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8R-Lisuride Is a Potent Stereospecific Histamine H_1-Receptor Partial Agonist

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ABSTRACT

The human histamine H_1 receptor (H_1R) is an important, well characterized target for the development of antagonists to treat allergic conditions. Many neuropsychiatric drugs are known to potently antagonize the H_1R, thereby producing some of their side effects. In contrast, the tolerability and potential therapeutic utility of H_1R agonism is currently unclear. We have used a cell-based functional assay to evaluate known therapeutics and reference drugs for H_1R agonist activity. Our initial functional screen identified three ergot-based compounds possessing heretofore-unknown H_1R agonist activity. 8R-lisuride demonstrated potent agonist activity in various assays including receptor selection and amplification technology, inositol phosphate accumulation, and activation of nuclear factor-κB with pEC_{50} values of 8.1, 7.9, and 7.9, respectively, and with varying degrees of efficacy. Based on these assays, 8R-lisuride is the most potent stereospecific partial agonist for the human H_1R yet reported. Investigation of the residues involved in histamine and lisuride binding, using H_1R mutants and molecular modeling, have revealed that although these ligands are structurally different, the lisuride-binding pocket in the H_1R closely corresponds to the histamine-binding pocket. The discovery of a potent stereospecific partial H_1R agonist provides a valuable tool to further characterize this important therapeutic target in vitro.

Histamine is a well characterized proinflammatory mediator of allergic responses (Majno and Palade, 1961; Assanasen and Naclerio, 2002). The $G\alpha_{q/11}$-coupled histamine H_1 receptor (Gutowski et al., 1991; Leopoldt et al., 1997), one of four known human histamine receptor subtypes, is expressed in a variety of cells and tissues, where it mediates many of the histamine-induced symptoms of allergic reactions. Pharmacological blockade of peripheral H_1 receptors effectively attenuates these actions, resulting in clinical efficacy against allergic reactions (Simons and Simons, 2002). Consequently, H_1-receptor antagonists have become one of the most widely prescribed drug families in Western countries (Woosley, 1996).

In contrast to the well defined clinical utility of H_1R antagonism, the therapeutic value of H_1R agonism remains unclear. Betahistine, N^{α}-methyl-2-(pyridine-2-yl) ethyl-1-amine (N^{α}-methyl-PEA), a H_1-receptor agonist with micromolar potency that also exhibits H_3-receptor antagonist properties (Arrang et al., 1985), has been used in the treatment of Ménière's disease, but the therapeutic action has not been clearly linked to its H_1R agonism. Activation of H_1-receptors may play a specific role in seizures (Scherkl et al., 1991; Yokoyama et al., 1994), where H_1R agonists may act as

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ABBREVIATIONS: PEA, 2-(pyridine-2-yl) ethyl-1-amine; H_nR, histamine *n* receptor, where *n* is 1 to 4; R-SAT, receptor selection and amplification technology; NF-κB, nuclear factor-κB; HBSS, Hanks' balanced salt solution; 8R-lisuride, (*R*)-(+)-N′-[(8α)-9,10-didehydro-6-methylergolin-8-yl]-*N*,*N*-diethylurea hydrogen maleate; 8S-lisuride, (*S*)-(−)-*N*′-[(8α)-(−)-9,10-didehydro-6-methylergolin-8-yl]-*N*,*N*-diethylurea; 8R-terguride, (*R*)-(+)-terguride; 8S-terguride, (*S*)-(−)-terguride maleate; LY-53,857, 6-methyl-1-[1-methylethyl]-ergoline-8β-carboxylic acid 2-hydroxy-1-methylpropyl ester maleate; LSD, lysergic acid diethylamide; Org 3770, (±)-1,2,3,4,10,14b-hexahydro-2-methylpyrazino-[2,1-a]pyrido[2,3-c][2]benzazepine; PCR, polymerase chain reaction; DMEM, Dulbecco's modified Eagle's medium; CRC, contractile response curves; APT, aminopotentidine; MD, molecular dynamics.

anticonvulsants (Tuomisto and Tacke, 1986; Yokoyama et al., 1992), whereas H_1R antagonists may induce (Schwartz and Patterson, 1978; Yokoyama et al., 1993) or exacerbate (Iinuma et al., 1993) seizures. In addition, there is increasing evidence of H_1R involvement in arousal, cognition, and memory (Malmberg-Aiello et al., 2000; Malmberg-Aiello et al., 1998; Tashiro et al., 2002). Although histamine H_1-receptor agonists have attracted attention as pharmacological tools during the last years, highly potent and specific H_1 receptor agonists have been lacking. Testing of compounds for H_1R activity has usually been performed on isolated tissue preparations, such as the ileum or aorta from the guinea pig (Zingel et al., 1995), despite pronounced species-dependent pharmacological characteristics (see Seifert et al., 2003). These efforts have yielded the specific H_1R agonists PEA (Walter et al., 1941), the substituted 2-phenylhistamines, such as 2-(3-trifluoromethylphenyl)histamine (Zingel et al., 1995), and recently the more active histaprodifens (Elz et al., 2000b) (see Fig. 1), none of which have yielded viable therapeutics.

We have used radioligand binding and multiple functional assays to further explore the clinical pharmacology of the human H_1R. Interestingly, a broad functional screen has identified lisuride (Fig. 1), a drug used for the treatment of Parkinson's disease, as a potent stereospecific H_1R agonist. Lisuride potently activated the cell based functional assay R-SAT, phosphatidylinositol hydrolysis, and an NF-κB–driven reporter-gene assay with varying degrees of efficacy and consequently demonstrated antagonistic actions in calcium mobilization and guinea pig ileum contraction experiments, consistent with its potent partial agonist properties at the histamine H_1 receptor. Finally, molecular modeling and radioligand binding and functional studies using structurally related compounds suggest that lisuride, although structurally dissimilar to histamine, interacts with the human H_1R via a ligand-binding pocket that closely resembles the histamine-binding pocket.

Materials and Methods

Materials. Cell culture media, penicillin, bovine calf serum, High-Fidelity Platinum *Taq* DNA Polymerase, and streptomycin were obtained from Invitrogen (Carlsbad, CA), fetal calf serum from Integro (Zaandam, The Netherlands), G-418 disulfide from Calbiochem (Amsterdam, The Netherlands), Hanks' buffered saline solution (HBSS) from Invitrogen, Eagle's minimal essential medium from Cambrex Bio Science Verviers S.p.r.l. (Verviers, Belgium), and Cyto-SF_3 from Kemp Laboratories (Frederick, MD). [^{3}H]Mepyramine (20 Ci/mmol), [N^{α}-methyl-^{3}H]histamine (82 Ci/mmol), [^{3}H]histamine (12.4 Ci/mmol), and [myo-2-^{3}H]inositol (21 Ci/mmol) were purchased from PerkinElmer Life and Analytical Sciences (Zaventem, Belgium). pNF-κB-Luc was obtained from Stratagene (La Jolla, CA), pSI from Promega (Madison, WI), the TOPO 2.1 vector from Invitrogen, and Superfect from QIAGEN (Dusseldorf, Germany).

The sources of many of the drugs used in this study have been reported previously (Wellendorph et al., 2002). In addition, dihydroergotamine mesylate, methysergide maleate, methylergonovine maleate (Methergine), and dihydroergocristine mesylate were purchased from Tocris (Avonmouth, UK). Lysergol (97%) and D-lysergic acid hydrate (95%) were purchased from Acros Organics (Geel, Belgium). 5′(α)-2-Bromo-12′-hydroxy-2′-(1-methylethyl)-5′-(2-methylpropyl)ergotaman-3′,6′,18trione mesylate, ketotifen fumarate, (*R*)-(+)-N′-[(8α)-9,10-didehydro-6-methylergolin-8-yl]-*N*,*N*-diethylurea hydrogen maleate (8R-lisuride), (*S*)-(−)-*N*′-[(8α)-(−)-9,10-didehydro-6-methylergolin-8-yl]-*N*,*N*-diethylurea (8S-lisuride), (*R*)-(+)-terguride (8R-terguride), and (*S*)-(−)-terguride maleate (8S-terguride) were purchased from ICN Biomedicals, Inc. (Zoetermeer, The Netherlands). Aminopotentidine, ATP disodium salt, bovine serum albumin, chicken egg albumin, chloroquine diphosphate, cholera toxin, DEAE-dextran (chloride form), cimetidine, doxepin hydrochloride, histamine (2-[4-imidazolyl]ethylamine hydrochloride), pyrilamine maleate, *N*-CBZ-[(8β)-1,6-dimethylergolin-8-yl]-methylamine, LY-53,857, lysergic acid diethylamide (LSD), 8β-[(methylthio)methyl]-6-propylergoline mesylate (>98%), polyethylenimine, probenecid, (*S*)-(α)-fluoromethylhistidine, tripelennamine hydrochloride, triprolidine hydrochloride, and Tween 20 were purchased from Sigma/RBI (Natick, MA). D-Luciferin was obtained from Duchefa Biochemie BV (Haarlem, The Netherlands), Fluo-4 acetoxymethyl ester

