Antimuscarinic Effects of Antihistamines: Quantitative Evaluation by Receptor-Binding Assay

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Abstract—Quantitative evaluation of antimuscarinic effects of antihistamines (H_1 - and H_2 -receptor antagonists) was carried out using a receptor-binding assay. The inhibition constants (K_i values) of twenty seven H_1 -receptor antagonists, one related antidepressant and three H_2 -receptor antagonists at H_1 -receptors and muscarinic receptors in the bovine cerebral cortex were determined. All the H_2 -receptor antagonists examined showed very low affinity for the muscarinic receptors. On the other hand, some H_1 -receptor antagonists (mequitazine, cyproheptazine, clemastine, diphenylpyraline, promethazine, homochlorcyclizine and alimemazine) had high affinity for the muscarinic receptors ($K_i=5.0$ –38 nM). Another group of H_1 -receptor antagonists (mepyramine, terfenadine, metapyrilen, azelastine, hydroxyzine and meclizine) had low affinity for the muscarinic receptors ($K_i=3,600$ –30,000 nM). Thus, a broad range of antimuscarinic potencies among the antihistamines was demonstrated. These results should provide helpful information with regard to the clinical and experimental use of antihistamines.

Histamine has been shown to stimulate two different types of receptors which are referred to as H_1 - and H_2 -receptors. H_1 -receptor mediates histamine-induced contraction of smooth muscles of the small intestine and bronchi. H_2 -receptor mediates the action of histamine in stimulating gastric acid secretion. The receptors are defined pharmacologically from the antagonists which selectively block the response of these tissues to histamine stimulation (1, 2).

Some of the classical H_1 -receptor antagonists such as diphenhydramine (3) and antazoline (4) were reported to display substantial atropine-like activity which was assessed by a bioassay system. In the following years, numerous antihistamines of much greater potency and specificity either at H_1 - or H_2 -receptors were synthesized. While some of these drugs are thought to possess antimuscarinic activities which may cause certain adverse effects in clinical use, quantitative evaluation of the antimuscarinic effects has not been reported. Recently, receptor-

binding studies have provided a new method for quantitative evaluation of the affinities of some drugs for various receptors (5). In this study, we determined the affinities of a large series of antihistamines for H_1 - and muscarinic receptors of bovine cerebral cortex using a receptor-binding assay and showed that there is a broad range of antimuscarinic potencies among the antihistamines.

Materials and Methods

Membrane preparations: Bovine brains were obtained from a local slaughterhouse, and stored at -80°C until use. Membrane preparations were prepared as described previously (6). Briefly, the cerebral cortex was homogenized in 20 volumes of ice-cold 50 mM Tris-HCl (pH 7.4) with a Kinematika Polytron PT-10 (setting 7, 20 sec). The homogenates were centrifuged three times for 10 min at $50,000\times g$ at 4°C with resuspension of the intermediate pellet from each centrifugation in the ice-cold Tris buffer. The final pellets were used immediately or stored at -80°C until assayed.

Radioligand-binding assay: ^3H -mepyra-

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mine and ^3H -quinuclidinyl benzilate (QNB) binding assays were performed as described previously (7, 8). Briefly, the membrane preparations were added to siliconized glass test tubes containing ^3H -mepyramine or ^3H -QNB and various drugs in the Tris buffer, with a final assay volume of 0.6 ml. The assay tubes were incubated for 30 min at 25°C for ^3H -mepyramine binding and for 60 min at 25°C for ^3H -QNB binding. The incubation was terminated by rapid filtration over poly-etylenimine-treated glass fiber filters (9) under vacuum. The filters were then rinsed rapidly with 20 ml of ice-cold Tris buffer. Radioactivity trapped on the filters was counted by liquid scintillation spectroscopy. Nonspecific bindings were determined in the presence of $2\text{ }\mu\text{M}$ triprolidine for ^3H -mepyramine binding and $1\text{ }\mu\text{M}$ atropine for ^3H -QNB binding. Protein was assayed by the method of Lowry et al. (10).

