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Biochemical characterization of desloratadine, a potent antagonist of the human histamine H_1 receptor

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Abstract

We have characterized desloratadine (5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine, 8-chloro-6,11-dihydro-11-(4-piperidinylidene), CAS 100643-71-8) as a potent antagonist of the human histamine H_1 receptor. [^{3}H]Desloratadine bound to membranes expressing the recombinant human histamine H_1 receptor in Chinese hamster ovary cells (CHO-H_1) in a specific and saturable manner with a K_d of 1.1 ± 0.2 nM, a B_{max} of 7.9 ± 2.0 pmol/mg protein, and an association rate constant of 0.011 nM^{-1} min^{-1}. The K_d calculated from the kinetic measurements was 1.5 nM. Dissociation of [^{3}H]desloratadine from the human histamine H_1 receptor was slow, with only 37% of the binding reversed at 6 h in the presence of 5 μM unlabeled desloratadine. Seventeen histamine H_1-receptor antagonists were evaluated in competition-binding studies. Desloratadine had a K_i of 0.9 ± 0.1 nM in these competition studies. In CHO-H_1 cells, histamine stimulation resulted in a concentration-dependent increase in $[Ca^{2+}]_i$ with an EC_{50} of 170 ± 30 nM. After a 90-min preincubation with desloratadine, the histamine-stimulated increase in $[Ca^{2+}]_i$ was shifted to the right, with a depression of the maximal response at higher concentrations of antagonist. The apparent K_b value was 0.2 ± 0.14 nM with a slope of 1.6 ± 0.1. The slow dissociation from the receptor and noncompetitive antagonism suggests that desloratadine may be a pseudoirreversible antagonist of the human histamine H_1 receptor. The mechanism of desloratadine antagonism of the human histamine H_1 receptor may help to explain the high potency and 24-h duration of action observed in clinical studies.

Keywords: Histamine H_1 receptor, human; Desloratadine

1. Introduction

Histamine is a biogenic amine that has natural physiologic functions, including smooth muscle contraction, microvascular permeability, catecholamine release, and neuromodulatory roles in the brain and central nervous system (Hill et al., 1997). Histamine is released upon stimulation of mast cells and is one of the major mediators of the pathophysiology of the allergic response (Slater et al., 1999).

The pharmacology of histamine is mediated through four distinct G-protein coupled receptors classified as H_1, H_2, H_3, and H_4. All four human histamine receptors have been cloned, as have bovine and rat histamine H_1 receptors (DeBacker et al., 1993; Moguilevsky et al., 1994; Gantz et al., 1991; Yamashita et al., 1991; Lovenberg et al., 1999; Oda et al., 2000; Morse et al., 2001). Histamine binding to the histamine H_1 receptor results in increases in intracellular calcium ($[Ca^{2+}]_i$) mobilization (Kotlikoff et al., 1987) and is linked to the smooth muscle contraction and edema associated with the allergic response.

Histamine H_1 receptor antagonists inhibit the binding of histamine to the histamine H_1 receptor, prevent many of histamine's adverse effects, and are considered first-line therapy for the treatment of allergic diseases (Meltzer, 1998). Most of the older histamine H_1 receptor antagonists have the potential for adverse central nervous system effects and are characterized by poor receptor specificity. They bind to muscarinic receptors resulting in marked anticholinergic effects (Meltzer, 1998). Newer antihistamines, including

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loratadine, cetirizine, azelastine, and fexofenadine, have improved side-effect profiles, but some, notably cetirizine and azelastine, can also cause sedation (Slater et al., 1999). In addition, the histamine H_1-receptor antagonists, terfenadine and astemizole, demonstrated severe cardiovascular liabilities (Woosley et al., 1993; Craft, 1986), including torsades de pointes, a potentially fatal cardiac abnormality characterized by ventricular tachycardia (Monahan et al., 1990; Saviuc et al., 1993). This is believed to be due to blockade of the I_{Kr} channel, which inhibits the rapid delayed rectifier K^+ current and prolonged the action potential (Woosley et al., 1993).

Desloratadine is a new histamine H_1 receptor antagonist for the treatment of seasonal allergic rhinitis (Handley, 1999; Salmun et al., 2000). Preclinical studies showed desloratadine to be a potent and selective histamine H_1-receptor antagonist with relatively low affinities for muscarinic (K_i values of 50, 47, 104 and 320 nM at the human muscarinic M_1, M_2, M_4 and M_5 receptors, respectively) and histamine H_2 (K_i value of 353 nM) receptors (Handley et al., 1997; Kreutner et al., 2000). Desloratadine does not interact with the human ether-a-go-go-related gene (HERG) channel at concentrations up to 10 μM, suggesting a low potential for ventricular arrhythmias. In addition, desloratadine does not cross the blood–brain barrier and does not cause drowsiness or sedation (Hey et al., 2000). Clinical studies have demonstrated that desloratadine effectively improves nasal and nonnasal symptoms of allergic rhinitis and is not associated with adverse cardiovascular or central nervous system effects (Salmun et al., 2000). In this report, we have characterized the affinity, potency, and mechanism of the antagonism of desloratadine in receptor-binding and functional assays at the human histamine H_1 receptor cloned from human lung and expressed in Chinese hamster ovary (CHO) cells.