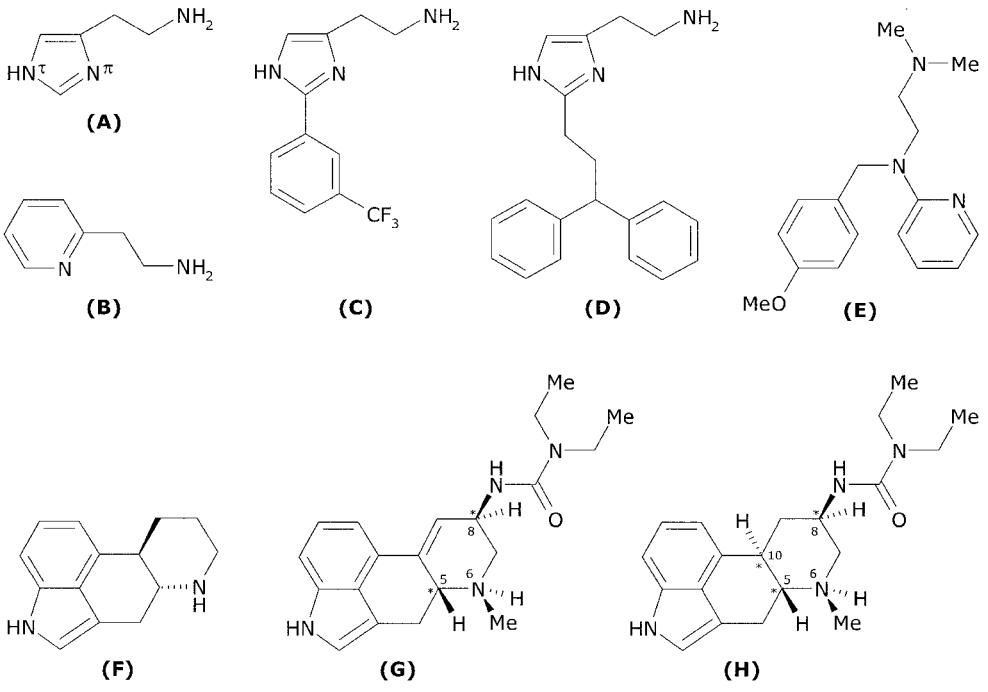


Fig. 1. Chemical structures of various H_1R ligands. Structures are shown for the agonists histamine (A), PEA (B), 2-(3-trifluoromethylphenyl)histamine (C), and histaprodifen (D), the H_1R inverse agonist mepyramine (E), and the ergoline skeleton (F) (Mantegani et al., 1999), 8R-lisuride (G), and 8R-terguride (H). 8R-Lisuride has two stereogenic centers (5*R* and 8*R*, indicated with asterisks in structure G). 8R-Terguride has three stereogenic centers (5*R*, 8*R*, and 10*R*, indicated with asterisks in structure H). Both lisuride and terguride have a basic nitrogen that is capable of inversion. The N-configuration shown in structures E and F yields the best interaction energies in the hH_1R model.

from Molecular Probes (Leiden, The Netherlands), glycerol from Riedel-de-Haën (Seelze, Germany), and Triton X-100 from Fluka (Zwijndrecht, The Netherlands). PEA, clobenpropit, and thioperamide were taken from our own stock. Gifts of acrivastine (The Wellcome Foundation Ltd, United Kingdom), astemizole (Janssen Pharmaceutica NV, Beerse, Belgium), cyproheptadine hydrochloride (MSD, Haarlem, The Netherlands), *d*-chlorpheniramine maleate (A. Beld, Nijmegen, The Netherlands), diphenhydramine hydrochloride (Brocades, The Netherlands), levocabastine (Janssen Pharmaceutica), loratadine (Schering Plough, Bloomfield, NJ), mianserin hydrochloride and mirtazapine (Org 3770; Organon NV, The Netherlands), pcDEF$_3$ (Dr. J. Langer, Robert Wood Johnson Medical School, Piscataway, NJ), ranitidine dihydrochloride (Glaxo, UK), and of the cDNAs encoding the human histamine H$_1$R [Dr. H. Fukui, University of Tokushima, Tokushima, Japan (Fukui et al., 1994)], the human histamine H$_2$ receptor [Dr. I. Gantz, University of Michigan Medical Center, Ann Arbor, MI (Gantz et al., 1991)], the mutant human histamine H$_1$Rs [H$_1$ Trp^{103}Ala, H$_1$ Asp^{107}Ala, H$_1$ Lys^{191}Ala, H$_1$ Thr^{194}Ala, and H$_1$ Asn^{198}Ala (Moguilevsky et al., 1995, 1998) (UCB Pharma, Braine-l'Alleud, Belgium)], and the S-KN-MC cell lines stably expressing the human H$_3$ and H$_4$ receptors [Dr. T. Lovenberg, Robert Wood Johnson Pharmaceutical Research Institute, San Diego, CA (Lovenberg et al., 1999; Liu et al., 2001)] are greatly appreciated.

Molecular Cloning. The human H$_1$ receptor was cloned by PCR using the following oligodeoxynucleotides primers: 5′-gct act aag tgg cca ctc atc acc caa gtc-3′ and 5′-caa cac aca ggc ctg cgg ccg cta ttt cct tg-3′). PCR conditions employed 100 ng (~125 pmol) of each primer, 250 μM dNTPs, 80 ng of human genomic DNA, 2 mM $MgSO_4$, 1× High Fidelity buffer (Invitrogen) and 1.75 units of High-Fidelity Platinum *Taq*DNA Polymerase. PCR reactions conditions were: 94°C for 5 min; 30 cycles of 94°C for 30 s, 60°C for 35 s, and 72°C for 1 min, 35 s; followed by a final 10-min extension at 72°C. The resultant PCR product was subcloned into the TOPO 2.1 vector according to the manufacturer's protocols and subsequently subcloned into the mammalian expression vector pSI (Promega, Madison, WI) for R-SAT based functional studies. All receptor constructs were fully sequence-verified by dideoxy chain termination methods. The sequence of the human H$_1$ receptor used in this study corresponds to GenBank accession number D14436. All plasmid DNA used for transfections was prepared using resin-based mega-prep purifications following the manufacturer's protocols (QIAGEN Inc.).

Cell Culture and Transfection. NIH-3T3 and HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 2 mM L-glutamine, 1% penicillin and streptomycin, and 10% bovine calf serum and maintained at 37°C in a humidified 5% CO_2/95% air atmosphere. African green monkey kidney COS-7 cells were maintained at 37°C in a humidified 5% CO_2/95% air atmosphere in DMEM containing 2 mM L-glutamine, 50 IU/ml penicillin, 50 μg/ml streptomycin, and 5% (v/v) fetal calf serum. SK-NM-C cells expressing human H$_3$ (445-amino acid isoform) (Lovenberg et al., 1999) or human H$_4$ (Liu et al., 2001) receptors were maintained in Eagle's minimal essential medium supplemented with 10% fetal calf serum, 50 IU/ml penicillin, nonessential amino acids solution, 2 mM L-glutamine, 50 μg/ml streptomycin, and 50 μg/ml sodium pyruvate under the selection of 600 μg/ml G-418 disulfide.

NIH-3T3 cells were transiently transfected using the Superfect transfection reagent following the manufacturer's protocols. COS-7 cells were transiently transfected using the DEAE-dextran method as described previously (Bakker et al., 2001). The total amount of DNA transfected was maintained constant by addition of pcDEF$_3$.

Receptor Selection and Amplification Technology Assays. R-SAT assays were performed as described previously (Weiner et al., 2001). Briefly, on day 1, NIH-3T3 cells were plated into 96-well cell culture plates at a density of 7500 cells/well. On day 2, cells were transfected with 10 to 25 ng/well of H$_1$R DNA, and 20 ng/well of plasmid DNA encoding β-galactosidase. On day 3, the medium was replaced with DMEM supplemented with 1% penicillin and streptomycin, 2% Cyto-SF$_3$, and varying drug concentrations. After 5 days of cell culture, medium was removed, and the cells were incubated in phosphate-buffered saline containing 3.5 mM *O*-nitrophenyl-β-D-galactopyranoside and 0.5% Nonidet P-40 detergent. The 96-well plates were incubated at room temperature for up to 8 h, and the resulting colorimetric reaction was measured by spectrophotometric analysis at 420 nm on an automated plate reader (Biotek Instruments Inc., Burlington, VT). Data were analyzed by a nonlinear, least-squares curve-fitting procedure using Prism software (GraphPad Software, Inc., San Diego, CA). All data shown are expressed as mean ± S.D.

Reporter-Gene Assay. COS-7 cells transiently cotransfected with pNFκ B-Luc (125 μg/1 × 10^7 cells) and either pcDEF$_3$ or pcDEF$_3$hH$_1$ (25 μg/1 × 10^7 cells) were seeded in 96-well blackplates (Costar, Cambridge, MA) in serum-free culture medium and incubated with drugs. After 48 h, cells were assayed for luminescence by aspiration of the medium and the addition of 25 μl/well luciferase assay reagent [0.83 mM ATP, 0.83 mM D-luciferin, 18.7 mM $MgCl_2$, 0.78 μM $Na_2H_2P_2O_7$, 38.9 mM Tris, pH 7.8, 0.39% (v/v) glycerol, 0.03% (v/v) Triton X-100, and 2.6 μM dithiothreitol]. After 30 min, luminescence was measured for 3 s/well in a Victor^2 multilabel counter (PerkinElmer Life and Analytical Sciences). All data shown are expressed as mean ± S.D.

[^{3}H]Inositol Phosphate Formation. Transiently transfected COS-7 cells were seeded in 24-well plates; 24 h after transfection, they were labeled overnight in inositol-free culture medium supplemented with 1 μCi/ml [myo-2-^{3}H]inositol. Subsequently, the medium was aspirated and cells were incubated with drugs for 1 h at 37°C in DMEM containing 25 mM HEPES, pH 7.4, and 20 mM LiCl. Incubations were stopped by aspiration of the culture medium and the addition of ice-cold 10 mM formic acid. After 90-min incubation at 4°C, [^{3}H]inositol phosphates were isolated by anion exchange chromatography and counted by liquid scintillation counting. All data shown are expressed as mean ± S.D.