Materials: ^3H -mepyramine (24.1 Ci/mmol) and ^3H -QNB (30.1 Ci/mmol) were obtained from New England Nuclear. Other reagents were purchased from Sigma. Examined compounds were as follows: mepyramine, metapyrilen, tripeleennamine, dimenhydrinate and antazoline (Sigma); triprolidine and diphenhydramine (Tanabe Pharmaceutical); promethazine (Shionogi Pharmaceutical); d-chlorphenilamine (Shering); doxepin, hydroxyzine and meclizine (Taito-Pfizer); clemastine and ketotifen (Sandoz); homochlorcyclizine, diphenylpyraline and azelastine (Eisai); mequitazine and ranitizine (Shin-nypon Jistugyo); cyproheptazine (Merck Sharp & Dohme); alimemazine (Dai-ichi Pharmaceutical); pyratiazine (Upjohn); carbinoxamine (Taisho Pharmaceutical); dimethindene (Ciba-Geigy Japan); diphetol (Toyama Chemical); isothipendyl (Sumitomo Chemical); mebhydroline (Bayer); astemizole (Janssen Pharmaceutical); terfenadine (Dow Chemical); cimetidine (SK&F Fujisawa); famotidine (Yamanouchi Pharmaceutical).

Results

^3H -mepyramine binding: Specific ^3H -mepyramine binding to the membrane preparations from bovine cerebral cortex was saturable (Fig. 1A). Scatchard plot of specific

^3H -mepyramine binding was linear and gave a K_d value of $1.16 \pm 0.21\text{ nM}$ and a B_{max} value of $95.4 \pm 5.2\text{ fmol/mg protein}$ (Fig. 1A). These values are in good agreement with data in the literature (11, 12). As shown in Fig. 2A, the affinities of various H_1 -receptor antagonists and other drugs for the H_1 -receptor in the bovine cerebral cortex were determined according to the inhibition of ^3H -mepyramine binding. The inhibition constants

Fig. 1. Characterization of histamine H_1 - and muscarinic receptors in bovine cerebral cortex. A) Saturation of ^3H -mepyramine binding in bovine cerebral cortex. ^3H -mepyramine binding was measured as described in "Materials and Methods" using various concentrations of ^3H -mepyramine in the presence (for nonspecific binding) and absence (for total binding) of $2\text{ }\mu\text{M}$ triprolidine. Specific binding was the difference between total and nonspecific binding. The experiment was repeated three times with similar results. Inset: Scatchard plot. B) Saturation of ^3H -QNB binding in bovine cerebral cortex. ^3H -QNB binding was performed using various concentrations of ^3H -QNB in the presence and absence of $1\text{ }\mu\text{M}$ atropine. The experiment was replicated three times with similar results. Inset: Scatchard plot.

Fig. 2. Competition by antihistamines for ^3H -mepyramine (A) and ^3H -QNB (B) binding to the membrane preparations from bovine cerebral cortex. The concentration of ^3H -mepyramine and ^3H -QNB were 1 nM and the concentrations of unlabelled compounds were varied as indicated. (●) mepyramine, (○) diphenylpyraline, (■) triprolidine, (□) mequitazine, (▲) diphenhydramine, (△) antazoline. The experiment was repeated three times with similar results.

(K_i) were calculated from the equation $K_i = \text{IC}_{50} / (1 + C/K_d)$, where IC_{50} = concentration causing 50% inhibition of specific binding which was obtained from the displacement experiments, C = ^3H -ligand concentration used in the displacement experiment, and K_d = dissociation constant from the Scatchard analysis of the equilibrium experiments. Calculated K_i values of H_1 -receptor antagonists for ^3H -mepyramine binding were in the range of 0.24 nM (mepyramine) to 160 nM (antazoline, diphenhydramine) (Table 1). Doxepin, an antidepressant, exhibited the most potent H_1 -receptor antagonism, and all the three H_2 -receptor antagonists examined

showed very low affinities for the H_1 -receptor (Fig. 2A and Table 1).

