European Journal of Pharmacology, 78 (1982) 279–285
Elsevier Biomedical Press

HIGH-AFFINITY BINDING OF [^{3}H]DOXEPIN TO HISTAMINE H_1-RECEPTORS IN RAT BRAIN: POSSIBLE IDENTIFICATION OF A SUBCLASS OF HISTAMINE H_1-RECEPTORS

JOHN E. TAYLOR * and ELLIOTT RICHELSON **

Departments of Psychiatry and of Pharmacology, Mayo Foundation, Rochester, MN 55905, U.S.A.

Received 13 October 1981, accepted 23 November 1981

J.E. TAYLOR and E. RICHELSON, *High-affinity binding of [^{3}H]doxepin to histamine H_1-receptors in rat brain: possible identification of a subclass of histamine H_1-receptors,* European J. Pharmacol. 78 (1982) 279–285.

The binding of the radioactively labeled tricyclic antidepressant, [^{3}H]doxepin, to rat brain tissue was examined. Scatchard plots of specific [^{3}H]doxepin binding indicated the presence of two distinct binding sites. The equilibrium dissociation constant (K_D) of the high-affinity site was 0.020 nM with a maximal binding capacity (B_{max}) of 13.7 fmol/mg protein. The corresponding values for the low-affinity site were 3.6 nM and 740 fmol/mg protein, respectively. The high-affinity site was sensitive to competition by pharmacologically relevant concentrations of histamine H_1 antagonists such as pyrilamine ($K_D = 1.0$ nM), diphenhydramine ($K_D = 20$ nM), d-chlorpheniramine ($K_D = 1.7$ nM), and l-chlorpheniramine ($K_D = 97$ nM). The B_{max} for [^{3}H]doxepin binding to the high-affinity H_1-receptor, however, was approximately 10% of the B_{max} obtained using [^{3}H]pyrilamine to label the H_1-receptor. Various tricyclic antidepressants were very potent inhibitors at the high-affinity [^{3}H]doxepin site. Their potencies, however, did not correlate with their potencies previously reported for the H_1-receptor. The regional distribution of [^{3}H]doxepin high-affinity sites correlated with the known distribution of H_1-receptors in the rat brain. These results suggest that [^{3}H]doxepin is binding to a subclass of histamine H_1-receptors.

Tricyclic antidepressants Antihistamines Histamine H_1-receptors

1. Introduction

Two types of histamine receptors (H_1 and H_2), distinguished by their differential sensitivities to various agonists and antagonists, have been identified as mediating the cellular actions of histamine (Ash and Schild, 1966; Black et al., 1972), and a variety of biochemical and physiological studies (Schwartz et al., 1980; Taylor and Richelson, 1981) suggest that both types are present in the mammalian brain. Recently, the application of radioligand binding assays to the identification and characterization of histamine H_1-receptors in the mammalian brain has been accomplished, both in vitro and in vivo, using the classical H_1-receptor antagonist, [^{3}H]pyrilamine (mepyramine) (Hill et al., 1978; Tran et al., 1978; Chang et al., 1979; Palacios et al., 1979; Taylor and Richelson, 1980a). Several of these radioligand binding studies (Tran et al., 1978; Chang et al., 1979; Taylor and Richelson, 1980a), as well as the reports of Richelson (1978, 1979) and Figge et al. (1979), who used biological assays of H_1-receptor binding, have shown that the tricyclic antidepressants are very potent antagonists of the histamine H_1-receptor, and that doxepin may be the most potent H_1-antagonist known. Direct evidence that doxepin can be used as a radioligand for H_1-receptors was recently obtained by ourselves (Taylor and Richelson, 1980b) and others (Tran et al., 1981).

In the present paper we extend our studies on the properties of the in vitro binding of [^{3}H]doxepin to histamine H_1-receptors of rat brain. The results suggest that [^{3}H]doxepin is binding to a subpopulation of histamine H_1-receptors.

* Present address: Indiana University School of Medicine, Evansville, IN 47732, U.S.A.
** To whom all correspondence should be addressed.