2. Materials and methods

2.1. Synthesis of [^{3}H]desloratadine

[^{3}H]Desloratadine was prepared by Tris-triphenylphosphine ruthenium (II) chloride catalyzed exchange with tritiated water (Fig. 1). Desloratadine (20 mg) and Tris-triphenylphosphine ruthenium (II) chloride (2 mg) were suspended in dioxane (100 μl) in a thick-wall glass ampule. Tritiated water (90 at.%, 20 Ci) was distilled in, and the ampule was capped, frozen in liquid nitrogen, evacuated, and flame sealed. The tube was heated for 3 h at 120 °C, after which it was cooled to room temperature and the contents were diluted with methanol and evaporated to dryness. The evaporation process was repeated a second time. A total yield of 3.2 Ci was obtained at a radiochemical purity of 60%. A 100-mCi portion of the batch was purified on a 9.4-mm × 25-cm YMC Basic column with a mobile phase of 0.05 M triethylammonium acetate, acetonitrile pH 4, (75:25) at a flow rate of 4 ml/min and detection at 280 nm. [^{3}H]Desloratadine had a retention time of 10.2 min. Fractions containing [^{3}H]desloratadine were pooled, evaporated to dryness, and stored in ethanol. Specific activity was 43.1 Ci/mmol.

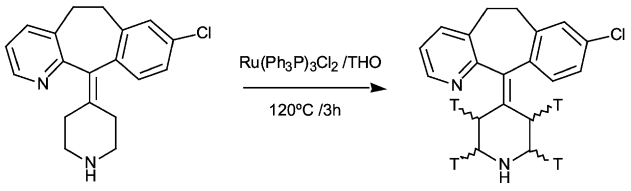

Fig. 1. Synthesis of [^{3}H]desloratadine. [^{3}H]Desloratadine was prepared by Tris-triphenylphosphine ruthenium (II) chloride catalyzed exchange with tritiated water.

2.2. Cloning and sequencing of the human histamine H_1 receptor

Oligonucleotides derived from the sequence of the human histamine H_1 receptor mRNA (Moguilevsky et al., 1994; Genbank accession number Z34897) was used to amplify a 1514-bp DNA fragment from human lung cDNA (Clontech Labs, Palo Alto, CA). The sequence of the sense primer was:

5′ CTGCAGATGAGCCTCCCCAATTCCTC – 3′

and the antisense primer was:

5′ GTCAAGCTTGTTGGACATCATAAGGAT – 3′.

Polymerase chain reaction amplification was carried out using KlenTaq polymerase and TaqStart antibody (hot-start) from Clontech, and the resulting polymerase chain reaction-product was cloned into the pNoTA/T7 shuttle vector (5 Prime/3 Prime, Boulder, CO). The insert was restriction digested with *Xba*I and subcloned into the pSRα-SPORT expression vector. The insert sequence was determined and found to be identical to that of the reported human histamine H_1 receptor.

2.3. Subcloning into pRc/RSV vector for establishment of a stable histamine H_1 receptor CHO cell line

The histamine H_1 receptor insert was digested with *Xba*I from the pSRα-SPORT vector and cloned into the *Xba*I multiple cloning site in the pRc/RSV vector. Clones were screened for orientation using the vector-specific pREP forward primer and the 3-prime gene-specific primer. An extraneous ATG codon upstream to the *Xba*I site was deleted using a *Pme*I and *Spe*I double digestion. The resulting plasmid was used to transform CHO cells, and a single stable cell line with high histamine H_1 receptor expression (CHO-H_1) was established by G418 selection.

2.4. Cell culture of CHO-H_1

CHO-H_1 cells as well as untransfected CHO cells were cultured in Dulbecco's minimal essential media containing

10% fetal calf serum (HyClone, Logan, UT), 1% nonessential amino acids, glutamine, and penicillin/streptomycin at 37 °C in a humidified atmosphere of 5% CO_2.

2.5. Membrane preparation and H_1-receptor-binding assay

CHO-H_1 cells were thawed at room temperature, resuspended in 5 volumes of ice-cold 50 mM Tris–HCl, pH 7.5, and left on ice to swell for 30 min. The cells were then disrupted with a Polytron at setting 6 for 30 s. The particulate material was centrifuged at $1000 \times g$ (3000 rpm, SS-34 rotor) for 10 min to remove nuclei and unbroken cells. The supernatant was centrifuged for 10 min at $50{,}000 \times g$ (20,000 rpm, SS-34 rotor). The high-speed pellet was suspended in a volume of Tris buffer equal to the original volume, a sample was taken for BCA protein assay (Pierce), and the suspension was centrifuged. The pellet was suspended in Tris at a protein concentration of 1 mg/ml and stored frozen at −70 °C.

Membrane (5 to 10 μg of protein) was incubated with various concentrations of [^{3}H]desloratadine (saturation binding experiments) or 2 nM [^{3}H]pyrilamine or [^{3}H]desloratadine (competition binding) without or with inhibitor compounds in a total volume of 200 μl of binding buffer (50 mM Tris–HCl, pH 7.5). Nonspecific binding was determined in the presence of 10^{-6} M chlorpheniramine. Assay mixtures were incubated for 60 min at room temperature in polypropylene, 96-well, deep-well plates and then filtered through 0.3% polyethylenimine-soaked GF/B filters. These were washed three times with 1.2 ml Tris buffer, dried in a microwave oven, impregnated with Meltilex wax scintillant, and counted at 40% efficiency in a Betaplate scintillation counter (Wallac).