^3H -QNB binding: Specific ^3H -QNB binding to the membrane preparations from bovine cerebral cortex was also saturable, and the Scatchard analysis gave a K_d value of 0.24 ± 0.02 nM and a B_{max} value of 1240 ± 270 fmol/mg protein (Fig. 1B). These values are in agreement with data in the literature (13, 14). The Scatchard plot of specific ^3H -QNB binding was linear, thereby indicating a single class of high affinity binding sites in the bovine cerebral cortex (Fig. 1B). As shown in Fig. 2B and Table 1, a group of H_1 -receptor antagonists (mequitazine, cyproheptazine, clemastine, diphenylpyraline, promethazine, homochlorcyclizine and alimemazine) showed a potent antagonism for the muscarinic receptor ($K_i = 5.0$ –38 nM). Another group of H_1 -receptor antagonists (mepyramine, terfenadine, metapyrilen, azelastine, hydroxyzine and meclizine) exhibited a weak antagonism of the muscarinic receptor ($K_i = 3,600$ –30,000 nM). Other H_1 -antagonists showed moderate affinity for the muscarinic receptors. Doxepin also showed high affinity for the muscarinic receptor, and three H_2 -antagonists had little cross-reactivity (Table 1). Hill coefficients for all antihistamines studied were essentially equal to unity (Table 1), suggesting the antagonistic nature of these compounds for muscarinic receptors (15).

The ratio of the K_i value with ^3H -mepyramine binding to that with ^3H -QNB binding for each drug was calculated in order to quantitatively evaluate the selectivity (Table 1). Except for several drugs (mepyramine, metapyrilen, ketotifen, dimethindene, azelastine and hydroxyzine, K_i value ratio = 590–130,000), most H_1 -receptor antagonists seemed to have substantial interaction with the muscarinic receptors. That is, thirteen compounds out of twenty-seven H_1 -antagonists had a ratio of less than 100, indicating considerably low selectivity. For example, mequitazine showed almost equal affinities for the H_1 - and the muscarinic receptors (K_i value ratio = 1.1).

Discussion

This paper presents the data of the binding

Table 1. Inhibition constants of antihistamines and related compounds for histamine H₁-receptors and muscarinic acetylcholine receptors in bovine cerebral cortex

	Inhibition of ³ H-QNB binding (K _{ia} , nM)	(nH)	Inhibition of ³ H-mepyramine binding (K _{ib} , nM)	K _i value ratio (K _{ia} /K _{ib})
H₁-antagonists				
Mequitazine	5.0±0.2	0.90±0.06	4.4 ±0.6	1.1
Cyproheptazine	8.8±0.4	0.92±0.04	2.6 ±0.2	3.4
Clemastine	16 ±2	0.97±0.06	0.26±0.03	62
Diphenylpyraline	20 ±2	0.92±0.05	0.84±0.09	24
Promethazine	22 ±2	0.82±0.05	2.6 ±0.2	8.5
Homochlorcyclizine	26 ±2	0.96±0.03	1.8 ±0.1	14
Alimemazine	38 ±3	0.95±0.04	0.72±0.10	53
Isothipendyl	74 ±8	0.92±0.05	0.42±0.04	180
Dimenhydrinate	160 ±10	0.93±0.07	8.4 ±0.4	19
Mebhydroline	180 ±20	0.88±0.10	7.6 ±0.6	24
Pyrazithiazine	220 ±10	0.89±0.04	1.3 ±0.2	170
Ketotifen	260 ±20	0.94±0.04	0.32±0.04	810
Diphenhydramine	280 ±50	1.0 ±0.1	14 ±2	20
d-Chlorpheniramine	300 ±20	0.96±0.06	2.6 ±0.3	120
Dimethindene	320 ±40	0.92±0.03	0.46±0.04	700
Triprolidine	500 ±50	0.95±0.06	2.2 ±0.4	230
Carbinoxamine	740 ±40	0.97±0.12	3.4 ±0.4	220
Tripelennamine	1300 ±200	0.92±0.05	7.4 ±0.6	180
Astemizole	2400 ±300	0.94±0.08	8.0 ±1.1	300
Dipheterol	3000 ±400	0.87±0.09	160 ±10	19
Antazoline	3000 ±400	0.82±0.09	160 ±10	19
Meclizine	3600 ±120	0.98±0.04	140 ±10	26
Hydroxyzine	3800 ±100	0.90±0.09	6.4 ±1.0	590
Azelastine	4200 ±500	1.1 ±0.1	6.4 ±0.7	660
Metapyrilene	5000 ±300	1.0 ±0.1	2.8 ±0.5	1800
Terfenadine	12000 ±1000	0.97±0.10	52 ±8	230
Mepyramine	30000 ±2000	0.99±0.04	0.24±0.03	130000
Antidepressant				
Doxepin	40 ±2	0.96±0.03	0.12±0.02	330
H₂-antagonists				
Famotidine	28000 ±1000	0.92±0.10	4000 ±200	7
Cimetidine	72000 ±560	1.0 ±0.1	26000 ±800	2.8
Ranitidine	120000 ±10000	1.0 ±0.1	90000 ±5000	1.3

