

# Pharmacological Characterization of the Novel Histamine H<sub>3</sub>-Receptor Antagonist *N*-(3,5-Dichlorophenyl)-*N'*-[[4-(1*H*-imidazol-4-ylmethyl)phenyl]-methyl]-urea (SCH 79687)

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Received January 21, 2003; accepted March 7, 2003

## ABSTRACT

We present the pharmacological and pharmacokinetic profiles of a novel histamine H<sub>3</sub> receptor antagonist, *N*-(3,5-dichlorophenyl)-*N'*-[[4-(1*H*-imidazol-4-ylmethyl)phenyl]-methyl]-urea (SCH 79687). The H<sub>3</sub>-receptor binding *K*<sub>i</sub> values for SCH 79687 were 1.9 and 13 nM in the rat and guinea pig (GP), respectively. The *K*<sub>i</sub> values for SCH 79687 at histamine H<sub>1</sub> and H<sub>2</sub> receptors were greater than 1 μM. SCH 79687 showed a 41- and 82-fold binding selectivity for the H<sub>3</sub> receptor over α<sub>2A</sub>-adrenoceptors and imidazoline I<sub>2</sub>, and >500-fold H<sub>3</sub> selectivity compared with over 60 additional receptors. The pA<sub>2</sub> value for SCH 79687 in the GP ileum electrical field-stimulated (EFS) contraction was 9.6 ± 0.3. Similar H<sub>3</sub> antagonist activity was observed in the EFS cryopreserved and fresh tissue isolated human saphenous vein (HSV) assays (p*K*<sub>b</sub> = 9.4 ± 0.3 and 10.1 ± 0.4). SCH 79687 (30 nM) did not block clonidine-induced inhibition of EFS-induced contractions in HSV. SCH 79687 (ED<sub>50</sub> = 0.3 mg/kg i.v.) attenuated (*R*)-α-methylhistamine inhibition of sympathetic hypertensive responses in the GP. At the time of activity evaluation, the GP plasma SCH 79687 concentration was 25 ng/ml

at the dose of 0.3 mg/kg i.v. In feline nasal studies, combined administration of SCH 79687 (3 mg/kg i.v.) and the H<sub>1</sub>-antagonist loratadine (3 mg/kg i.v.), at individual doses that do not produce decongestion, inhibited the compound 48/80-induced congestion by 47%. The α-adrenergic agonist phenylpropanolamine (PPA; 1 mg/kg i.v.) also attenuated compound 48/80 nasal responses by 42%. Unlike the H<sub>3</sub>/H<sub>1</sub> combination that did not affect blood pressure (BP), PPA (1 mg/kg i.v.) significantly increased BP compared with control animals by a maximum of 31 mm Hg. Orally, SCH 79687 (10 mg/kg) plus loratadine (10 mg/kg) also produced decongestion without effects on BP. In pharmacokinetic studies, oral dosing with SCH 79687 in the rat (10 mg/kg) and monkey (3 mg/kg) achieved plasma C<sub>max</sub> and area under the curve values greater than 1.5 and 12.1 μg · h/ml, respectively. SCH 79687 is an orally active H<sub>3</sub> antagonist with a good pharmacokinetic profile that, in combination with an H<sub>1</sub> antagonist, demonstrates decongestant efficacy comparable with oral sympathomimetic decongestants but without hypertensive liabilities.

Allergic rhinitis is a highly prevalent and chronic disease that affects a significant portion of the U.S. population (Naclerio, 1991; Blaiss, 2000). Symptoms of the disease are pruritus, sneezing, rhinorrhea, mucosal inflammation, and nasal congestion (Turner and Foreman, 1999; Corey et al., 2000). Mast cell histamine is a principal mediator in nasal allergic responses in sensitized people (Babe and Serafin, 1996; Baraniuk, 1998; Park and Baraniuk, 2002). Histamine

exerts numerous local and systemic effects by activation of pharmacologically distinct histamine receptors, namely, H<sub>1</sub>, H<sub>2</sub>, H<sub>3</sub>, and possibly through a newly discovered H<sub>4</sub> receptor (Hirschowitz, 1979; Leurs et al., 1995; Baroody and Naclerio, 2000; Nakamura et al., 2000; Alves-Rodrigues et al., 2001; Morse et al., 2001; Nguyen et al., 2001; Stark et al., 2001). Many of the allergic nasal responses to histamine such as mucus secretion, increased vascular permeability and pruritus are mediated by H<sub>1</sub> receptors (Babe and Serafin, 1996). A significant contribution of histamine H<sub>2</sub> receptors to nasal allergic responses has not been demonstrated and a role for H<sub>4</sub> receptors, which are found predominantly on inflammatory cells, in upper airway allergic responses remains to be identified.