2.6. Association experiments

CHO-H_1 membranes were incubated with various concentrations of [^{3}H]desloratadine or [^{3}H]pyrilamine in a total volume of 200 μl of binding buffer without or with 10^{-6} M chlorpheniramine (nonspecific binding) at room temperature. Assay mixtures were incubated in polypropylene, 96-well, deep-well plates and then filtered through 0.3% polyethylenimine-soaked GF/B filters at various times and processed as previously described.

2.7. Dissociation experiments

CHO-H_1 membranes were incubated with 2 nM [^{3}H]desloratadine or [^{3}H]pyrilamine in a total volume of 200 μl of binding buffer without or with 10^{-6} M chlorpheniramine (nonspecific binding) at room temperature for either 60 min ([^{3}H]desloratadine)or 30 min ([^{3}H]pyrilamine). Dissociation of [^{3}H]desloratadine was initiated by the addition of 5 μM desloratadine, and samples were filtered at various times up to 6 h. Dissociation of [^{3}H]pyrilamine was initiated by the addition of 5 μM pyrilamine, and samples were filtered at various times up to 40 min. These filters were processed as previously described.

2.8. Calcium flux assay

Cells were seeded in clear, flat-bottomed, black-wall, 96-well plates 1 to 3 days prior to assay at a density of 50,000 cells/well. On the day of the assay, the media was removed and cells were incubated for 1 h at 37 °C in a buffer (Hank's buffered saline solution, 20 mM HEPES, 0.4% bovine serum albumin, 2.5 mM probenecid) containing 4 μM Fluo-3 A.M. (Molecular Probes, Eugene, OR). Cells were washed four times with buffer utilizing a Labsystems Cellwash plate washer, leaving 100 μl of buffer in each well. Solutions containing the appropriate concentration of antagonist or buffer were added to the cells and incubated at 37 °C for 90 min before the addition of various concentrations of histamine in 50 μl volume. In a typical experiment, triplicate determinations of four drug concentrations were measured in the presence of 10 concentrations of histamine. Changes in intracellular calcium were measured with a fluorometric imaging plate reader (FLIPR) by excitation of the calcium-sensitive fluorescent dye with an argon laser at 488 nm and measuring emission in the 500–560-nm range. Data are presented as percentage of maximum peak height relative to that with 100 μM histamine.

2.9. Data analysis

Specific binding was defined as the difference between total and nonspecific binding. Data from saturation and competition binding experiments were analyzed by PRISM software (GraphPad Software, San Diego, CA) using a nonlinear least squares regression analysis. PRISM determined K_i values based on the Cheng and Prusoff (1973) equation of $K_i = IC_{50}/(1+(L)/K_d)$. Association of [^{3}H]desloratadine and [^{3}H]pyrilamine was determined by measuring the kinetics of association at four ligand concentrations. Specific binding data were analyzed by PRISM and observed rate constant values (K_{obs}) were plotted against the various radioligand concentrations, and linear regression was performed to obtain the slope (k_1).

For functional studies, agonist potency is expressed as an EC_{50} (concentration of histamine causing a 50% increase relative to the maximal response). The EC_{50} value was calculated using PRISM software. Antagonist affinity is expressed as an apparent K_b value. This value was obtained by experimentally determining the agonist dose ratio (A′/A) where A′ and A are the equieffective agonist concentrations in the presence (A′) and absence (A) of the antagonist. The log of the dose ratio minus 1 ($\log[A'/A - 1]$) was graphed on the y-axis versus the negative log of the antagonist concentration on the x-axis, and a linear regression was performed to determine the slope of the line. Mean values and the S.E.M. were calculated using the program StatView (Abacus Concepts, Berkeley, CA).

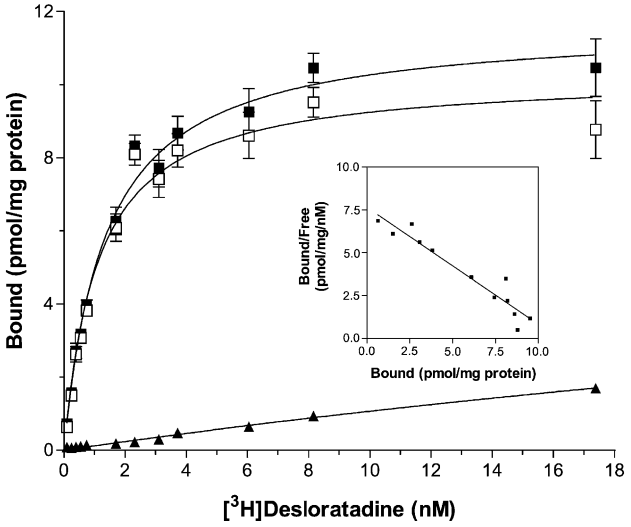

Fig. 2. Saturation isotherm of [^{3}H]desloratadine binding to CHO-H_1 cell membranes. Membrane was incubated with various concentrations of [^{3}H]desoratadine as described in Materials and methods in the absence (■) or presence (▲) of 10^{-6} M chlorpheniramine. Specific binding (□) was defined as the difference between total and nonspecific binding. Data are the mean and S.E.M. from triplicate determinations from a typical experiment performed three times. Inset: Scatchard transformation of specific binding.