Measurement of $[Ca^{2+}]_i$ by Fluorescence Microplate Reader. HeLa cells endogenously expressing the human histamine H$_1$ receptor were used for the measurement of changes in intracellular calcium concentrations. Hela cells were loaded for 15 min at room temperature with 4 μM fluo-4 acetoxymethyl ester in HBSS containing 20 mM HEPES and 2.5 mM probenecid. Cells were washed three times with HBSS containing 20 mM HEPES and 2.5 mM probenecid, plated at approximately 50.000 cells/well of a 96-well plate (180 μl/well), and transferred to a Novostar (BMG Labtechnologies Inc., Offenburg, Germany) after a 1-h incubation at 37°C. Fluorescence was measured at room temperature for 50 s after the addition of 20 μl of a ligand solution (bringing total volume to 200 μl). Changes in calcium concentrations ($[Ca^{2+}]_i$) were calculated using the following formula: $[Ca^{2+}]_i = K_D(F - F_{min})/(F_{max} - F)$, where K_D is the dissociation constant with which Fluo-4 binds Ca^{2+} (345 nM; Molecular Probes, Eugene, OR), F_{max} is the fluorescence at maximum Ca^{2+} concentrations as determined by the addition of 25 μl of 5% (v/v) Triton X-100 in HBSS to the stimulated cells, and F_{min} is the fluorescence at minimum free Ca^{2+} concentration as determined by the addition of 25 μl of 0.1 M EGTA in HBSS.

H$_1$ Receptor Mediated Contraction of Guinea Pig Ileum. Male guinea pigs (350–450 g) were killed by a blow to the head and their ilea were rapidly removed and washed in carbogenated Krebs buffer (117.5 mM NaCl, 5.6 mM KCl, 1.18 mM $MgSO_4$ 2.5 mM $CaCl_2$, 1.28 mM NaH_2PO_4, 25 mM $NaHCO_3$, and 5.5 mM D-glucose). Ilea were cut in segements (1–1.5 cm) which were mounted longitudinally under 0.5 g of tension on a Hugo Sachs Hebel-Messovorsatz TL-2/HF-modem (Hugo Sach Electronik, Hugstetten, Germany) in a 20-ml tissue bath containing Krebs buffer at 37°C, which was continuously aerated with 95% O_2/5% CO_2. After equilibration for at least 40 min with replacement of fresh Krebs buffer every 10 min, cumulative dose-contractile response curves (CRC) were recorded using half-log increments of histamine. At least two equal CRC of histamine were made, and the last CRC was considered the reference curve. The contractile response produced by histamine was reevaluated after

the addition (including a 30-min equilibration period) of 8R-lisuride. Every CRC was followed by an adequate washing using Krebs buffer.

H_1R Binding Studies. Cells used for radioligand binding-studies were harvested 48h after transfection and homogenized in ice-cold H_1-binding buffer (50 mM Na_2/K^+-phosphate buffer, pH 7.4). The cell homogenates were incubated for 30 min at 25°C in a total volume of 400 μl of H_1-binding buffer with 1 nM [^{3}H]mepyramine. The nonspecific binding was determined in the presence of 1 μM mianserin. The incubations were stopped by rapid dilution with 3 ml of ice-cold H_1-binding buffer. The bound radioactivity was separated by filtration through Whatman GF/C filters that had been treated with 0.3% polyethylenimine. Filters were washed twice with 3 ml of buffer, and radioactivity retained on the filters was measured by liquid scintillation counting. All binding data were evaluated by a nonlinear, least-squares curve-fitting procedure using Graphpad Prism. Protein concentrations were determined according to the method of Bradford (1976), using bovine serum albumin as a standard. All data shown are expressed as mean ± S.D.

H_2R Binding Studies. The affinity of 8R-lisuride for the human H_2 receptor was determined by [^{125}I]aminopotentidine (^{125}I-APT) displacement studies using cell homogenates of COS-7 cells transiently transfected with cDNA encoding the human H_2 receptor ($pcDNA_3$-H_2) as described previously (Leurs et al., 1994). Cell homogenates were incubated for 90 min at 30°C in a total volume of 400 μl 50 mM Na_2/K^+-phosphate buffer containing gelatin (0.1%; pH 7.4 at 30°C) with approximately 0.5 nM ^{125}I-APT. The incubations were stopped by rapid dilution with ice-cold H_2 wash buffer (20 mM Na_2/K^+-phosphate buffer supplemented with 0.1% chicken egg albumin, pH 7.4 at 4°C), filtered through Whatman GF/C filters that were washed three times with H_2 wash buffer. Radioactivity retained on the filters was measured and binding data were evaluated using a K_D value of ^{125}I-APT for the human H_2 receptor of 0.43 nM.

H_3R Binding Studies. The affinity of 8R-lisuride for the human H_3 receptor was determined by [N^α-methyl-^{3}H]histamine displacement studies using cell homogenates of SK-NM-C cells stably expressing the H_3 receptor (445-amino acid isoform) (Lovenberg et al., 1999) that were incubated for 40 min at 25°C in a total volume of 400 μl of 50 mM Na_2PO_4, pH 7.4 at 25°C, with approximately 1 nM [N^α-methyl-^{3}H]histamine. The incubations were stopped by rapid dilution with ice-cold H_3 wash buffer (25 mM Tris-HCl, 145 mM NaCl, pH 7.4 at 4°C), filtered through Whatman GF/C filters that were washed three times with H_3 wash buffer. Radioactivity retained on the filters was measured and binding data were evaluated using a K_D value of [N^α-methyl-^{3}H]histamine for the human H_3 receptor of 2.85 nM.

H_4R Binding Studies. The affinity of 8R-lisuride for the human H_4 receptor was determined by [^{3}H]histamine displacement studies using cell homogenates of SK-NM-C cells stably expressing the H_4 receptor (Liu et al., 2001) that were incubated for 60 min at 37°C in a total volume of 400 μl of 50 mM Tris-HCl, pH 7.4 at 37°C, with approximately 10 nM [^{3}H]histamine. The incubations were stopped by rapid dilution with ice-cold 50 mM Tris-HCl, pH 7.4 at 4°C, filtered through Whatman GF/C filters that were washed three times with 50 mM Tris-HCl, pH 7.4 at 4°C. Radioactivity retained on the filters was measured and binding data were evaluated using a K_D value of [^{3}H]histamine for the human H_4 receptor of 7.69 nM.

Molecular Modeling. The chemical structures of 8R- and 8S-lisuride and 8R-terguride were built from their crystal structures in the Cambridge Structural Database (CSD entries WEVTUM, FEPQIA, PIKMEB). The configurations *R* and *S* indicate the stereochemistry at stereogenic center at atom 8, but there are additional stereogenic centers in these alkaloid derivatives (see Fig. 1). For example, the absolute configuration at carbon atom 5 of these compounds is *R*, as observed in crystal structures of lisuride, terguride and their close analogs LSD and ergotamine (CSD entries LSDIBZ, YOTSAB, HICCUR, and LIJNIB) (Husak et al., 1998). Concerning the chirality of carbon atom 10, both the 10*R*- and 10*S*-configuration of terguride can be found in the CSD (entries FEPQIA and PIKMEB, respectively). The *S* and *R* designations represent the absolute configuration of the stereogenic center at atom 8 (see Fig. 1 and Fig. 7). Diverse ring conformations of 8R-lisuride (21 conformers) and 8R-terguride (39 conformers) were generated using the Confort conformation analysis tool in Sybyl. Relative energies of these conformations were calculated after minimization with the Tripos force field and Gasteiger Hückel partial charges.

The three-dimensional model of the human histamine H_1R was constructed based on the crystal structure of rhodopsin (Palczewski et al., 2000; PDB code 1F88.pdb) using the homology-modeling program Modeler 4.0. Molecular dynamics simulations were performed with AMBER 5.0 (Cornell et al., 1995). Point charges of histamine, lisuride, and terguride in the protonated state were calculated with GAMESS U.S. (Schmidt et al., 1993) and the RESP program following the method of Bayly et al. (1993). Molecular dynamics simulation runs at constant temperature (300 K) lasted 100 ps using a step size of 1 fs. A distance restraint of 3 Å with a force constant of 50 kcal · $mol^{-1}Å^{-2}$ was applied between the protonated nitrogens of the agonists and the carboxylate group of Asp^{107}. Similar restraints were applied to the backbone H-bonds in the transmembrane domains of the receptor model.

Results

Evaluation of H_1R Agonist Activity of Various Therapeutics Using R-SAT

We screened a library of clinically relevant therapeutic drugs for functional activity at the human H_1R using R-SAT (R. A. Bakker, M. W. Goodman, T. T. Smith, E. S. Burstein, U. Hacksell, H. Timmerman, R. Leurs, M. R. Brann, and D. M. Weiner, manuscript in preparation). H_1R mediated responses in R-SAT reflect, in part, G_q-mediated signaling cascades, as evidenced by augmentation of basal receptor activity by cotransfections of the $G\alpha_q$ subunit (Weiner et al., 2001). As depicted in Fig. 2. three compounds, lisuride, terguride, and methylergonovine maleate displayed reasonable potency as H_1R agonists. The dopamine/serotonin receptor agonist 8R-lisuride behaved as a full H_1R agonist, with an observed EC_{50} of 8 nM, which is 4- to 6-fold more potent than histamine itself. The structurally related compound 8R-terguride behaved as a partial agonist with an EC_{50} of 363 nM (α = 0.49 ± 0.07). The serotonergic reference compound methylergonovine maleate also behaved as a weak partial H_1R agonist, with an EC_{50} of 457 nM (α = 0.57 ± 0.04; Fig. 1). The serotonin/dopamine drugs methysergide and quinpirole possess very weak agonist activity at human H_1Rs, with potencies greater than 10 μM (data not shown).