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Article, publication date, and citation information can be found at <http://jpet.aspetjournals.org>.  
DOI: 10.1124/jpet.103.049254.

**ABBREVIATIONS:** CNS, central nervous system; SCH 79687, *N*-(3,5-dichlorophenyl)-*N'*-[[4-(1*H*-imidazol-4-ylmethyl)phenyl]-methyl]-urea; DMSO, dimethyl sulfoxide; GPI, guinea pig ileum; HSV, human saphenous vein; EFS, electrical field stimulation(ed); HPLC-API/MS/MS, high-performance liquid chromatography-atmospheric pressure ionization tandem mass spectrometry; PK, pharmacokinetic; PPA, phenylpropanolamine.

Histamine H<sub>3</sub> receptors are widely distributed in the CNS and in the peripheral autonomic nervous system (Arrang et al., 1983; Coruzzi et al., 1991; Yanai et al., 1994; Göthert et al., 1995). Peripheral H<sub>3</sub> receptors may serve as a target for the development of novel antiallergy drugs, in particular, mechanism-based decongestants (McLeod et al., 1999, 2001b). These receptors prejunctionally modulate sympathetic neurotransmission and attenuate a variety of organ responses governed by sympathetic nervous system regulation (Koss and Hey, 1992; McLeod et al., 1993; Yu et al., 2001). Moreover, *in vivo* histamine H<sub>3</sub> receptors play a physiological role in the modulation of nasal vascular tone, a regulator of nasal patency (Bolser et al., 1994; McLeod et al., 2001a). Additionally, we have observed that in an experimental nasal congestion model that histamine H<sub>3</sub> antagonists when administered in combination with an H<sub>1</sub> antagonist can evoke decongestant activity in addition to the antiallergy effects that arise from sole blockade of H<sub>1</sub> receptors (McLeod et al., 1999).

In the present study, we characterized the preclinical *in vitro* and *in vivo* pharmacological activity and pharmacokinetic profile of SCH 79687 (Aslanian et al., 2002), a novel, selective, and potent histamine H<sub>3</sub> receptor antagonist. In addition, we studied the oral nasal decongestant effect of SCH 79687 (Fig. 1) alone and in combination with the histamine H<sub>1</sub> antagonist loratadine in a feline experimental model of nasal congestion.

## Materials and Methods

### Materials

*N*-(3,5-Dichlorophenyl)-*N'*-[[4-(1*H*-imidazol-4-ylmethyl)phenyl]-methyl]-urea (SCH 79687 dihydrochloride), cimetidine, and chlorpheniramine maleate were supplied by Chemistry, Schering-Plough Research Institute (Kenilworth, NJ). [<sup>3</sup>H]*N*<sup>α</sup>-Methylhistamine (81 Ci/mmol) was purchased from DuPont (Wilmington, DE). (*R*)- $\alpha$ -Methylhistamine dihydrochloride, thioperamide maleate, clonidine dihydrochloride, *l*-norepinephrine bitartrate, prazosin hydrochloride, and compound 48/80 were obtained from Sigma/RBI (Natick, MA). Concentrated stock solutions of chlorpheniramine maleate, cimetidine, *l*-norepinephrine, clonidine, and KCl were prepared in water. Concentrated stock solutions of SCH 79687 and thioperamide were prepared in dimethyl sulfoxide (DMSO) and either further diluted in DMSO (guinea pig ileum experiments) or water (human saphenous vein) before addition to the baths. Final concentrations of DMSO did not exceed 0.06% (guinea pig ileum, GPI) or 0.01% (human saphenous vein, HSV) by volume in the bath. Sterile-filtered, heat-inactivated fetal bovine serum, sucrose, and KCl were obtained from Sigma-Aldrich (St. Louis, MO). For intravenous *in vivo* studies, loratadine was dissolved in 30% DMSO, 40% ethyl alcohol, and physiological saline (0.9%) and given slowly over 2 min. SCH 79687 was dissolved in 30% DMSO and saline.