2.10. Materials

[^{3}H]Pyrilamine (23 Ci/mmol) was obtained from Dupont Life Sciences NEN (Boston, MA). [^{3}H]Desloratadine (43.1 Ci/mmol) was synthesized by the Department of Radiochemistry at Schering-Plough Research Institute (Kenilworth, NJ). Racemic chlorpheniramine maleate (*N*-(4-chloro-phenyl)-*N*′,*N*′-dimethyl-*N*-pridin-2-yl-ethane-1,2-diamine) and pyrilamine *N*-[(4-methoxyphenyl)methyl]-*N*′,*N*′-dimethyl-*N*-2-pyridinyl-1,2-ethanediamine) were obtained from RBI (Natick, MA). Diphenhydramine (2-(diphenylmethoxy)-*N*,*N*-dimethyl-ethanamine), hydroxyzine (2-{2-[4-(4-chloro-benzhydryl)-piperazin-1-yl}-ethoxy}-ethanlol), and terfenadine (1-4-*tert*-butyl-phenyl)-4-[4-(hydroxy-diphenyl-methyl)-piperidin-1-yl]-butan-1-ol) were obtained from Sigma (St. Louis, MO). Astemizole (1-(4-fluorobenzyl)-2((1-(*p*-methoxyphenethyl)-4-piperidyl)amino)-benzimidazole, azelastine (4-[(4-chlorophenyl)methyl]-2-(hexadhydro-1-methyl-1*H*-azepin-4-yl)-1(2*H*)-phthalazinone, carebastine (alpha,alpha-dimethyl-4-[4-[4-(dipheylmethoxy)-1-piperidinyl]-1-oxobutyl]benzene acetic acid, cetirizine ([2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]acetic acid), desloratadine, ebastine (1-[4-(1,1-dimethylethyl)phenyl]-4-[4-(diphenylmethoxy)-1-piperidinyl]-1-butanone), epinastine (3-amino-9-13*b*,dihydro-1*H*-dibenz[*c*,*f*]imidazo[1,5-*a*]azepine), fexofenadine (4-[1-hydroxy-4-[4-(hydroxy-diphenylmethyl)-1-pipiridinyl]butyl]alpha,alpha-dimethylbenzene acetic acid), ketotifen (4-(1-methyl-4-piperidylidene)-4*H*benzo(4,5)cyclohepta(1,2*B*)thiophene-10-(9*H*)-one), loratadine (4-(8-chloro-5,6-dihydro-11*H*-benzo[5,6]-cyclohepta[1,2-*b*]pyridin-11-ylidene)-1-piperidine carboxylic acid ethyl ester), and mizolastine (2-[[1-[-[(4-flurophenyl)methyl]-1*H*-benzimidazol-2-yl]-4-pipiridinyl]methylamino]-4-pyrimidinol) were obtained from the Department of Chemistry at Schering-Plough Research Institute.

3. Results

3.1. Saturation-binding studies

The affinity of [^{3}H]desloratadine for the human histamine H_1 receptor was determined by saturation-binding analysis. The binding of [^{3}H]desloratadine ($K_d = 1.1 \pm 0.2$ nM; $B_{max} = 7.9 \pm 2$ pmol/mg protein) to the human histamine H_1 receptor expressed in CHO cells was specific, saturable, and of high affinity (Fig. 2). Scatchard transformation of the [^{3}H]desloratadine saturation isotherm demonstrated that the binding was to a single population of receptor sites (Fig. 2). No specific binding of [^{3}H]desloratadine was detected on membranes from untransfected CHO cells (data not shown).

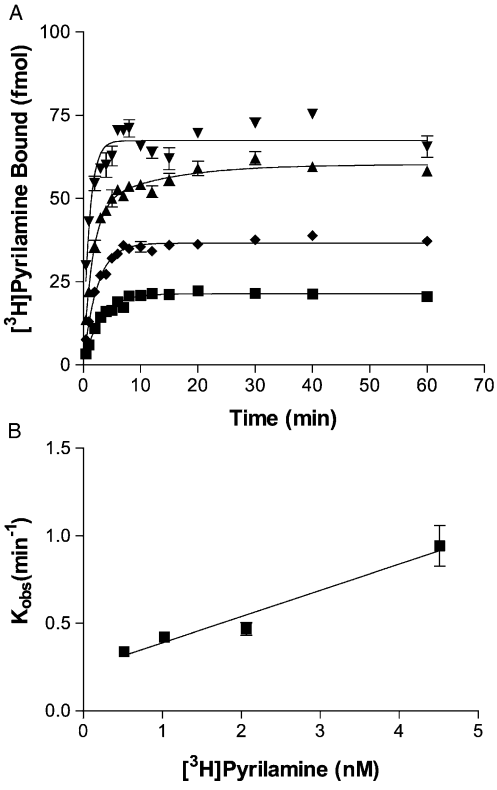

Fig. 3. Association of [^{3}H]pyrilamine with the human histamine H_1 receptor. (A) Specific binding of [^{3}H]pyrilamine (■, 0.5 nM; ◆, 1.0 nM; ▲, 2.1 nM; ▼, 4.5 nM) at various times. (B) Correlation between the observed rate constant (K_{obs}) at each concentration of ligand was determined as described in Data analysis. Data presented are a representative experiment performed in triplicate.