Detailed Analysis of the H_1R Agonist Properties of Various Ergolines

H_1R Agonist Properties of 8R-Lisuride. The intriguing finding that 8R-lisuride displayed potent H_1R agonist activity stimulated us to further characterize this compound. We determined the effects of lisuride in the NF-κB reporter-gene and inositol phosphate accumulation assays using COS-7 cells transiently expressing the human H_1R, as well as the affinity of 8R-lisuride for the H_1R using radioligand binding techniques. In agreement with our findings using R-SAT, 8R-lisuride displays high affinity for the human H_1R ($pK_{i,}$ = 7.9 ± 0.1, Table 1) and stimulates NF-κB and inositol phosphate accumulation with high potency [pEC_{50} = 7.6 ± 0.1 (α = 0.63 ± 0.03) and pEC_{50} = 7.9 ± 0.1 (α = 0.46 ± 0.01), respectively]. The agonist effects of 8R-lisuride are blocked by the inverse H_1R agonist mepyramine in R-SAT assays

(data not shown), inositol phosphate accumulation assays (Fig. 3), and NF-κB reporter-gene assays (Fig. 4). Yet, in contrast to the R-SAT assay, 8R-lisuride behaves as a high-affinity partial H_1R agonist in these assays (see also Table 1). Consequently, 8R-lisuride is able to partially inhibit histamine-induced accumulation of [^{3}H]inositol phosphates (Fig. 3).

We have previously reported the competitive antagonism of histamine-induced [^{3}H]inositol trisphosphate accumulation and NF-κB activation by mepyramine (Bakker et al., 2001). Mepyramine was also found to competitively inhibit 8R-lisuride–induced H_1R responses in the NF-κB reporter-gene assay (Fig. 4A). Schild plot analysis of the competitive antagonism by mepyramine of the 8R-lisuride-induced human H_1R mediated NF-κB activity yielded a pA_2 value for mepyramine of 8.1 (slope = 0.95 ± 0.1, r^2 = 0.92; Fig. 4B), which is in agreement with the pA_2 value of 8.0 (slope = 0.94 ± 0.1, r^2 = 0.93) for mepyramine antagonizing histamine-induced NF-κB activation (Bakker et al., 2001) (see also Fig. 4, C and D).

Stereospecific H_1R-Agonist Properties of Lisuride. 8S-Lisuride, which differs from 8R-lisuride in the orientation of the substituent at position 8 (see Fig. 1), also behaves as a partial H_1R agonist in these assays albeit with a much lower potency [pEC_{50} = 6.3 ± 0.1 (α = 0.58 ± 0.03) and pEC_{50} = 6.5 ± 0.1 (α = 0.17 ± 0.02) in the NF-κB and inositol phosphate accumulation assay, respectively], and also possesses a lower affinity for the H_1R (pK_i = 6.3 ± 0.1, Table 1; see also Fig. 5). Therefore, lisuride displays a significant degree of stereospecificity toward the H_1R in favor of 8R-lisuride, having almost 2 log orders of magnitude higher affinity for the H_1R than its *S*-diastereoisomer.

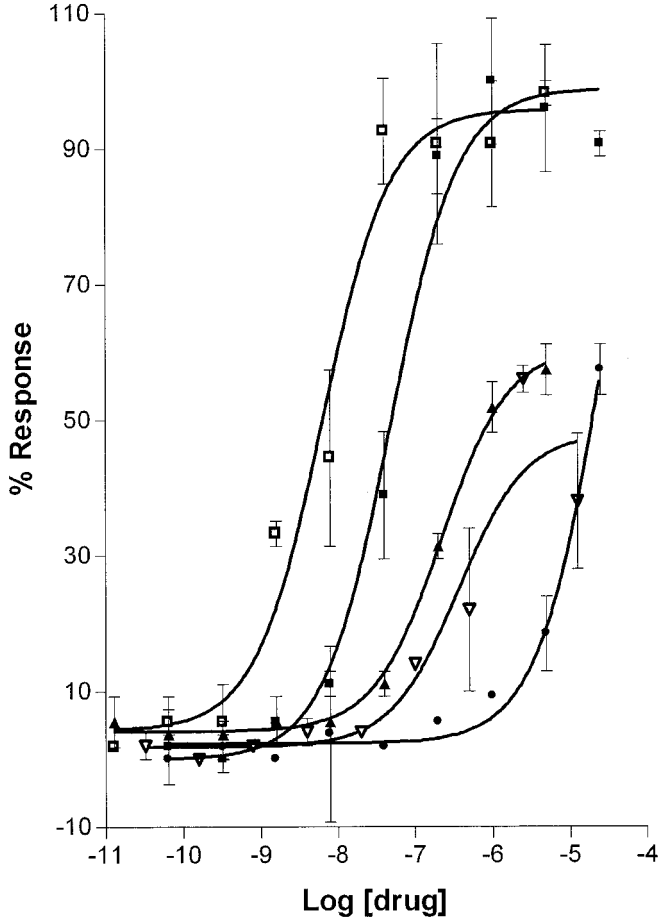


Fig. 2. Pharmacology of the human histamine H_1R observed in R-SAT. Functional agonist responses observed for histamine (■), methysergide (●), methylergonovine maleate (▲), 8R-lisuride (□), and 8R-terguride (▽) at the human H_1R as determined by R-SAT. Data are reported as a percentage of the total response determined by the full agonist histamine. Data are from representative nine-point concentration response curves performed in duplicate, which yielded the average potencies and intrinsic activities as reported in the text.

Evaluation of 8R-Lisuride in Assay Systems Using Endogenously Expressed H_1Rs. We examined the effects of the partial H_1R agonist 8R-lisuride on the H_1R mediated increase in intracellular Ca^{2+} ($[Ca^{2+}]_i$) using HeLa cells endogenously expressing human H_1Rs (Simons and Simons, 2002). Stimulation of HeLa cells with histamine (10 μM; Fig. 6A) gives a robust increase in $[Ca^{2+}]_i$, whereas the same concentration of 8R-lisuride is without a measurable agonistic effect. Mepyramine (10 μM) was able to completely inhibit the histamine-induced effects. 8R-Lisuride (10 μM) also inhibits the histamine induced changes in $[Ca^{2+}]_i$ (Fig. 6B). The inhibition of the histamine-induced effects by 8R-lisuride, however, is not complete. These effects may be attributable to a lack of equilibrium of histamine and 8R-lisuride with H_1Rs within the short duration of rapid transient effects of histamine on intracellular calcium. 8R-Lisuride did not affect the ATP (10 μM)-induced increase in $[Ca^{2+}]_I$ (Fig. 6C), indicating that the inhibitory effect of 8R-lisuride on histamine induced responses is not caused by interference with calcium signaling.

We also evaluated the effects of lisuride on the contraction of guinea pig ileum. When tested alone, neither 8R- nor 8S-lisuride induced contraction of the guinea pig ileum. However, the spontaneous motility of the ileum was increased at high concentrations concomitant with a small increase in basic tonus (5–10% of the maximal histamine induced contraction). When tested in the presence of histamine, preincubation with 8R-lisuride resulted in a clear rightward shift of cumulative dose-contractile response induced by histamine (see Fig. 6D). Estimation of the antagonist potency of 8R-lisuride by Schild analysis results in a pA_2 value of approximately 8.1 (data not shown). At high concentrations of 8R-lisuride, histamine is no longer able to produce the full response, indicating that 8R-lisuride may act as an noncompetitive antagonist under these experimental conditions.

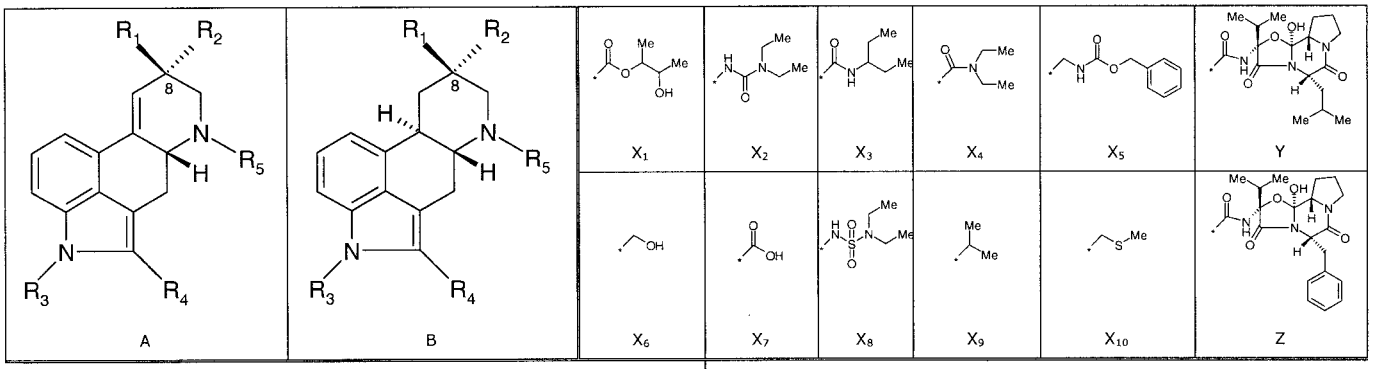
Examination of the Affinity of 8R-Lisuride for Other Histamine Receptors. The high affinity of 8R-lisuride for the human histamine H_1 receptor prompted us to investigate the affinity of lisuride for the other known histamine receptor subtypes. Radioligand displacement studies using COS-7 cells transiently transfected with cDNA encoding the human H_2 receptor using ^{125}I-APT as a radioligand yielded a pK_i value of 8R-lisuride for the H_2 receptor of 6.4 ± 0.1 (n = 3). Similarly, radioligand binding studies using cell homogenates of SK-NM-C cells stably expressing either the human H_3 (445 amino acid isoform) or the human H_4 receptor using [N^{α}-methyl-^{3}H]histamine and [^{3}H]histamine, respectively, yielded pK_i values of 8R-lisuride for the human H_3 receptor of 5.0 ± 0.1 (n = 3) and for the human H_4 receptor of 5.4 ± 0.1 (n = 3).