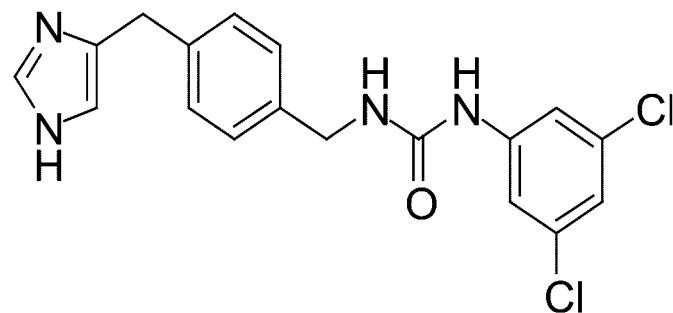


Fig. 1. Chemical structure of SCH 79687.

All drug doses refer to their respective free base. For oral cat decongestant studies, drugs were administered in gelatin capsules (size 0; Torpac, Inc., Fairfield, NJ). All control animals were given appropriate vehicle controls.

### Animal Care and Use

These studies were performed in accordance to the National Institutes of Health Guide to the Care and Use of Laboratory Animals and the Animal Welfare Act in an Association for the Assessment and Accreditation of Laboratory Animal Care program.

### Guinea Pig Histamine H<sub>3</sub> Receptor Binding Assay

Histamine H<sub>3</sub> receptor binding was performed according to West et al. (1990). Guinea pig brains were obtained frozen, thawed at room temperature, and homogenized in 10 volumes (w/v) of ice-cold 50 mM Tris-HCl, pH 7.5, buffer and disrupted with a Polytron (PTA 10 tip, 30 s at setting 5). After low-speed centrifugation (10 min, 1000g), the supernatant was centrifuged 10 min at 50,000g. The high-speed pellet was resuspended in the original volume of buffer, a sample was taken for protein assay (bicinchoninic acid; Pierce Chemical, Rockford, IL), and the suspension was centrifuged again at 50,000g. Pellets were removed and frozen at  $-80^{\circ}\text{C}$  until use.

Membrane (300  $\mu\text{g}$  of protein) was incubated with [<sup>3</sup>H]*N*<sup>α</sup>-methylhistamine (0.5 nM) without or with inhibitor compounds in a total volume of 200  $\mu\text{l}$  of buffer. Nonspecific binding was determined in the presence of 10  $\mu\text{M}$  thioperamide. Assay mixtures were incubated for 30 min at 30 $^{\circ}\text{C}$  in polypropylene, 96-well, deep-well plates then filtered through 0.3% polyethylenimine-soaked GF/B filters (Whatman, Maidstone, UK). These were washed three times with 1.2 ml of buffer, dried in a microwave oven, impregnated with Meltilex scintillant, and were counted at 40% efficiency in a Betaplate scintillation counter (PerkinElmer Wallac, Gaithersburg, MD). Curves were fit to the guinea pig data for SCH 79687 with Prism nonlinear least-squares curve-fitting program (GraphPad Software, Inc., San Diego, CA). One- and two-site fits were tested.

### Additional Receptor Binding Characterization

SCH 79687 was run against a panel of 65 receptor binding assays (MDS Pharma Services, Taipei, Taiwan). Methods used in all receptor assays were adapted from the scientific literature and are available upon request from MDS Pharma Services. SCH 79687 was tested in duplicate at 1  $\mu\text{M}$ .

### Histamine H<sub>3</sub> Receptor Antagonist Activity in Isolated Guinea Pig Ileum and Human Saphenous Vein

Discarded HSVs from coronary artery bypass graft patients (male and female, age = 53–80 years; Hackensack University Medical Center Institute for Biomedical Research, Hackensack, NJ) stored and shipped in 4 $^{\circ}\text{C}$  heparinized autologous blood were received within 30 h of removal. HSV rings (3–7 mm in diameter  $\times$  5 mm) were used fresh upon arrival or cryopreserved. Cryopreservation and thawing were as described previously (Valentine et al., 1999) except for a 10-min cryofreezing container equilibration at 4 $^{\circ}\text{C}$  before its transfer to  $-70^{\circ}\text{C}$ .

The general methods used for the isolated GPI and HSV assays, including guinea pig strain, sex, and size, were as described in Valentine et al. (1999). The assays were performed in the presence of chlorpheniramine maleate (1  $\mu\text{M}$ ) alone (GPI) or combined with cimetidine (1  $\mu\text{M}$ , HSV) to block H<sub>1</sub>- and H<sub>2</sub>-mediated effects, respectively (Leurs et al., 1995).