The inhibition of specific binding of ³H-mepyramine (1 nM) and ³H-QNB (1 nM) binding was determined with seven to ten concentrations of competing drugs assayed in triplicate as shown in Fig. 2. The mean inhibitory concentration (IC₅₀) values were determined from the log-probit analysis, and K_i values were calculated from the equation described in "Results". The Hill coefficient (nH) was calculated as described by Bennett and Yamamura (15). Values are the means±S.E. from three independent experiments.

potencies of a large series of antihistamines at the muscarinic acetylcholine receptor and shows that there is a broad range of antimuscarinic potencies (K_i=5.0–30,000 nM) among the antihistamines. Except for several drugs, many classical and new antihistamines

were shown to have antimuscarinic effects. However, the antimuscarinic and antihistaminic potencies for the twenty-seven H₁-receptor antagonists studied were not significantly correlated (r=0.65), indicating that the structural requirement for the anti-

muscarinic effect is different from that for the antihistaminic effect. In general, ethanolamines (prototype: diphenhydramine) and phenothiazines (prototype: promethazine) were potent H_1 -receptor antagonists that possess significant antimuscarinic activity. On the other hand, ethylenediamines (prototype: mepyramine), alkylamines (prototype: chlorpheniramine) and piperazine (prototype: hydroxyzine) were selective H_1 -antagonists with no considerable antimuscarinic activity. Seventeen out of twenty-seven H_1 -receptor antagonists have K_i values for muscarinic receptors in the nanomolar range (5.0–740 nM); and in spite of its high K_i value (3,000 nM), antazoline was reported to have an atropine-like effect (4), probably due to its low selectivity for the H_1 -receptor (K_i value ratio=19). Consequently, compounds at the top of the lists (Table 1) will likely block muscarinic receptors at the blood concentrations achieved in clinical practice. Some H_1 -receptor antagonists with potent antimuscarinic activity are clinically used to combat motion sickness. These include ethanolamines (dimenhydrinate and diphenhydramine) and piperazines (meclizine and others). Since scopolamine was reported to be a potent drug for the prophylaxis and treatment of motion sickness (16), the anti-motion sickness activity of some of the H_1 -receptor antagonists seems to be related to their antimuscarinic ability. As the histamine H_1 -receptor blockade is suggested to be associated with the sedative activity (17), use of a drug which has both antimuscarinic and antihistaminic effects may be more effective in the treatment of motion sickness. The ranking of these drugs (Table 1) as antimuscarinic agents also can be used clinically to predict the likelihood that these drugs will cause certain adverse effects in patients such as dry mouth, blurred vision, urinary retention and constipation.

Thus, these results will provide helpful information with regard to the clinical and experimental use of antihistamines.

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