Examination of H_1R Functional Activities of Lisuride Analogs. We evaluated a set of commercially avail-

TABLE 1

H_1R agonist activity of clinically relevant drugs and several structural analogues.

The pEC50 and intrinsic activity (a) values as determined by NF-kB assays are reported and are expressed as means ± S.D. of at least three separate experiments, each performed in triplicate.



Compound	Structure	R_1	R_2	R_3	R_4	R_5	[^{3}H]Mepyramine pK_i	NF-κB Assay pEC_{50}/pIC_{50}	NF-κB Assay α
8R-Lisuride	A	X_2	H	H	H	CH_3	7.9 ± 0.1	7.9 ± 0.1	0.59 ± 0.03
8S-Lisuride	A	H	X_2	H	H	CH_3	6.3 ± 0.1	6.3 ± 0.1	0.58 ± 0.03
8R-Terguride	B	X_2	H	H	H	CH_3	6.1 ± 0.1	<5	—
8S-Terguride	B	H	X_2	H	H	CH_3	6.1 ± 0.1	<5	—
Methysergide	A	X_3	H	CH_3	H	CH_3	N.D.	5.6 ± 0.3	0.24 ± 0.01
Methergine	A	X_3	H	H	H	CH_3	N.D.	5.7 ± 0.1	0.51 ± 0.07
Lysergol	A	X_6	H	H	H	CH_3	5.6 ± 0.1	N.E.	<0.05
D-Lysergic acid	A	H	X_7	H	H	CH_3	5.7 ± 0.1	<4	—
Mesulergine	B	H	X_8	CH_3	H	CH_3	5.4 ± 0.1	5.0 ± 0.1	−0.56 ± 0.01
Bromocriptine	A	Y	H	H	Br	CH_3	N.D.	5.6 ± 0.1	−0.57 ± 0.25
LSD	A	X_4	H	H	H	CH_3	5.7 ± 0.1	<5	—
Metergoline	B	X_5	H	CH_3	H	CH_3	6.4 ± 0.1	6.0 ± 0.1	−0.96 ± 0.09
LY-53,857	B	X_1	H	X_9	H	CH_3	4.8 ± 0.1	6.1 ± 0.2	−0.65 ± 0.03
Dihydroergotamine	B	Z	H	H	H	H	4.9 ± 0.1	N.E.	—
Dihydroergocristine	B	Z	H	H	H	CH_3	5.0 ± 0.1	N.E.	—
Pergolide	B	X_{10}	H	H	H	$(CH_2)_2CH_3$	N.D.	5.8 ± 0.2	−0.59 ± 0.01

—, could not be determined; N.E., no effect; N.D., not determined.

able ergoline derivatives for their H_1R activity and affinity (see Fig. 5 and Table 1). Testing of 8R- and 8S-terguride, the dihydro-analogs of 8R- and 8S-lisuride, respectively, and a variety of other derivatives revealed that 8R-lisuride is by far the most potent H_1R agonist identified in this study (Fig. 5 and Table 1). These data indicate that the molecular structure of 8R-lisuride is optimal for H_1R activation compared with the other tested compounds, which is in agreement with binding data reported in literature for rat or guinea pig H_1Rs (Beart et al., 1986; Millan et al., 2002). Interestingly, lysergol, an analog of lisuride in which the double bond is conserved, seems to lack intrinsic H_1R activity, although it shows moderate H_1R affinity ($pK_i = 5.6 \pm 0.1$, Table 1).

Characterization of the Binding Pocket of Lisuride in the H_1R

Mutational Analysis of the H_1R. To explore the ligand-binding pocket of 8R-lisuride in the human H_1R, we tested the effects of 8R-lisuride on several mutant human H_1Rs. Previous mutagenesis studies following a bacteriorhodopsin-based homology model of the H_1R have been invaluable in identification of the histamine-binding pocket in the H_1R (Moguilevsky et al., 1995). Table 2 reports the observed changes in binding affinities, agonist, and inverse agonist activities of selected compounds upon expression of the mutant H_1Rs in which Trp^{103}, Asp^{107}, Lys^{191}, Thr^{194}, or Asn^{198} has been changed into alanine (Moguilevsky et al., 1995, 1998). As expected from existing literature (Ohta et al., 1994), $H_1Asp^{107}Ala$ displays no [^{3}H]mepyramine binding and failed to mediate functional responses upon stimulation with either histamine or 8R-lisuride. Mutation of either Trp^{103}, Lys^{191}, or Asn^{198} into Ala resulted in a great reduction of the affinity and potency of both histamine and 8R-lisuride for the H_1R, potentially implicating these residues in the histamine, 8R-lisuride, and 8R-terguride binding site(s) in the H_1R.

Molecular Modeling of the H_1R Agonist Binding Pocket. Because lisuride has a very rigid structure (see Fig. 7), it is very well suited for molecular modeling studies. We generated a molecular model of the H_1R that is based upon the crystal structure of rhodopsin (Palczewski et al., 2000) to perform molecular modeling studies for the identification of potential interactions of histamine and lisuride with the H_1R.

Molecular dynamics simulation runs using this rhodopsin-based model of the H_1R identified several residues that are known to be involved in the binding of histamine to the H_1R, such as Asp^{107} and Asn^{198} (Moguilevsky et al., 1995, 1998) as interaction points in the histamine binding-pocket in the H_1R (Fig. 7). However, the MD simulation runs also indicate that Ser^{111} and Glu^{181} may be involved in the binding of histamine to the human H_1R (Fig. 7). Additional experiments need to be performed to evaluate whether these residues are involved in the binding of histamine to the H_1R.

In MD simulation runs, the tetracyclic ring system of 8R-

lisuride remains parallel with respect to the transmembrane helices of the H_1R in an orientation very similar to that found for histamine. As observed for histamine, and in agreement with our mutagenesis data (Table 2), 8R-lisuride forms a salt bridge with Asp^{107} and a hydrogen bond with Asn^{198} (Table 3 and Fig. 7). In addition, 8R-lisuride can make hydrogen bonds secondary to the orientation of the amide group in the side chain with residues Glu^{181} and Lys^{179} in the second extracellular loop of the histamine H_1R (Fig. 7), explaining the relatively high potency of this compound. The modeling results are different for 8S-lisuride. The steric bulk of the C8-substituent of 8S-lisuride, which is pseudoaxially located, prevents 8S-lisuride from adopting a position similar to that of 8R-lisuride relative to the H_1R. Therefore, the tetracyclic moiety of 8S-lisuride ends up in an orientation, after MD simulation, in which no hydrogen bonds are formed with Glu^{181} and Lys^{179} (Table 3). This inability of 8S-lisuride to form two energetically favorable hydrogen bond interaction with the H_1R provides a rationale for the significantly lower affinity of 8S-lisuride compared with 8R-lisuride. The importance of hydrogen bonding to Lys^{179} and Glu^{181} is further supported by the much lower H_1R activities of close analogs of lisuride that have the amide functionality inverted (e.g., methysergide and methylergonovine maleate) or the carbonyl group at another position (e.g., LSD and LY-53,875) (Table 1).

Discussion

Although a large variety of H_1R antagonists have been developed based on the role of the H_1R in mediating allergic responses, highly potent receptor agonists have not yet been available. Interestingly, our functional screen has identified potent H_1R agonist activity of the structurally related ergolines lisuride and terguride. We confirmed these findings using radioligand binding as well as multiple functional assays. These functional assays demonstrate the potent H_1-receptor agonist activity of these compounds with varying agonist efficacies. Our experiments on H_1R-mediated calcium mobilization and guinea pig ileum contractions indicate that 8R-lisuride may act as an antagonist in vivo, These discrepancies between assays are not surprising given the dependence of agonist efficacies on such factors as receptor expression levels and differences in the degree of receptor occupancy required for generating measurable agonist responses. These data may be consistent with the idea that different agonists may produce an array of active receptor states that differentially activate signal transduction cascades (Watson et al., 2000). Conversely, there may be differences in the second-messenger pathways in these assays. The discovery of the partial agonistic properties of 8R-lisuride provides a useful tool to further investigate these phenomena and advocates a detailed investigation of signal transduction cascades that may be activated by the H_1R.