Whole longitudinal GPI segments (2 cm) equilibrated at 0.3-g passive tension were contracted repetitively (1-min intervals) using submaximal EFS, pulse duration, and voltage adjusted to attain 60 to 80% of the reference contraction to 25 Hz, 8 V, 1-ms pulse duration, and 1-s train/min. Antagonist was added 5 min before the initiation of repetitive submaximal EFS trains. After the first stimulus train, rising cumulative additions of (*R*)- $\alpha$ -methylhistamine (1

nM–100  $\mu$ M, half-log increments) were performed 1 min before each succeeding stimulus train.

Fresh and cryopreserved HSV rings equilibrated at 1.0-g passive tension were contracted using submaximal EFS (16 Hz, 1-ms pulse duration and submaximal voltage). A 45-min antagonist equilibration preceded a series of 30-s train EFS-induced contractions performed at 15-min intervals. After the control EFS-train, rising cumulative additions of (*R*)- $\alpha$ -methylhistamine (0.01 nM–100  $\mu$ M, log increments) were performed 10 min before each succeeding stimulus train. Prazosin (1  $\mu$ M), an  $\alpha_1$ -adrenoceptor antagonist (McGrath et al., 1989), applied 10 min before the final stimulus supplied the maximum inhibition of the  $\alpha$ -adrenergic component of the EFS response used to normalize (*R*)- $\alpha$ -methylhistamine responses. For studies of SCH 79687 (30 nM), effects on prejunctional  $\alpha_2$ -adrenergic receptor modulation of EFS, clonidine, an  $\alpha_2$ -adrenergic agonist (McGrath et al., 1989), replaced (*R*)- $\alpha$ -methylhistamine. To evaluate SCH 79687 effects on postjunction-mediated contractility, cumulative applications of *l*-norepinephrine (0.1–100  $\mu$ M), a nonspecific  $\alpha$ -adrenergic agonist (McGrath et al., 1989), and an application of KCl (80 mM) preceded a 1-h equilibration with 3 or 10  $\mu$ M SCH 79687 and a repeat of the *l*-norepinephrine and KCl applications.

### In Vivo Studies

**Histamine H<sub>3</sub> Antagonist Activity in the Guinea Pig.** The procedure for evaluating the in vivo histamine H<sub>3</sub> receptor activity has been described previously (Hey et al., 1992). The in vivo histamine H<sub>3</sub> antagonist activity of SCH 79687 was evaluated by measuring its ability to block the inhibitory effects of (*R*)- $\alpha$ -methylhistamine on the sympathetic hypertensive responses evoked by electrical stimulation of the medulla oblongata. Male Hartley guinea pigs were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and surgically prepared for catheterization of the trachea, jugular vein, and carotid artery. Animals were mechanically ventilated (tidal volume = 4 ml, frequency = 45 breaths/min) and paralyzed with gallamine triethiodide (2 mg/kg i.v.) and pretreated with ipratropium bromide (10  $\mu$ g/kg i.v.) to block cholinergic cardiopulmonary responses. Animals were instrumented for measurement of blood pressure, heart rate, and pulmonary insufflation pressure. All physiological parameters were recorded continuously on a physiograph. Animals were positioned in a stereotaxic apparatus and implanted with electrodes for stimulation of medullary cardiopressor areas. The stimulation parameters used were 32 Hz, 3- to 5-s trains, 0.5- to 1.5-ms square pulses, and varying intensities from 25 to 100  $\mu$ A. The cardiopressor responses to CNS stimulation were evaluated before and after the test drug (or vehicle). SCH 79687 (0.03–3.0 mg/kg i.v.) was given 5 min before (*R*)- $\alpha$ -methylhistamine (0.3 mg/kg i.v.). Peak cardiopressor responses were recorded to each stimulus intensity (25–100  $\mu$ A) approximately 30 min after (*R*)- $\alpha$ -methylhistamine was given. The dose of (*R*)- $\alpha$ -methylhistamine was chosen based on its ability to produce maximal H<sub>3</sub> receptors mediated inhibition of CNS-induced hypertension (Hey et al., 1992). For determination of the inhibitory effect, the stimulus intensity of 75  $\mu$ A was selected because it elicits consistent, submaximal increases in blood pressure.