0014-2999/82/0000-0000/$02.75

2. Materials and methods

Whole rat brain was homogenized in 50 vol of 50 mM Na-K phosphate buffer (pH 7.4) with a Polytron homogenizer and centrifuged at 48000 × *g* for 10 min. The pellet was suspended in the same volume of buffer and the centrifugation was repeated. The final pellet was suspended in the Na-K phosphate buffer to obtain a protein concentration of approximately 5 mg/ml. The incubation mixture contained 50 mM Na-K phosphate buffer (pH 7.4), [^{3}H]doxepin, and approximately 0.5 mg protein of the tissue suspension (within the linear range of binding) in a final volume of 2 ml. The incubation was for 30 min at 37°C and terminated by filtration under vacuum on Whatman GF/B filters which were washed three times with 5 ml of ice-cold buffer before the radioactivity trapped on the filters was counted by liquid scintillation spectrometry. All assays were done in triplicate and the specific binding was defined as the total amount of [^{3}H]doxepin bound minus that bound in the presence of 100 nM doxepin or 1 μM pyrilamine. [^{3}H]Pyrilamine binding was determined as previously described (Taylor and Richelson, 1980a) using 5 μM d-chlorpheniramine to define nonspecific binding. Protein concentrations were determined by the method of Lowry et al. (1951).

[^{3}H]Doxepin, 41 Ci/mmol, was purchased from New England Nuclear, and [^{3}H]pyrilamine, 24.1 Ci/mmol, was obtained from Amersham. The sources of drugs were as follows: d- and l-chlorpheniramine, Schering Corporation; doxepin, Pfizer; imipramine, CIBA-Geigy; amitriptyline and protriptyline, Merck Sharp and Dohme; nortriptyline, Eli Lilly; desipramine, USV; and metiamide, Smith Kline and French; diazepam, Roche; naloxone, Endo. All other drugs were purchased from commercial suppliers.

Scatchard plots were analyzed with the use of a non-linear least squares regression program on a Hewlett-Packard 9845B computer. We assumed a two-site binding model as described by Feldman (1972).

3. Results

The specific binding of [^{3}H]doxepin was measured at concentrations between 0.01 and 5 nM in five independent experiments. Scatchard analysis of the binding data yielded plots which were curvilinear, suggesting the presence of two ligand binding sites (fig. 1). Analysis of these plots by a non-linear least squares regression, assuming a two-site model (Feldman, 1972), showed that the equilibrium dissociation constant (K_D) for the site of higher affinity was 20 pM ± 9 (±S.E.M., N = 5) and that the maximal binding capacity for this site (B_{max}) was 13.7 ± 4.5 fmol/mg protein. The K_D for the lower affinity site was 3.6 nM ± 1.3, and the B_{max} was 740 ± 210 fmol/mg protein. By comparison, the B_{max} for [^{3}H]pyrilamine binding to histamine H_1-receptors was found to be 175 ± 13 fmol/mg protein (n = 3). At a concentration of 0.08 nM, specific [^{3}H]doxepin binding reached equilibrium by 20 min at 37°C (fig. 2A), and the calculated association rate constant (k_1) was 1.38 × 10^9 $M^{-1} \cdot min^{-1}$. The half-time for dissociation at 37°C was 18 min and the calculated dissociation rate constant (k_{-1}) was 0.038 min^{-1} (fig. 2B). The K_D determined from the ratio k_{-1}/k_1 was 27 pM.

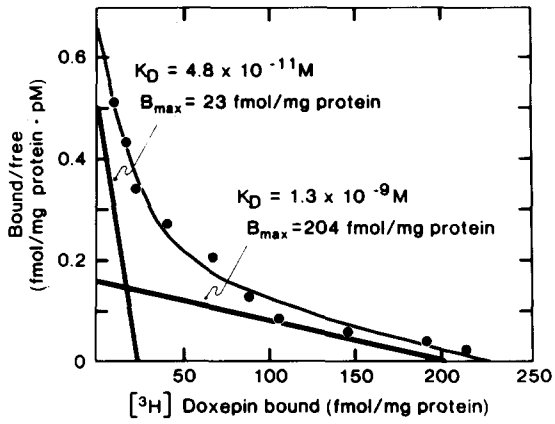

Fig. 1. Scatchard analysis of [^{3}H]doxepin binding. Specific binding was assayed as described in Materials and methods using concentrations of [^{3}H]doxepin between 0.02 and 5 nM and approximately 0.5 mg protein per assay. Each point is the mean of triplicate determinations from a single experiment. The data were fit to a two-site model (Feldman, 1972) as described in Materials and methods.