Several imidazole-containing derivatives of histamine have been evaluated for their H_1R activity ever since the first synthesis of histamine in the early 1900s (Windaus and Vogt,

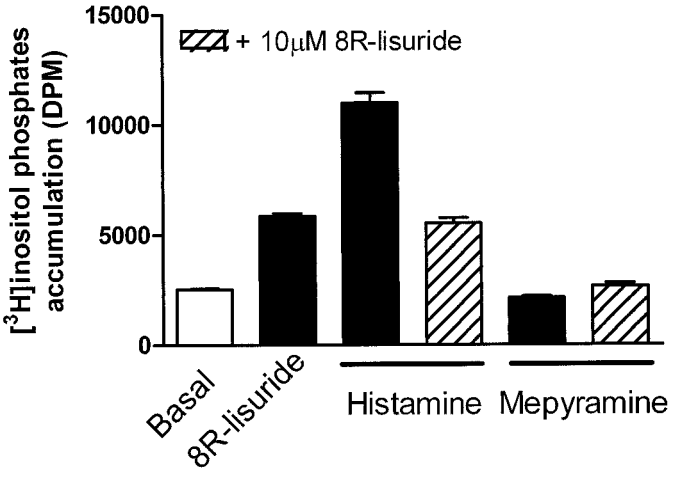


Fig. 3. Effects of 10 μM 8R-lisuride on H_1-receptor mediated [^{3}H]inositol phosphate accumulation in transiently transfected COS-7 cells. Also shown are the inhibitory effects of 8R-lisuride on 10 μM histamine-induced effects and the inhibition of 8R-lisuride–mediated effects by the inverse H_1-receptor agonist mepyramine (10 μM). [^{3}H]Inositol phosphates are counted by liquid scintillation and expressed as disintegrations per minute (DPM).

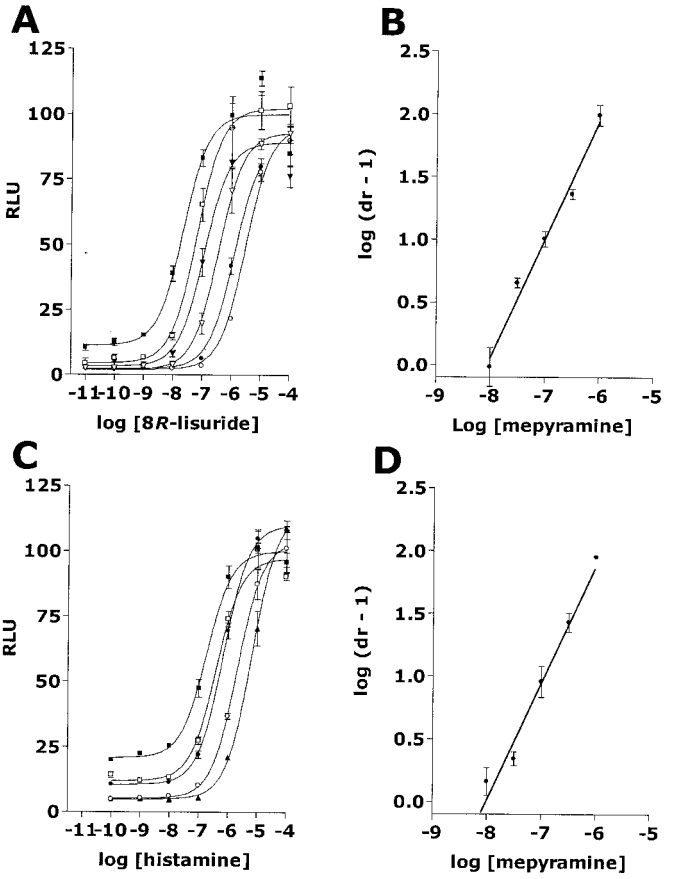


Fig. 4. Histamine H_1R pharmacology. A, 8R-lisuride H_1R pharmacology; competitive antagonism of H_1R mediated NF-κB responses to 8R-lisuride (■) by increasing concentrations of mepyramine [10 nM (□), 30 nM (▼), 100 nM (▽), 300 nM (●), or 1 μM (○)]. Shown is a representative example of three independent experiments, each performed in triplicate. B, Schild plot analysis of the combined data set of three independent experiments of the competitive antagonism by mepyramine of the 8R-lisuride–induced H_1R NF-κB responses (pA_2 value = 8.1; slope = 0.95 ± 0.06; r^2 = 0.92). C, competitive antagonism of H_1R-mediated NF-κB responses to histamine (■) by increasing concentrations of mepyramine [10 nM (□), 30 nM (●), 100 nM (○), or 300 nM (▲)]. Shown is a representative example from three independent experiments, each performed in triplicate. D, Schild plot analysis of the combined data set of three independent experiments of the competitive antagonism by mepyramine of the histamine-induced H_1R NF-κB responses (pA_2 value = 8.0; slope = 0.94 ± 0.06; r^2 = 0.93) (Bakker et al., 2001).

1907). In line with our findings of the H_1R agonist activity of 8R-lisuride, the H_1R agonist activity of PEA (Fig. 1) has demonstrated that the imidazole nucleus is not a structural requirement of H_1R agonists (Walter et al., 1941). Nonetheless, development of selective H_1R agonists has focused mainly on the substitution of the imidazole ring. For many years the substituted 2-phenylhistamines such as 2-(3-trifluoromethylphenyl)histamine (Fig. 1) have been the most selective H_1R agonists with a relatively high affinity (Zingel et al., 1995). The most potent H_1R agonists described so far are various derivatives of histaprodifen (see Fig. 1) (Elz et al., 2000a,b; Malinowska et al., 1999), such as methylhistaprodifen, dimethylhistaprodifen, and N^{α}-imidazolylethylhistaprodifen (suprahistaprodifen) (Schlicker et al., 2001), which are potent H_1R agonists when tested on guinea pig ileum and rat aorta in vitro. Evaluation of suprahistaprodifen on the human H_1R transiently expressed in COS-7 cells, however, yielded a pK_i value of 5.8 ± 0.1 and a pEC_{50} value of 6.4 ± 0.2 in the NF-κB reporter-gene assay (data not shown; see also Seifert et al., 2003), in agreement with the recently reported affinities of this class of H_1R agonists (Seifert et al., 2003).

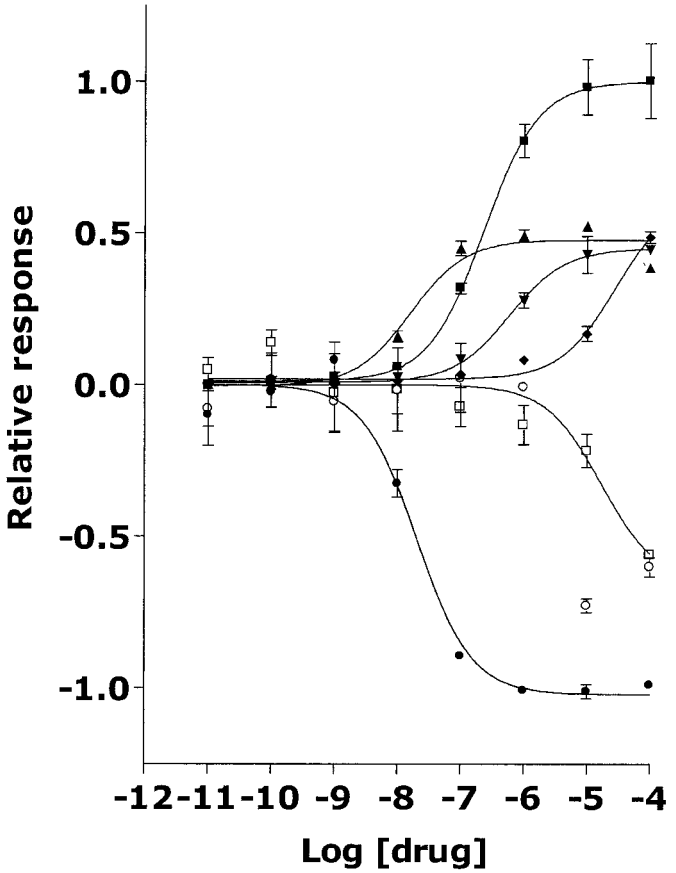


Fig. 5. H_1R pharmacology of various clinically relevant drugs as determined by the NF-κB reporter-gene assay, performed in COS-7 cells expressing the human H_1R (3.2 ± 0.4 pmol/mg protein): 8R-lisuride (▲), 8S-lisuride (▼), 8R-terguride (♦), 5′α)-2-bromo-12′-hydroxy-2′-(1-methylethyl)-5′-(2-methylpropyl)ergotaman-3′,6′,18-trione mesylate (○), mesulergine (□), and histamine (■), and mepyramine (●) as a full agonist (histamine) and inverse agonist (mepyramine) reference compound, respectively. Data are reported as fractions of the response yielded by the full agonist histamine ($\alpha = 1$) for the agonists and as fractions of the response yielded by the full inverse agonist mepyramine ($\alpha = -1$) for the inverse agonists. Shown is a representative example of at least three independent experiments, each performed in triplicate.

Therefore, 8R-lisuride represents the most potent agonist yet identified for the human H_1R, with H_1R affinity 2 log-units higher than that of the histaprodifen analogs.