**Effect of SCH 79687 Plus Loratadine on Increases in Nasal Resistance Due to Compound 48/80 in the Cat.** Acoustic rhinometry measurements of evaluating decongestant activity in anesthetized cat has been described previously (McLeod et al., 1999). Briefly, pentobarbital (35 mg/kg i.p.) anesthetized cats were mechanically ventilated (volume = 25 ml, rate = 20 breaths/min) with room air. The left nostril was sealed with repositil (Dentsply International Inc., Milford, DE). A cuffed endotracheal tube was advanced from the upper esophagus to the nasopharynx. A constant flow (1.7 l/min) of air was passed through the right nasal airway via the endotracheal tube. Nasal pressure values were converted to nasal resistance using the formula resistance = pressure/flow. Continuous blood pressure measurements were recorded on a Grass chart recorder.

The effects of combined H<sub>1</sub> and H<sub>3</sub> receptor blockade with loratadine (3 mg/kg i.v.) and SCH 79687 (3 mg/kg i.v.), loratadine alone,

SCH 79687 alone, PPA (1 mg/kg i.v.), or vehicle were studied on the increases in nasal resistance produced by exposure to compound 48/80 (1%, aerosolized for 45 s). The doses of loratadine (3 mg/kg i.v.) and PPA (3 mg/kg i.v.) were chosen to match previously validated doses of these drugs in the cat nasal congestion model (McLeod et al., 1999). Similarly, the dose of SCH 79687 was comparable with doses of thioperamide previously evaluated in the cat (McLeod et al., 1999, 2001a). Drugs were given 30 min before administration of compound 48/80. Pharmacological responses were observed 40 min after drug treatment.

**Effect of SCH 79687 Plus Loratadine on Decreases in Nasal Cavity Volume Due to Compound 48/80.** Estimates of nasal volumes were determined according to the methods of McLeod et al. (1999) in the anesthetized cat using acoustic rhinometry equipment purchased from NADAR (Aarhus, Denmark). Sound waves produced from a spark generator were propagated down a rigid wave tube and entered the nasal cavity through an airtight 3.2-cm nose-piece. Reflected sound waves from the nose were amplified and recorded. The sampling frequency was 100 kHz. The data obtained were converted to area-distance function curves and were used to provide estimates of cross-sectional areas and nasal volumes. The distance measured from the nostril opening into the nasal cavity was 3.0 cm and was based on measurements of cast impressions made of the cat nasal passageways.

The effect of either the combination of loratadine (10 mg/kg p.o.) and SCH 79687 (10 mg/kg p.o.) or loratadine alone, SCH 79687 alone, PPA (10 mg/kg p.o.), or vehicle given 2 h before compound 48/80 (1.0%, 50  $\mu$ l) was instilled into the left nares was evaluated. The right nares was given saline. Effects on the ratios of the volumes of the left to right nasal passage were evaluated at 3 h after drug treatment.

### Pharmacokinetic Studies

**Pharmacokinetic Profile in the Guinea Pig.** A separate study was conducted in guinea pigs using the SCH 79687 ED<sub>50</sub> value to determine systemic exposure at this dose. Blood samples were collected 1, 5, 15, 20, 30, and 40 min post i.v. administration and then centrifuged to isolate plasma.

**Pharmacokinetic Profile in the Rat.** Four male Sprague-Dawley rats were dosed p.o. at 10 mg/kg using a 0.4% (w/v) methylcellulose suspension of SCH 79687 as the micronized crystalline dihydrochloride salt. Blood samples were collected into tubes containing heparin at 0.5, 1, 3, 5, 7, and 24 h post dose. After settling on ice, plasma was isolated and placed into sample tubes. The tubes were stored at –20°C until assayed via high-performance liquid chromatography-atmospheric pressure ionization tandem mass spectrometry (HPLC-API/MS/MS).

**Pharmacokinetic Profile of SCH 79687 Administered to Monkeys.** Four male cynomolgus monkeys were dosed p.o. at 3 mg/kg using a 0.4% (w/v) methylcellulose suspension of SCH 79687 as the micronized crystalline dihydrochloride salt. Blood samples were collected into tubes containing heparin at 0.25, 0.5, 1, 2, 4, 6, 7.5, 12, and 24 h post dose. Plasma was isolated and placed into sample tubes. The tubes were stored at –20°C until assayed via HPLC-API/MS/MS.

