histamine receptors? Phenothiazine neuroleptics compete for mepyramine binding with potencies almost as great as those of the tricyclics. Although some phenothiazines relieve agitated depression, they appear to act largely through their calming effects (20), whereas the tricyclic antidepressants additionally exert a unique psychic energizing action.

Tricyclic antidepressants inhibit a brain histamine H_2-linked adenylate cyclase (6, 8) with potencies 0.03% to 50% of those in competing for [^{3}H]mepyramine binding. In blocking H_2 receptors in brain homogena, neuroleptics tend to be more potent than tricyclic antidepressants in one study (8), but similar in another (21). In blocking amine uptake (18) and histamine H_1 and H_2 (17, 21) receptors, some tricyclics differ 100-fold or more in potencies and yet are employed clinically at similar doses.

Supported by U.S. Public HEalth Service Grant MH-18501 and grants of the Hartford and McKnight Foundations.

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