3.2. Kinetic studies

Kinetic analysis of [^{3}H]pyrilamine binding (Fig. 3A and B) indicated that binding was rapid, concentration dependent, and reached steady state as a function of ligand concentration. The K_{obs} measured for 0.51, 1.0, 2.1, and 4.5 nM [^{3}H]pyrilamine were 0.34, 0.42, 0.47, and 0.94 min^{-1}, respectively. The association rate constant (k_1) calculated from the slope of this line was 0.15 nM^{-1} min^{-1}, and the dissociation rate constant (k_{-1}) determined from the y-intercept was 0.24 min^{-1}. The K_d calculated from these kinetic measurements (k_{-1}/k_1) was 1.6 nM, consistent with the K_d of 1.2 nM determined from saturation-binding experiments.

Association kinetic analysis of [^{3}H]desloratadine binding (Fig. 4A and B) indicated that binding was concentration dependent, and reached steady state as a function of ligand concentration. The K_{obs} measured for 0.35, 0.73, 1.6, and 3.9 nM [^{3}H]desloratadine were 0.015, 0.027, 0.032, and 0.056 min^{-1}, respectively. The k_1 calculated from the slope of this line was 0.011 nM^{-1} min^{-1}, and the k_{-1} determined from the y-intercept was 0.015 min^{-1}. The K_d calculated from these kinetic measurements was 1.4 nM, consistent with the K_d of 1.1 nM determined from saturation-binding experiments.

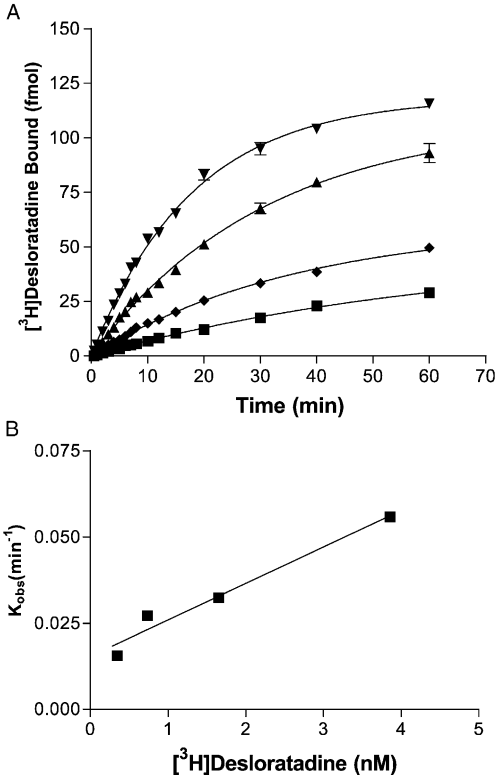

Fig. 4. Association of [^{3}H]desloratadine with the human histamine H_1 receptor. (A) Specific binding of [^{3}H]desloratadine (■, 0.35 nM; ♦, 0.73 nM; ▲, 1.7 nM; ►, 3.86 nM) at various times. (B) Correlation between the observed rate constant (K_{obs}) at each concentration of ligand was determined as described in Data analysis. Data presented are a representative experiment performed three times in triplicate.

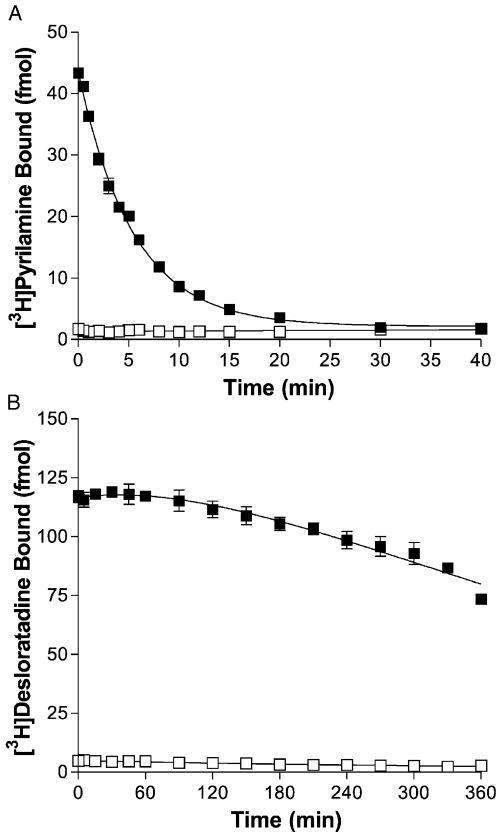

Fig. 5. Dissociation of [^{3}H]pyrilamine (A) and [^{3}H]desloratadine (B) from the human histamine H_1 receptor. Ligand was incubated with the human histamine H_1 receptor as described in Materials and methods for either 60 min ([^{3}H]desloratadine) or 30 min ([^{3}H]pyrilamine), and dissociation was initiated with the addition of 5 μM of unlabeled ligand. Data are from a representative experiment done in triplicate performed three times.