Two male cynomolgus monkeys were dosed i.v. at 3 mg/kg using a 20% (w/v) 2-hydroxypropyl- $\beta$ -cyclodextrin solution of SCH 79687 as the micronized crystalline dihydrochloride salt. Blood samples were collected into tubes containing heparin at 0.12, 0.25, 0.5, 1, 2, 4, 6, 7.5, 12, and 24 h post dose. After settling on ice, plasma was isolated and placed into sample tubes. The tubes were stored at –20°C until assayed via HPLC-API/MS/MS.

**HPLC-API/MS/MS Assay of Plasma Samples.** Plasma samples were assayed for concentration of SCH 79687 using the technique of HPLC-API/MS/MS as described previously (Bryant et al., 1997). Plasma (40  $\mu$ l) was added to a microcentrifuge tube and subjected to protein precipitation with 100  $\mu$ l of acetonitrile containing 0.2 ng/ $\mu$ l of an internal standard, SCH 66336 (4-[2-[4-(3,10-dibromo-8-chloro-



6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2b]pyridin-11-yl)-piperidin-1-yl]-2-oxo-ethyl)-piperidine-1-carboxylic acid amide; Liu et al., 1998). After vortexing for 30 s and centrifugation at 12,000g for 8 min, the supernatant was transferred into HPLC injection vial. The HPLC system consisted of a Shimadzu LC-10AD pump and PerkinElmer series 200 autosampler. Chromatographic separation of SCH 79687 and the internal standard was achieved with an Inertsil ODS-2 column (4.6 × 50 mm) using an isocratic solvent system containing 50% methanol in water (4 mM ammonium acetate) at a flow rate of 0.8 ml/min. The effluent from the HPLC system was connected directly to a PE-Sciex API 365 triple quadrupole mass spectrometer equipped with an atmospheric pressure chemical ionization interface. Multiple reaction monitoring was used for quantitation of SCH 79687 (MH<sup>+</sup> *m/z* 375 to a fragment ion *m/z* 171). Calibration samples (5–5000 ng/ml) were prepared by spiking drug-free guinea pig plasma with known concentrations of SCH 79687 and then processed with the study samples. The calibration curve was constructed using least-squares linear regression with 1/*x*<sup>2</sup> weighting and used peak area ratios of the analyte and internal standard. Quality control samples at three specified concentrations were also run with the study to ensure accuracy of the calibration curve.

**Data Analysis and Statistics.** Contractions were measured as grams of tension increase over baseline, normalized as percentage of reference EFS response for GPI EFS-induced contraction and as percentage of KCl (80 mM) response for contractions to *l*-norepinephrine. The inhibition of EFS-induced contraction, normalized as percentage of (*R*)- $\alpha$ -methylhistamine maximum (GPI) or percentage of prazosin response (HSV), represented prejunctional agonist activity. Agonist EC<sub>50</sub> (half-maximal concentration) was estimated using linear regression analysis of individual agonist concentration-response curves and EC<sub>50</sub> or pD<sub>2</sub> (–log<sub>10</sub> of the EC<sub>50</sub>) was used to express potency. H<sub>3</sub> antagonist activity was represented by shift in the agonist EC<sub>50</sub>, from which an agonist dose ratio (DR = A'/A, where A' and A are the EC<sub>50</sub> values estimated in the presence and absence of the antagonist, respectively, was calculated (Tallarida, 1988). Antagonist affinity was estimated as pA<sub>2</sub> (–log<sub>10</sub> of the antagonist molar concentration that produces a DR = 2) or apparent pK<sub>b</sub> (= –log<sub>10</sub> of K<sub>b</sub>) (Tallarida and Murray, 1981; Tallarida, 1988). The pA<sub>2</sub> was calculated using Analysis I: Schild Plot of Tallarida and Murray (1981) and individual dose ratios from antagonist concentrations that yielded mean DR ≥ 2. Apparent K<sub>b</sub> was estimated using K<sub>b</sub> = [B]/(A/A – 1), where [B] is the concentration of antagonist tested (Tallarida, 1988) and individual dose ratios ≥ 2 from statistically active antagonist concentrations. Statistical significance was taken as *p* < 0.05 using a Kruskal-Wallis nonparametric multiple group analysis and/or a Mann-Whitney *U* two-group analysis comparing control and treated EC<sub>50</sub> values.