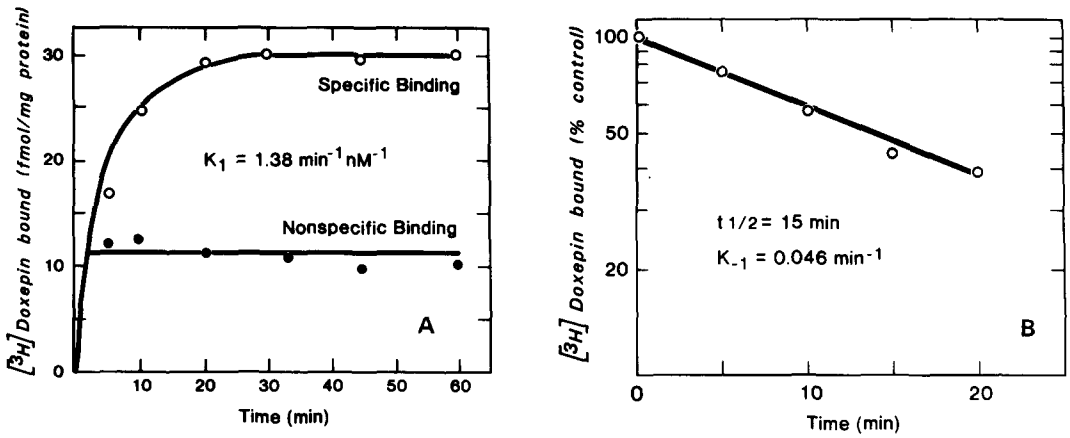

Fig. 2. Association (A) and dissociation (B) of specific [^{3}H]doxepin binding. (A) Specific binding of [^{3}H]doxepin (0.08 nM) was measured at the indicated times after the addition of tissue. Each point is the mean of triplicate determinations from two independent experiments. (B) The rate of dissociation was determined by incubating the tissue with [^{3}H]doxepin (0.08 nM) for 30 min, followed by the addition of unlabeled doxepin at a final concentration of 10 nM. The assay was then terminated at the indicated times. Each point is the mean of triplicate determinations from two independent experiments.

Histamine and histamine H_1-receptor antagonists were effective at competing for [^{3}H]doxepin high-affinity binding sites (table 1). Pyrilamine was the most potent of the classical H_1-antagonists tested, followed by d-chlorpheniramine and diphenhydramine. d-Chlorpheniramine was approximately 60 times more potent in inhibiting the high-affinity [^{3}H]doxepin binding than was l-chlorpheniramine. These values obtained from the inhibition of [^{3}H]doxepin binding were very simi-

TABLE 1

Competition for specific, high-affinity binding of [^{3}H]doxepin and [^{3}H]pyrilamine to rat brain membranes.

Drug	K_D (±S.E.M.) (M) [a,b]	
	[^{3}H]Doxepin	[^{3}H]Pyrilamine
Pyrilamine	$1.0\pm0.3\times10^{-9}$	$1.5\pm0.2\times10^{-9}$
d-Chlorpheniramine	$1.7\pm0.4\times10^{-9}$	$6.3\pm0.4\times10^{-9}$
l-Chlorpheniramine	$9.7\pm2.3\times10^{-8}$	$5.7\pm2.6\times10^{-7}$
Diphenhydramine	$2.0\pm1.0\times10^{-8}$	–
Histamine	$1.8\pm0.7\times10^{-5}$	4.3×10^{-5}
Atropine	$4.1\pm0.6\times10^{-7}$	–
Metiamide	$7.3\pm0.5\times10^{-6}$	–
Doxepin	$1.8\pm0.6\times10^{-11}$	$2.6\pm0.7\times10^{-11}$
Amitriptyline	$2.0\pm0.8\times10^{-11}$	$1.0\pm0.1\times10^{-10}$
Nortriptyline	$5.6\pm2.1\times10^{-10}$	$1.3\pm0.9\times10^{-9}$
Protriptyline	$2.0\pm0.5\times10^{-9}$	$6.6\pm0.7\times10^{-8}$
Imipramine	$2.9\pm0.1\times10^{-9}$	$2.7\pm0.1\times10^{-8}$
Desipramine	$2.9\pm0.8\times10^{-8}$	$2.3\pm0.9\times10^{-7}$