Evaluation of a set of derivatives of the ergoline skeleton (see Table 1) indicates critical structural and stereochemical factors for potent H_1R agonism. Either hydrogenation of lisuride, yielding the dihydro analogs 8R- and 8S-terguride, or substitution at the 8 position provides compounds devoid of potent H_1R agonism. Both mutational analysis and molecular modeling studies of the human H_1R binding pocket indicated that 8R-lisuride could possibly interact with residues Asp^{107} and Asn^{198}. The human H_1R model that we used in this study was generated based upon the crystal structure of rhodopsin (Palczewski et al., 2000) and suggests that in addition to the residues known to be involved in the binding of histamine to the human H_1R, such as Asp^{107} and Asn^{198} (Moguilevsky et al., 1995, 1998), Ser^{111} and Glu^{181} may also interact with histamine. Additional experiments need to be performed to evaluate whether 8R-lisuride also interacts with Glu^{181} and Lys^{179} as suggested by the molecular modeling. The bioactive conformer of 8R-lisuride bound to the H_1R found using Amber 5.0 MD simulations is a stretched conformation identical to the global energy minimum found in a Tripos FF molecular mechanics conformation analysis, which supports the high H_1R affinity of this compound. The bioactive conformation of 8R-terguride in the H_1R seems to be very similar to that of 8R-lisuride (Fig. 7, B and C). However, in contrast to 8R-lisuride, the more flexible 8R-terguride lacks one double bond, allowing for a stable folded global minimum conformation with an intramolecular hydrogen bond between the carbonyl group and the protonated tertiary amine. Consequently, the bioactive conformation of 8R-terguride in the H_1R has a high relative molecular mechanics energy of 8.7 kcal/mol relative to its global energy minimum. This may explain the lower H_1R affinity of 8R-terguride compared with the H_1R affinity of 8R-lisuride (pK_i values of 6.1 and 7.9, respectively; Table 1). So far, pronounced stereoselectivity has been demonstrated only for H_1R inverse agonists, such as cetirizine (Bakker et al., 2000, 2001). The highly stereo-selective H_1 receptor agonist lisuride may allow refining of the existing molecular model of the human H_1R to include molecular interactions/activation mechanisms of the receptor. In addition, ergolines closely related in their chemical structure display varied intrinsic activities (Table 1). Lysergol, an analog of lisuride in which the double bond is conserved, seems to lack intrinsic H_1R activity and may represent a weak ($pK_i = 5.6$), neutral H_1R antagonist. The analyses described herein argue that the binding pocket of lisuride is overlapping, if not identical to, the binding pocket of histamine (Fig. 7). Evaluation of the numerous lisuride analogs that are described in literature (Mantegani et al., 1999), combined with directed synthesis of new molecules, may harbor great potential for the identification of high-affinity H_1R ligands displaying a range of intrinsic activities.

The ergot alkaloids and derivatives have attracted great interest for their broad spectrum of pharmacological action, and these drugs find application in the treatment of a variety of clinical conditions. It has been suggested that their diverse biological properties may be best explained by assuming these compounds interact with a variety of receptors (reviewed in Mantegani et al., 1999). Indeed, it has been recog-

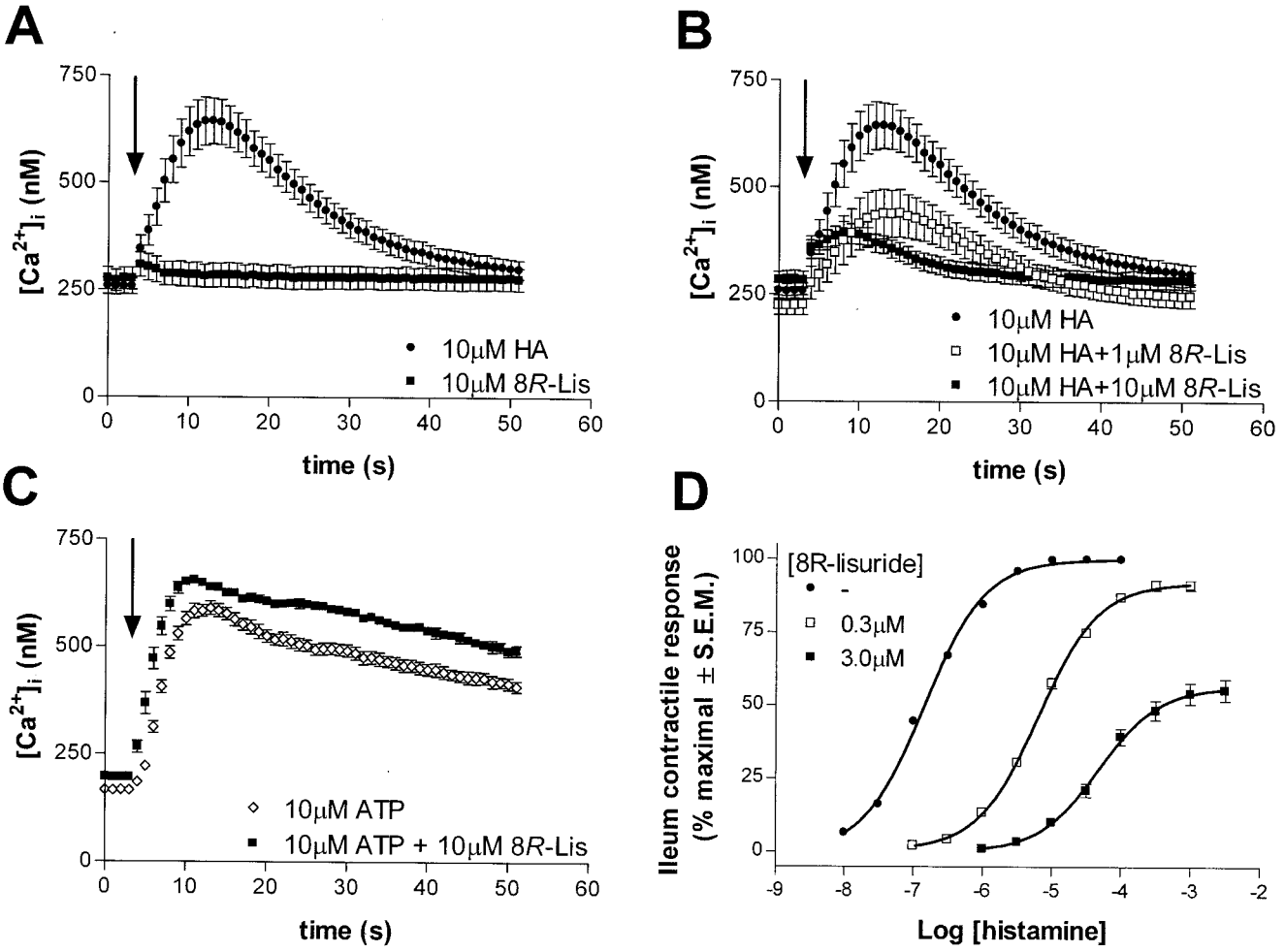


Fig. 6. Effects of 8R-lisuride on H_1-receptor mediated calcium mobilization using HeLa cells endogenously expressing human H_1 receptors (A–C) and guinea pig ileum contractions (D). A, effects of histamine (10 μM, ●) and 8R-lisuride (10 μM, ■) on calcium mobilization in Hela cells. B, effects of 1 μM (□) and 10 μM (■) 8R-lisuride on histamine (10 μM, ●) induced calcium mobilization. C, effects of 10 μM (■) 8R-lisuride on the calcium mobilization induced by 10 μM ATP (◇). D, effects of a 30-min pretreatment with either 0.3 (□) or 3.0 μM (■) 8R-lisuride on histamine (●)-induced contractions of the guinea pig ileum. The arrows in A through C indicate a single injection of an individual ligand or a mixture of ligands (simultaneous stimulations, no preincubations). Data shown for calcium mobilization assays are the average of eight individual measurements, and the data shown for the guinea pig ileum contractions are the average of at least three independent measurements.

TABLE 2

Effects of mutational analysis of the H_1R on the functional responses and binding affinities of various ligands compared with the wild-type receptor

Receptor affinities (pK_d and pK_i values) and receptor expression levels (B_{max}, pmol/mg of protein) were determined by radioligand-displacement studies using [^{3}H]mepyramine, functional responses were determined using the NF-κ B reporter-gene assay. The pIC_{50} and pK_i values obtained for the mutant H_1Rs are presented as the shift (Δ) compared with the values obtained for the wild-type receptor that are indicated in parentheses.

	[^{3}H]Mepyramine		Mepyramine		Histamine		8R-Lisuride			8R-Terguride
Receptor	pK_d	B_{max}	ΔpK_i	ΔpIC_{50}	ΔpK_i	ΔpEC_{50}	ΔpK_i	ΔpEC_{50}	α^a	ΔpK_i
H_1	8.7 ± 0.1	10.9 ± 6.4	(8.7 ± 0.1)	(7.9 ± 0.2)	(4.2 ± 0.1)	(6.7 ± 0.1)	(7.9 ± 0.1)	(7.9 ± 0.1)	0.60 ± 0.04	(6.1 ± 0.1)
H_1 Trp^{103}Ala	7.8 ± 0.1	2.6 ± 0.6	−0.9	−0.7	>−2	−2.1	−1.5	−1.8	0.82 ± 0.15	−1.3
H_1 Asp^{107}Ala	—[b]	—[b]	—[b]	—[b]	—[b]	—[c]	—[b]	—[c]	—[c]	—[b]
H_1 Lys^{191}Ala	N.D.	N.D.	N.D.	+0.4	N.D.	−1.6	N.D.	−1.4	0.57 ± 0.08	N.D.
H_1 Thr^{194}Ala	9.0 ± 0.1	4.0 ± 2.0	+0.3	+0.1	−0.5	−0.7	+0.1	−0.4	0.63 ± 0.07	0.0
H_1 Asn^{198}Ala	8.3 ± 0.1	0.9 ± 0.1	−0.4	+0.2	−1.2	>−2.7	−1.3	−1.8	0.23 ± 0.04[d]	−0.8

N.D., not determined.
[a] Data relative to responses of the full agonist histamine (for which a=1).
[b] No [^{3}H]mepyramine binding was observed.
[c] No functional effects were observed.
[d] Estimated from histamine response.