The kinetics of [^{3}H]pyrilamine and [^{3}H]desloratadine binding to the human histamine H_1 receptor was further characterized in dissociation experiments. Fig. 5A demonstrates that [^{3}H]pyrilamine dissociated from the human histamine H_1 receptor upon addition of 5 μM pyrilamine. In this experiment, the $t_{1/2}$ was 3.8 min and the k_{-1} was 0.18 min^{-1}. This value is in close agreement with the k_{-1} value of 0.24 min^{-1} obtained from the association experiments. The K_d calculated from these kinetic measurements (k_{-1}/k_1) was 1.2 nM.

Dissociation of [^{3}H]desloratadine from the human histamine H_1 receptor was slow, with only 37% of the ligand dissociated 6 h after the addition of 5 μM desloratadine (Fig. 5B).

3.3. Competition-binding studies

For a potency comparison among a number of antihistamines, competition-binding studies were conducted with [^{3}H]pyrilamine and [^{3}H]desloratadine as the labeled ligands. The competition curves are shown in Fig. 6, and the affinity

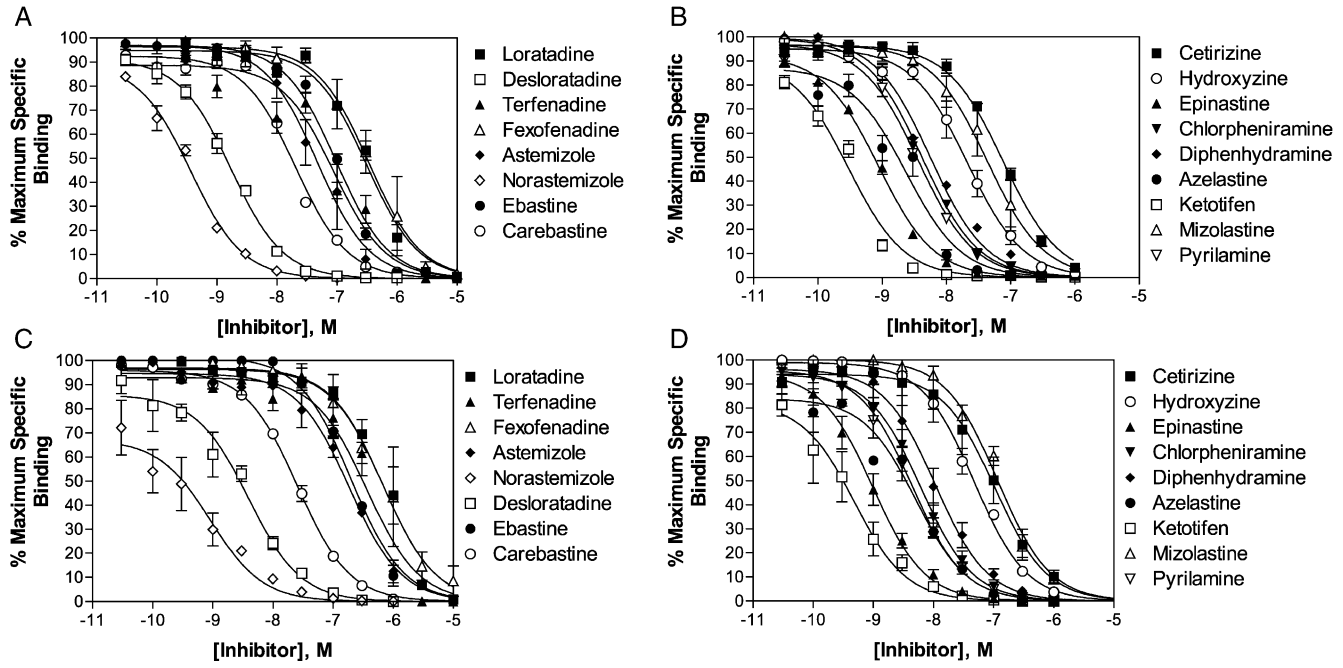

Fig. 6. Inhibition of [^{3}H]pyrilamine binding (A,B) or [^{3}H]desloratadine (C,D) to the cloned human histamine H_1 receptors expressed on CHO membranes by various antihistamines. Membranes from CHO-H_1 cells were incubated with 2.0 nM [^{3}H]pyrilamine or [^{3}H]desloratadine without or with various concentrations of inhibitor compounds. Data are the mean and S.E.M. from triplicate determinations from competition-binding experiments performed three or four times. Data are presented as a percentage of maximal specific binding in the absence of competitor.

constants (K_i) of each antihistamine are summarized in Table 1. Desloratadine, norastemizole, ketotifen, and epinastine were among the most potent compounds, with K_i values less than 1 nM when [^{3}H]pyrilamine was the ligand. Compared with other commonly used antihistamines, desloratadine was 52, 57, 194, and 153 times more potent than cetirizine, ebastine, fexofenadine, and loratadine, respectively. Similar K_i values were obtained when [^{3}H]desloratadine was used as the ligand in competition-binding studies (Table 1).