The nasal cavity volume data were expressed as the ratio of the volume of left-treated nares versus the right-untreated nares (McLeod et al., 1999). Values displayed in the table and the figures represent the mean ± S.E.M. For all in vivo studies, data were evaluated using a Kruskal-Wallis analysis in conjunction with a Mann-Whitney *U*. Statistical significance was set at *p* < 0.05.

## Results

**Receptor Binding Profile.** Competition of SCH 79687 versus [<sup>3</sup>H]*N*<sup>α</sup>-methylhistamine binding to guinea pig brain disclosed high-affinity inhibition of binding to an apparently single site. The K<sub>i</sub> value for SCH 79687 at guinea pig was 13 ± 6. A similar guinea pig K<sub>i</sub> affinity was obtained for thioperamide (12 ± 6 nM). Table 1 lists the K<sub>i</sub> values for SCH 79687 binding to a variety of receptor systems (MDS Pharma Services Counterscreen). In this analysis, SCH 79687 showed 41- and 82-fold selectivity for the H<sub>3</sub> receptor over the  $\alpha$ <sub>2A</sub> (human)-adrenergic- and imidazoline I<sub>2</sub> (rat)-receptor, re-

TABLE 1

Receptor binding characterization of SCH 79687<sup>a</sup>

Please note that SCH 79687 did not display significant activity in the following MDS Pharma enzymes and receptor binding assays: acetylcholinesterase, carbonic anhydrase, choline acetyltransferase, myeloperoxidase, phosphodiesterase (II, III, IV, V), calpain, protease-cathepsin G, collagenase IV, protease-elastase, neutral endopeptidase, EGF receptor Tyr kinase, HER2 tyrosine kinase, CD45 Tyr phosphatase, protein phosphatase, PTP1B, protein phosphatase, PTP1C, adenosine A<sub>3</sub>, adrenergic  $\alpha$ <sub>1A</sub>,  $\alpha$ <sub>2C</sub>,  $\beta$ <sub>1</sub>,  $\beta$ <sub>2</sub>,  $\beta$ <sub>3</sub>, bradykinin B<sub>1</sub>, calcitonin, Ca<sup>2+</sup> channel (L,N), cannabinoid (CB<sub>1</sub>, CB<sub>2</sub>), cholecystokinin (CCK<sub>A</sub>, CCK<sub>B</sub>), dopamine (D<sub>1</sub>, D<sub>2</sub>, D<sub>3</sub>, D<sub>4</sub>, D<sub>5</sub>), endothelin ET<sub>B</sub>, epidermal growth factor, glucocorticoid, interferon- $\gamma$ , interleukin-6, interleukin-8, leukotriene B<sub>4</sub>, muscarinic (M<sub>1</sub>, M<sub>2</sub>, M<sub>3</sub>, M<sub>4</sub>, M<sub>5</sub>), neuropeptide Y<sub>1</sub>, K<sup>+</sup> channel (K<sub>A</sub>), serotonin (5-HT<sub>1A</sub>, 5-HT<sub>6</sub>, 5-HT<sub>7</sub>), sigma ( $\sigma$ <sub>1</sub>,  $\sigma$ <sub>2</sub>) tachykinin (NK<sub>1</sub>, NK<sub>2</sub>), vasoactive intestinal peptide (VIP<sub>1</sub>).

Receptor Target	K <sub>i</sub>
Histamine H <sub>3</sub> (rat)	1.9 nM
Histamine H <sub>1</sub> (guinea pig)	>1 $\mu$ M
Histamine H <sub>2</sub> (guinea pig)	>1 $\mu$ M
Adrenergic $\alpha$ <sub>2A</sub> (human)	78 nM
Imidazoline I <sub>2</sub> (rat)	155 nM

<sup>a</sup> MDS Pharma Services Counterscreen profile.

spectively, and >500-fold higher affinity for the H<sub>3</sub> receptor compared with 65 additional receptors (data not shown).