[a] Determined from the equation $K_D = IC_{50}/(1+L/K_B)$ Cheng and Prusoff, 1973) where L refers to the concentration of [^{3}H]doxepin (0.08 nM) and K_B refers to the equilibrium dissociation constant for [^{3}H]doxepin (0.027 nM). The calculated K_D values represent the mean of three or four independent experiments. The K_D values for the tricyclic antidepresants are from Taylor and Richelson (1980a).

[b] The following compounds did not inhibit the binding [^{3}H]doxepin by 50% when present at 10 μM: hexamethonium, tubocurare, diazepam, naloxone, propranolol, picrotoxin and apomorphine.

lar to those derived from the inhibition of [^{3}H]pyrilamine (table 1). The histamine H_2-antagonist, metiamide, and the muscarinic cholinergic antagonist, atropine were relatively weak competitors. Nicotinic cholinergic antagonists such as tubocurare and hexamethonium were ineffective competitors, as were a number of non-histaminergic compounds such as propranolol, picrotoxin, apomorphine, naloxone and diazepam.

Among the most potent antagonists tested were the tricyclic antidepressants. Doxepin, amitriptyline, and nortriptyline were the most potent with K_D of 0.018, 0.02 and 0.56 nM, respectively. Desipramine was the weakest inhibitor with a K_D of 29 nM. The Hill coefficients for the tricyclics ranged from 0.76 to 0.90. Interestingly, except for doxepin, the other tricyclics were 5–10 times more potent in inhibiting [^{3}H]doxepin binding than in inhibiting [^{3}H]pyrilamine binding (table 1).

Histamine H_1-receptor antagonists were weak inhibitors of [^{3}H]doxepin binding at the low affinity site. When the assay was performed in the presence of 0.1 μM pyrilamine to eliminate completely high-affinity binding, the K_D for pyrilamine was 520 μM and d- and l-chlorpheniramine were equipotent with K_D of 280 and 290 nM, respectively. The K_D for the six tricyclics ranged from 50 nM for doxepin to 750 nM for desipramine.

The specific binding of high-affinity [^{3}H]doxepin to histamine H_1-receptors was highest in the hypothalamus intermediate in the midbrain, cerebral cortex and brainstem; and lowest in the corpus striatum, thalamus and cerebellum (table 2). The affinities for [^{3}H]doxepin binding were similar in all regions (data not shown). The regional distribution of [^{3}H]pyrilamine in the rat brain quite closely resembles the distribution of [^{3}H]doxepin to the high-affinity site.

TABLE 2

Regional distribution of specific [^{3}H]doxepin and [^{3}H]pyrilamine to histamine H_1-receptors in rat brain [a].

Region	% Hypothalamus ± S.E.M.	
	[^{3}H]Doxepin	[^{3}H]Pyrilamine
Hypothalamus	100	100
Brainstem	67 ± 4	72 ± 8
Midbrain	87 ± 11	79 ± 9
Cerebral cortex	81 ± 3	76 ± 11
Thalamus	58 ± 7	68 ± 4
Corpus striatum	63 ± 4	59 ± 4
Cerebellum	52 ± 7	57 ± 6

[a] The specific binding of [^{3}H]doxepin was defined as the total [^{3}H]doxepin bound minus that bound in the presence of 1 μM pyrilamine. Specific [^{3}H]pyrilamine binding was defined as the [^{3}H]pyrilamine bound minus that bound in the presence of 5 μM d-chlorpheniramine. Each value represents the mean of 4–5 independent experiments. The concentration of [^{3}H]doxepin was 0.08 nM and for the hypothalamus the binding was 20.1 ± 2.1 fmol/mg protein. The respective values for [^{3}H]pyrilamine were 4.0 nM and 170 ± 48 fmol/mg protein.