Table 1
Receptor-binding affinity of histamine antagonists for the human histamine H_1 receptor

Compound	K_i (nM)	
	[^{3}H]Pyrilamine	[^{3}H]Desloratadine
Astemizole	26.6 ± 11.3	83 ± 11
Azelastine	1.1 ± 0.3	3.1 ± 1
Carebastine	9.7 ± 0.9	11.2 ± 0.3
Cetirizine	47.2 ± 10	73 ± 8
Chlorpheniramine	2.0 ± 0.2	2.1 ± 0.2
Desloratadine	0.9 ± 0.08	1.6 ± 0.01
Diphenhydramine	2.5 ± 0.2	5.2 ± 1.1
Ebastine	51.7 ± 6.8	120 ± 20
Epinastine	0.4 ± 0.06	0.4 ± 0.1
Fexofenadine	175 ± 68	188 ± 26
Hydroxyzine	10 ± 1.8	19 ± 1.3
Ketotifen	0.14 ± 0.01	0.2 ± 0.03
Loratadine	138 ± 23	171 ± 38
Mizolastine	22 ± 5.9	30 ± 7
Norastemizole	0.2 ± 0.02	0.4 ± 0.1
Pyrilamine	1.7 ± 0.08	2 ± 0.3
Terfenadine	40 ± 4.6	48 ± 12

[^{3}H]Pyrilamine or [^{3}H]desloratadine was incubated with membranes from CHO-H_1 cells as described in Materials and methods. K_i values (mean ± S.E.M.) were obtained from three to five independent experiments performed in triplicate.

3.4. $[Ca^{2+}]_i$ flux studies

Histamine stimulated increase in $[Ca^{2+}]_i$ in CHO-H_1 cells was used to evaluate the potency and nature of the antagonism of desloratadine and pyrilamine. The increase in $[Ca^{2+}]_i$ due to histamine stimulation was rapid, attaining a maximum within 20 s, and remained elevated for several minutes. In the absence of extracellular calcium, there was a smaller and less sustained increase in $[Ca^{2+}]_i$. These data suggest that the sustained portion of $[Ca^{2+}]_i$ increase is due to the influx of extracellular calcium (data not shown).

Histamine induced an increase in $[Ca^{2+}]_i$ with an EC_{50} of 170 ± 30 nM. A time course to determine the optimal preincubation time for histamine H_1-receptor antagonists demonstrated that maximal inhibition was achieved with 90 min of preincubation (data not shown). Dose-ratio determinations were performed to determine the nature of the antagonism. Desloratadine produced rightward shifts of the histamine concentration curve with a suppression of the maximal response (Fig. 7A). The inability of higher concentrations of histamine to overcome the inhibition by desloratadine suggested that desloratadine inhibited histamine activation of the human histamine H_1 receptor in a noncompetitive manner. Schild analysis of the data demon-

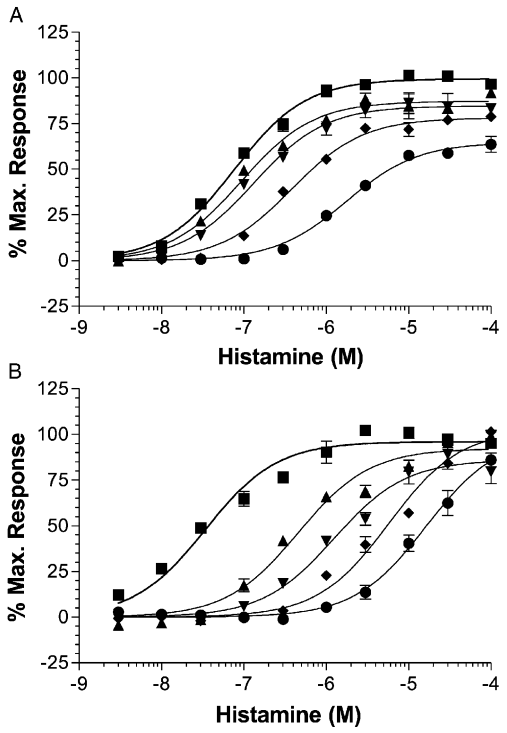

Fig. 7. Antagonism of histamine-stimulated increase in $[Ca^{2+}]_i$ in CHO-H_1 cells by desloratadine and pyrilamine. (A) Desloratadine (■, control; ▲, 0.3 nM; ►, 1 nM; ♦, 3 nM; ●, 10 nM) and (B) pyrilamine (■, control; ▲, 3 nM; ►, 10 nM; ♦, 30 nM; ●, 100 nM) were incubated for 90 min before the addition of histamine. Peak fluorescent responses induced by histamine are expressed as a percentage of the maximal response of 100 μM histamine.

strated a slope of 1.6, which is also consistent with noncompetitive interactions, and an apparent K_b of 0.2 nM. Pyrilamine (Fig. 7B) caused a shift of the histamine concentration curve without a suppression of the maximal response. Schild analysis of this data demonstrated a slope of 1.1 and a K_b of 1.3 nM, consistent with a competitive interaction of pyrilamine at the human histamine H_1 receptor.