nized that norepinephrine, dopamine, and serotonin may be viewed as structural elements of the ergoline ring system, which is shared by all the ergot alkaloids. Synthesis of various ergoline derivatives has thus yielded a variety of compounds with quite different pharmacological activities. Histamine can be seen as a structural element of the ergoline

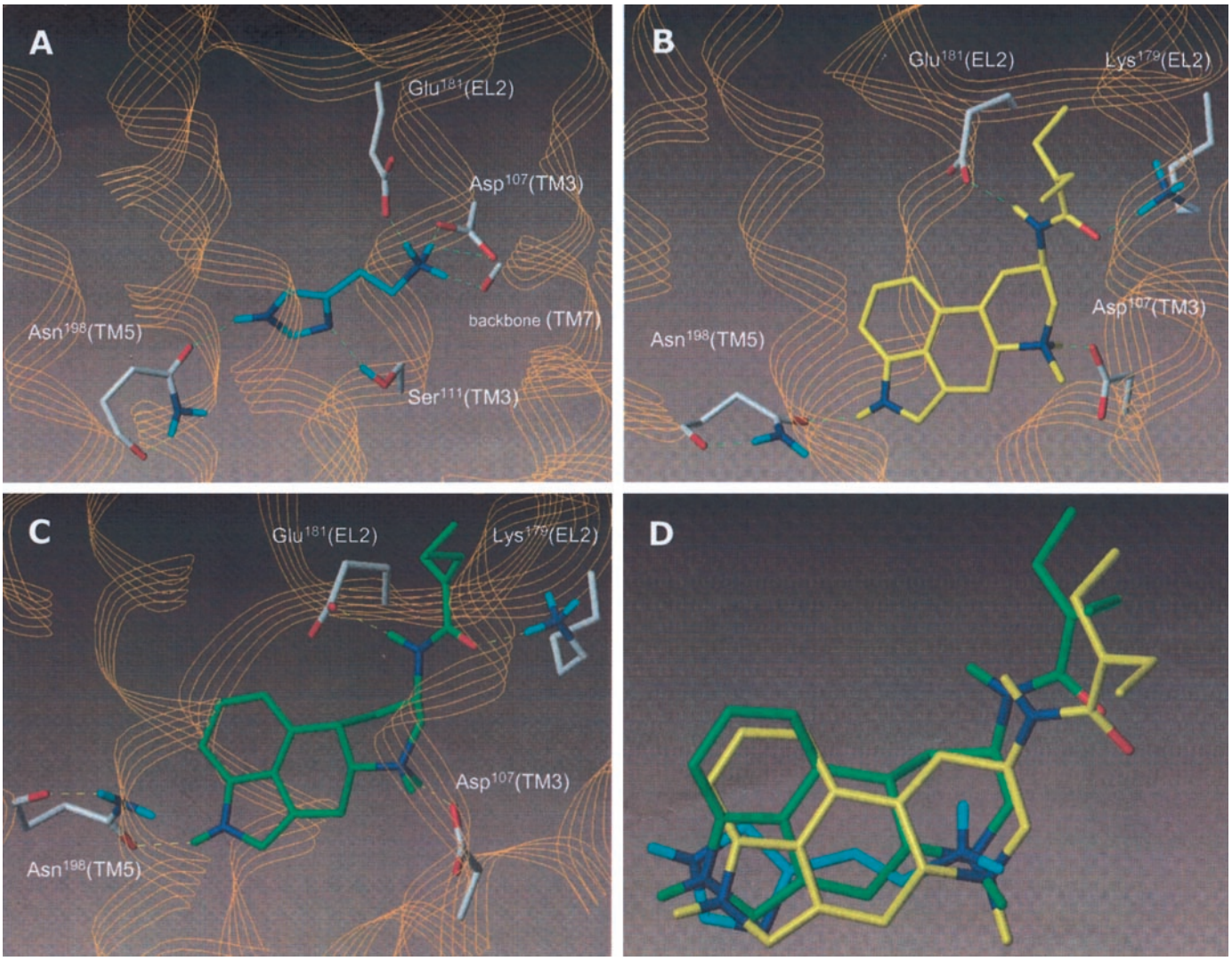


Fig. 7. Exploration of the H_1R agonist binding-pocket. Conformations of histamine (A, light-blue), 8R-lisuride (B, yellow), and 8R-terguride (C, green) in the histamine H_1R binding site after 100 ps of MD simulations. Hydrogen bonding with residues Asn^{198} and Asp^{107} is observed for all three agonists. The amide groups in the side chains of 8R-lisuride and 8R-terguride make additional hydrogen bonds with residues Glu^{181} and Lys^{179} in extracellular loop 2 of the histamine H_1R. An overlay of the binding sites of the three agonists is shown (D). TM, transmembrane domain; EL, extracellular loop.

ring system despite the presence of only two nitrogens in the ring system of the ergolines (Fig. 1), as has been recognized, for instance, in the case of LSD as an H_1R and H_2R agonist (Green et al., 1977; Batzri and Dyer, 1982; Topiol and Sabio, 1991), as well as for lisuride as an H_1R ligand (Beart et al., 1986; Millan et al., 2002). It is thus not surprising that 8R-lisuride also has affinity, albeit significantly less than that for the H_1R, for human H_2, H_3, and H_4 receptors. In addition, however, our data suggest that ergolines with defined structures can be recognized by the human H_1R and may act as potent H_1R agonists (see Table 1).

The identification of the heretofore unappreciated potent partial agonist activity of lisuride at the human H_1R raises a number of questions regarding the clinical relevance of this finding. Lisuride was initially developed for use as a dopamine receptor agonist for the treatment of Parkinson's disease, although its current use is limited secondary to the development of newer compounds with better therapeutic indices. It is efficacious in this indication; however, it has a high propensity to induce adverse effects, including sleep disorders, nausea, and vomiting (Herrmann et al., 1977; Allain et al., 2000; Marona-Lewicka et al., 2002; Stocchi et al., 2002), whereas first-generation H_1R antagonists are used as nonprescription remedies for insomnia (Richardson et al., 2002) and are also effective against nausea (Peggs et al., 1995). Lisuride, acting as a weak partial H_1R agonist, may act as an antagonist in vivo, as suggested by the effects of lisuride on Ca^{2+} signaling and ileum contractions using endogenously expressed H_1Rs; however, the extent to which the known side effects of lisuride might be caused by agonistic/antagonistic activity of lisuride at H_1Rs in vivo remains unknown.

Lisuride is far from selective for the H_1R (Beart et al., 1986; Boess et al., 1997; Egan et al., 1998; Marona-Lewicka et al., 2002; Millan et al., 2002). Herein, we reported not only the high H_1R affinity of lisuride with an average pK_i value of 7.9, which is in agreement with data reported in literature for rat or guinea pig H_1Rs (Beart et al., 1986; Millan et al., 2002), but also the high potency of lisuride as a partial H_1R agonist with an average pEC_{50} value of 8.0. When functionally profiled using R-SAT, lisuride displays significantly higher potency as an agonist at human D_2 and 5-HT_2 recep-

TABLE 3

Hydrogen bonding pattern during the last 50 ps of the MD run

In contrast to 5*R*,8*R*-lisuride, 5*R*,8*R*,10*R*-terguride and 5*R*,8*S*,10*R*-terguride, the compound 5*R*,8*S*-lisuride is not able to form hydrogen bonds with Lys^{179} and Glu^{181} in extracellular loop 2 of the histamine H1R.

Molecular Structure	% Hydrogen bonding with			
	Asn^{198}	Asp^{107}	Glu^{181}	Lys^{179}
5*R*,8*R*-Lisuride	99	97	97	87
5*R*,8*S*-Lisuride	99	94	0	0
5*R*,8*R*,10*R*-Terguride	100	81	85	100
5*R*,8*S*,10*R*-Terguride	100	72	70	100

tors, with nearly 100- to 400-fold selectivity for these sites over H_1Rs (data not shown). These data argue that the clinical effects of lisuride represent the sum total of the interactions with a number of different receptor sites and that it probably could, on the basis of absolute potency, occupy H_1Rs in vivo. Moreover, these data suggest that one should be cautious in using 2-iodolisuride in positron emission tomography (PET) or single photon emission computed tomography (SPECT) studies for monitoring D_2 receptors in vivo (Prunier et al., 2001) because H_1Rs, which are highly expressed in the central nervous system, may also be labeled to some extent.

In conclusion, we have identified 8R-lisuride as a high-affinity partial H_1R agonist that displays pronounced stereospecificity toward the receptor, providing both a valuable tool for the in vitro study of H_1Rs, as well as a promising template for the development of specific high affinity H_1R agonists. The future development of such brain-penetrating H_1R agonists will advance our understanding of the role of the H_1R in the central nervous system and address outstanding questions regarding tolerability and therapeutic utility of H_1R agonists.

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Correction to "8R-Lisuride is a potent stereospecific histamine H1-receptor partial agonist"

In the above article [Bakker RA, Weiner DM, ter Laak T, Beuming T, Zuiderveld OP, Edelbroek M, Hacksell U, Timmerman H, Brann MR, and Leurs R (2004) *Mol Pharmacol* **65:**538–549], the footnotes reporting the current addresses of two of the authors were incorrect. Those addresses should have been:

for T. ter Laak: CDCC/Computational Chemistry, Research Laboratories, Schering AG, Müllerstr. 178, 13342 Berlin-Wedding, Germany.

for T. Beuming: Department of Physiology and Biophysics, Mount Sinai School of Medicine, New York, NY 10029-6574.

We regret this error and apologize for any confusion or inconvenience it may have caused.