4. Discussion

Although the mechanism of action of the tricyclic antidepressants in the mammalian CNS is uncertain, these drugs have a number of pharmacological actions including the blockade of presynaptic re-uptake of norepinephrine and serotonin (Maas, 1975), antagonism of muscarinic cholinergic (Snyder and Yamamura, 1977; Richelson and Divinetz-Romero, 1977) α-adrenergic (U'Prichard et al., 1978; Tang and Seeman, 1980), histamine H_1 (Richelson, 1978, 1979; Tran et al., 1978; Figge et al., 1979; Taylor and Richelson, 1980a), histamine H_2 (Green and Maayani, 1977; Kanof and Greengard, 1978) and serotonin (Tang and Seeman, 1980) receptors.

Our laboratory has shown that some of these compounds are more potent as histamine H_1-antagonists than as effectors of any of the pharmacological actions mentioned above (Richelson, 1978; Richelson, 1979; Taylor and Richelson, 1980a). However, since there is no correlation between therapeutic blood levels and affinity for the histamine H_1-receptor for the tricyclics, it is likely that histamine H_1-receptor blockade is unrelated to the antidepressant actions of these drugs.

The present study describes the direct binding characteristics of doxepin, the most potent of the tricyclic antidepressants at the histamine H_1-receptor (Richelson, 1979; Taylor and Richelson, 1980), to rat brain tissue. It is evident from our

data that [^{3}H]doxepin binds to two distinct sites of widely differing affinities. Although our data show that H_1-receptors are not involved, the pharmacological specificity of the low affinity site ($K_D = 3.6$ nM) is not known at the present time. However, it is clear that the high-affinity site ($K_D \simeq 0.02$ nM) has the pharmacological characteristics of a histamine H_1-receptor.

For example, there is good agreement between the equilibrium dissociation constants for several classical H_1-antagonists obtained from this study as compared to those from our data and others using [^{3}H]pyrilamine binding to brain tissue of various mammalian species (Hill et al., 1978; Tran et al., 1978; Chang et al., 1979; Hill and Young, 1980). The stereoselectivity of the high-affinity binding site was demonstrated with the use of the steroisomers of the histamine H_1-antagonist chlorpheniramine. In our binding studies, the d-isomer was about 60 times more potent than the l-isomer in competing for [^{3}H]doxepin binding at the high-affinity site, a value similar to that found for the ratio of these isomers in our binding studies using [^{3}H]pyrilamine.

A variety of non-histaminergic compounds such as hexamethonium, tubocurare, diazepam, naloxone, picrotoxin, apomorphine and propranolol were virtually inactive in competing for the high-affinity binding site. The only other compounds that had any appreciable activity were atropine ($K_i \simeq 400$ nM) and the histamine H_2 antagonist, metiamide ($K_i \simeq 10000$ nM). These values are considerably higher than would be anticipated if the high-affinity [^{3}H]doxepin binding were to either muscarinic cholinergic (Richelson and Divinetz-Romero, 1977) or histamine H_2-receptors (Black et al., 1972; Green and Maayani, 1977).

The equilibrium dissociation constants (K_D) derived from our saturation and kinetic experiments for [^{3}H]doxepin binding at the high-affinity site are in quantitative agreement with the K_D calculated from the inhibition of H_1-receptor-mediated cyclic GMP formation in mouse neuroblastoma cells (Richelson, 1978; Richelson, 1979), inhibition of histamine-induced contractions of the guinea-pig ileum (Figge et al., 1979), and from the K_i values calculated from the in vivo (Palacios et al., 1979) and in vitro (Taylor and Richelson, 1980) competition for [^{3}H]pyrilamine binding to rat brain tissue.