4. Discussion

In this report, we have characterized the pharmacology of desloratadine at the human histamine H_1 receptor that was cloned from human lung tissue and recombinantly expressed in CHO cells. The human histamine H_1 receptor was originally cloned from a human lung cDNA library and expressed in CHO and COS cells. In those studies, [^{3}H]pyrilamine bound with a K_d of 3.7 nM (Moguilevsky et al., 1994) and 1.2 nM (DeBacker et al., 1993). This is the first report characterizing [^{3}H]desloratadine at the human histamine H_1 receptor. The K_d of [^{3}H]desloratadine (1.1 nM) determined from saturation experiments and K_i of 1.6 nM from competition binding are in agreement with the K_i values of 2.5 and 4.1 nM for desloratadine obtained from competition-binding studies with [^{3}H]pyrilamine in guinea pig lung and brain, respectively (Kreutner et al., 2000; Ter Laak et al., 1993). In the present receptor-binding studies, desloratadine was more potent than other commonly used antihistamines, including cetirizine, ebastine, fexofenadine, and loratadine.

[^{3}H]Pyrilamine dissociated rapidly from the human histamine H_1 receptor with a $t_{1/2}$ of 3.8 min. This is in agreement with a $t_{1/2}$ of 4 min obtained in guinea pig cerebellum (Treherne and Young, 1988). In contrast, desloratadine dissociated slowly from the human histamine H_1 receptor. In this regard, [^{3}H]desloratadine may represent a useful tool for evaluating histamine H_1 receptor biology due to the high specific binding, high affinity and the slow dissociation.

Histamine binding to the human histamine H_1 receptor endogenously expressed in human airway smooth muscle, astrocytoma 1321N1, and HeLa cells or recombinantly expressed in human embryonic kidney cells (HEK293) or CHO cells has been reported to result in an increase in $[Ca^{2+}]_i$, and the sustained phase of the increase was dependent on extracellular calcium (Murray and Kotlikoff, 1991; Miller et al., 1999; Ohuchi et al., 1998). The EC_{50} values for histamine-stimulated $[Ca^{2+}]_i$ increase were reported as 90 nM in HeLa S3, 190 nM in 1321N1 astrocytoma cells, and 14 and 74 nM when the human histamine H_1 receptor was recombinantly expressed in HEK293 cells. Furthermore, an EC_{50} of 40 nM was demonstrated for histamine in a [^{3}H]inositol phosphate assay in HEK293 cells transiently transfected with either the guinea pig or human histamine H_1 receptor (Wieland et al., 1999; Bakker et al., 2001). In this study, an EC_{50} for histamine-stimulated $[Ca^{2+}]_i$ in the CHO-H_1 cells was experimentally determined to be 170 nM, and the sustained phase of the increase was dependent on extracellular calcium. In the present experiments, histamine-stimulated $[Ca^{2+}]_i$ was utilized to determine the potency of desloratadine and pyrilamine. Pretreatment of CHO-H_1 cells with desloratadine resulted in a concentration-dependent rightward shift of the histamine concentration curve with suppression of the maximum at higher antagonist concentrations. Thus, the antagonism produced by desloratadine appears noncompetitive in this assay. However, desloratadine does not interact in an irreversible manner because this functional antagonism could be reversed slowly with washing (data not shown). In contrast, pyrilamine caused a concentration-dependent rightward shift of the histamine concentration curve without suppression of the maximum. These results demonstrated that pyrilamine is a competitive antagonist. In calcium assays, the response to histamine was within 20 s, and histamine would not be expected to attain a steady state with the receptor at this time (Miller et al., 1999; Christopoulos et al., 1999). Therefore, apparent K_b values were calculated for the antagonists although the equilibrium states of agonists were apparently not fulfilled under these conditions. Desloratadine had a K_b value of 0.2 nM, which was in close

agreement with the K_i value of 0.9 nM from competition-binding studies. Desloratadine, a metabolite of loratadine, is 153 times more potent that loratadine in receptor-binding studies at the human histamine H_1 receptor. Desloratadine is 65 times more potent than loratadine in receptor-binding studies with guinea pig lung (Ter Laak et al., 1993).

Recently, all histamine H_1 receptor antagonists evaluated have been demonstrated to behave as inverse agonists when the recombinant human histamine H_1 receptor is transiently expressed in COS cells measuring either inositol phosphate formation or NF-κB activation (Bakker et al., 2000; Bakker et al., 2001). We evaluated desloratadine and pyrilamine in the CHO cells that stably expressed the human histamine H_1 receptor using a [^{35}S]GTPγS assay. Histamine resulted in an increase in [^{35}S]GTPγS binding to membranes, which could be antagonized by desloratadine or pyrilamine. However, we were unable to detect a decrease in basal [^{35}S]GTPγS with desloratadine or pyrilamine under conditions used for this assay.

Insurmountable or pseudoirreversible antagonism in functional assays has been demonstrated for other G-protein-coupled receptors, including angiotensin II and neurokinin-1 receptors (Panek et al., 1995; Cascieri et al., 1997). In those studies, antagonists demonstrated a slow dissociation rate from the receptor and apparent noncompetitive interaction in functional assays. The high affinity and slow dissociation rate from the human histamine H_1 receptor and the long plasma half-life of desloratadine may be factors that account for the long duration of action demonstrated in vivo.

In summary, desloratadine has been shown to bind with high affinity to the human histamine H_1 receptor in saturation, kinetic, and competition-binding assays. Desloratadine antagonized histamine at the human histamine H_1 receptor in a functional assay in an insurmountable manner, suggesting that the high affinity and potency may be due to the slow dissociation of desloratadine from the human histamine H_1 receptor.

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