However, there are a number of differences between [^{3}H]doxepin binding and [^{3}H]pyrilamine binding to rat brain. First, the maximum concentration of high-affinity binding sites for [^{3}H]doxepin ($B_{max} \simeq 14$ fmol/mg protein) in whole brain was lower than the maximum number of sites identified with [^{3}H]pyrilamine ($B_{max} \simeq 175$ fmol/mg protein). In addition, the tricyclic antidepressants, amitriptyline, nortriptyline, protriptyline, imipramine and desipramine were at least 10 times more potent at competing for high-affinity [^{3}H]doxepin binding than at competing for [^{3}H]pyrilamine binding (Taylor and Richelson, 1980a), or antagonizing histamine H_1-receptor mediated cyclic GMP formation (Richelson, 1978) and guinea-pig ileum contractions (Figge et al., 1979). The rank order of potency was the same against either [^{3}H]ligand, however. Nevertheless, the regional distribution of high-affinity [^{3}H]-doxepin sites was similar to that for histamine H_1-sites identified by [^{3}H]pyrilamine.

Our results also differ in some respects from those reported by Tran et al. (1981) for [^{3}H]doxepin binding to rat brain. These workers found the K_D for binding at the high-affinity H_1 site to be approximately 10-fold higher than we report. Similar differences were found with respect to the inhibition of [^{3}H]doxepin binding by other tricyclic antidepressants. Interestingly, however, they found that [^{3}H]doxepin bound to 40% fewer sites than did [^{3}H]pyrilamine in rat brain, although this difference was not apparent in the guinea pig brain. This observation is relevant because of previous observations which suggest that there are marked differences in the ligand binding properties for [^{3}H]pyrilamine to rat and guinea pig brain homogenates (Hill and Young, 1980). Differences in our experimental techniques such as the use of a lower receptor concentration may explain, at least in part, the higher affinities that we observed in this study (cf., Taylor and Richelson, 1980a).

It is interesting that [^{3}H]doxepin binds to fewer H_1 sites than does [^{3}H]pyrilamine, yet our previous studies (Taylor and Richelson, 1980a) showed that doxepin is able to displace all of the [^{3}H]pyrilamine binding sites with approximately

the same affinity. The explanation for this apparent discrepancy may reside in the observation that the average pseudo Hill coefficient for doxepin inhibition of [^{3}H]pyrilamine (Taylor and Richelson, 1980a) is 0.55 indicating the possibility of a heterogeneity of histamine H_1-receptors, one of which is labeled by [^{3}H]doxepin with high-affinity and comprises only 10% of the sites; and a second site which is completely displaced by higher concentrations of doxepin. However, we feel that since the high-affinity component apparently composes approximately 10% of the sites, it would have been difficult to detect this site in our original [^{3}H]pyrilamine inhibition studies (Taylor and Richelson, 1980a). Interestingly, other tricyclic antidepressants as well as certain classical H_1 antagonists such as pyrilamine, methapyriline, and triprolidine (Hill et al., 1978) yield low Hill coefficients. However, the present study showed that the Hill coefficients for the inhibition of high-affinity [^{3}H]doxepin binding were in the range of 0.76–0.90. This suggests that at low concentrations, [^{3}H]doxepin is primarily bound to a single receptor type. One difficulty with this argument is that the overall K_D value for the doxepin inhibition of [^{3}H]pyrilamine binding is very close to the K_D calculated for the high-affinity binding site derived from the [^{3}H]doxepin isotherms. Thus, the differences between [^{3}H]doxepin and [^{3}H]pyrilamine binding to the H_1-receptor are somewhat contradictory and may not represent a true biological phenomenon.

In any event it is evident that the high-affinity binding site for [^{3}H]doxepin in the rat brain has the pharmacological specificity of a histamine H_1-receptor site, the differences in B_{max} and tricyclic antidepressant potencies, if real, suggest that this high-affinity site may be a subtype of the histamine H_1-receptor.

References

Ash, A.S.F. and H.D. Schild, 1966, Receptors mediating some actions of histamine, Br. J. Pharmacol. Chemother. 27, 427.

Black, J.W., W.A.M. Duncan, C.J. Durant, C.R. Ganellin and E.N. Parsons, 1972, Definition and antagonism of histamine H_2-receptor, Nature 236, 385.

Chang, R.S.L., V.T. Tran and S.H. Snyder, 1979, Heterogeneity of histamine H_1-receptors: Species variations in [^{3}H] mepyramine binding of brain membranes, J. Neurochem. 32, 1653.

Cheng, Y.-C. and W.H. Prusoff, 1973, Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (I_{50}) of an enzymatic reaction, Biochem. Pharmacol. 22, 3099.

Feldman, H.A., 1972, Mathematical theory of complex ligand-binding systems at equilibrium: Some methods for parameter fitting, Anal. Biochem. 48, 317.

Figge, J., P. Leonard and E. Richelson, 1979, Tricyclic antidepressants: Potent blockade of histamine H_1-receptors of guinea pig ileum, European J. Pharmacol. 58, 479.

Green, J.P. and S. Maayani, 1977, Tricyclic antidepressant drugs block histamine H_2 receptor in brain, Natue 269, 163.

Hill, S.J., P.C. Emson and J.M. Young, 1978, The binding of [^{3}H]mepyramine to histamine H_1-receptors in guinea-pig brain, J. Neurochem. 31, 997.

Hill, S.J. and J.M. Young, 1980, Histamine H_1-receptors in the brain of the guinea-pig and rat: Differences in ligand binding properties and regional distribution, Br. J. Pharmacol. 68, 687.

Kanof, P.D. and P. Greengard, 1978, Brain histamine receptors as targets for antidepressant drugs, Nature 272, 329.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951, Protein measurement with the folin phenol reagent, J. Biol. Chem. 193, 265.

Maas, J.W., 1975, Biogenic amines and depression: Biochemical and pharmacological separation of two types of depression, Arch. Gen. Psychiatr. 32, 1357.

Palacios, J.M., W.S. Young, III and M.J. Kuhar, 1979, Autoradiographic localization of H_1-histamine receptors in brain using [^{3}H]mepyramine: Preliminary studies, European J. Pharmacol. 58, 295.

Richelson, E., 1978, Tricyclic antidepressants block histamine H_1-receptors of mouse neuroblastoma cells, Nature 274, 176.

Richelson, E., 1979, Tricyclic antidepressants and histamine H_1-receptors, Mayo Clin. Proc. 54, 669.

Richelson, E. and S. Divinetz-Romero, 1977, Blockade by psychotropic drugs of the muscarinic acetylcholine receptor in cultured nerve cells, Biol. Psychiatr. 12, 771.

Schwartz, J.C., H. Pollard and T.T. Quach, 1980, Histamine as a neurotransmitter in mammalian brain: Neurochemical evidence, J. Neurochem. 35, 26.

Snyder, S.H. and H.I. Yamamura, 1977, Antidepressants and the muscarinic acetylcholine receptor, Arch. Gen. Psychiatr. 34, 236.

Tang, S.W. and P. Seeman, 1980, Effect of antidepressant drugs on serotonergic and adrenergic receptors, Naunyn-Schmiedeb. Arch. Pharmacol. 311, 255.

Taylor, J.E. and E. Richelson, 1980a, High affinity binding of tricyclic antidepressants to histamine H_1-receptors: Fact and artifact, European J. Pharmacol. 67, 41.

Taylor, J.E. and E. Richelson, 1980b, High affinity binding of [^{3}H]doxepin to rat brain tissue, Soc. Neurosci. Abstr. 6, 237.

Taylor, J.E. and E. Richelson, 1981, Neurotransmitter receptors, Part 2, in: Receptors and Recognition, Series B, eds. S.J. Enna and H.I. Yamamura (Chapman and Hall) Vol. 10, 73.

Tran, V.T., R.S.L. Chang and S.H. Snyder, 1978, Histamine H_1 receptor identified in mammalian brain with [^{3}H]mepyramine, Proc. Natl. Acad. Sci. U.S.A. 75, 6290.

Tran, V.T., R. Lebovitz, L. Toll and S.H. Snyder, 1981, [^{3}H]Doxepin interactions with histamine H_1-receptors and other sites in guinea pig and rat brain homogenates, European J. Pharmacol. 70, 501.

U'Prichard, D.C., D.A. Greenberg, P.P. Sheehan and S.H. Snyder, 1978, Tricyclic antidepressants: Therapeutic properties and affinity for α-noradrenergic receptor binding sites in the brain, Science 199, 197.