**Histamine H<sub>3</sub> Receptor Antagonist Activity in Isolated Guinea Pig Ileum and Human Saphenous Vein.** (*R*)- $\alpha$ -Methylhistamine inhibited EFS-induced cholinergic contractions of isolated GPI, demonstrating mean EC<sub>50</sub> = 8.4 ± 0.7 nM (pD<sub>2</sub> = 8.1; *n* = 61). The maximal inhibition by (*R*)- $\alpha$ -methylhistamine was nearly complete in this assay, amounting to 96.0 ± 1.0% of the baseline EFS response in a representative sample of tissues (*n* = 35; data not shown). SCH 79687 and thioperamide dose dependently inhibited (*R*)- $\alpha$ -methylhistamine activity, demonstrating pA<sub>2</sub> estimates in the sub- to low nanomolar range (Table 2). Schild plot slopes were –0.94 ± 0.25 (95% confidence limits, –1.5 to –0.4) and –0.8 ± 0.11 (95% confidence limits, –1.0 to –0.5) for SCH 79687 and thioperamide, respectively.

(*R*)- $\alpha$ -Methylhistamine inhibited EFS-induced sympathetic contractions of isolated HSV. Mean (*R*)- $\alpha$ -methylhistamine EC<sub>50</sub> value was 0.5 ± 0.2 nM (pD<sub>2</sub> = 9.3; *n* = 21) in cryopreserved tissue and 2.4 ± 1.7 nM (pD<sub>2</sub> = 8.6; *n* = 11) in fresh tissue. The potency of (*R*)- $\alpha$ -methylhistamine in fresh and cryopreserved HSVs was not significantly different. In contrast to GPI, maximal (*R*)- $\alpha$ -methylhistamine inhibition of EFS was partial in HSVs, amounting to 42.2 ± 3.2 and 29.1 ± 2.5% inhibition in samples of fresh and cryopreserved tissue, respectively (*n* ≥ 19; data not shown). In the same fresh and cryopreserved tissue samples, respectively, prazosin (1  $\mu$ M) produced 59.7 ± 5.7 and 49.9 ± 4.0% inhibition, thus maximal (*R*)- $\alpha$ -methylhistamine inhibition represented 71 and 58% of the prazosin-sensitive portion of the EFS twitch response. SCH 79687 and thioperamide dose dependently inhibited (*R*)- $\alpha$ -methylhistamine responses and exhibited sub- to low nanomolar pK<sub>b</sub> estimates in cryopreserved HSVs (Table 2). A pK<sub>b</sub> of 10.1 ± 0.4 was estimated for SCH 79687 against (*R*)- $\alpha$ -methylhistamine in fresh HSVs.

The specificity of SCH 79687 for the H<sub>3</sub> receptor was also

TABLE 2

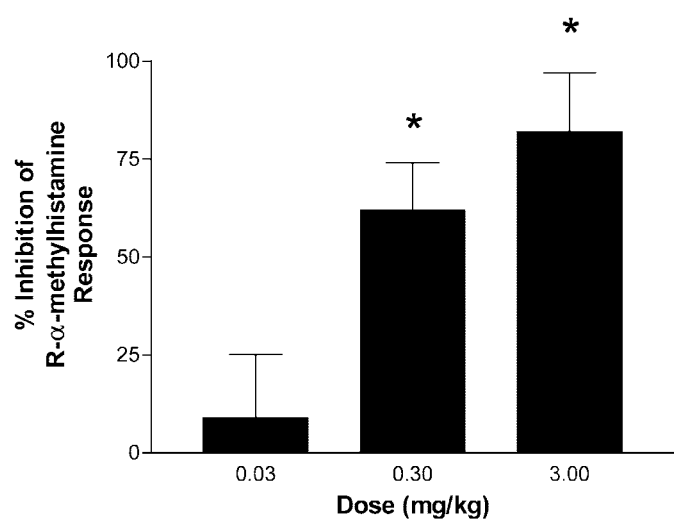
Histamine H<sub>3</sub> antagonist activity in the guinea pig ileum and cryopreserved isolated human saphenous vein

Compound	Guinea Pig Ileum pA <sub>2</sub>	Human Saphenous Vein pK <sub>b</sub> Estimate
SCH 79687	9.6 ± 0.3	9.4 ± 0.3
Thioperamide	8.8 ± 0.24	8.9 ± 0.5

investigated using pre- and postjunctional-mediated contractions of cryopreserved HSVs. Clonidine inhibited EFS-induced contractions of HSVs (mean  $pD_2 = 10.4 \pm 0.3$ ;  $n = 6$ ). The maximal clonidine inhibition of EFS twitch ( $28.8 \pm 4.4\%$  inhibition;  $n = 6$ ) was similar to the maximal inhibition obtained with (*R*)- $\alpha$ -methylhistamine in this tissue. No significant inhibition of clonidine modulation of EFS contractions was seen with 30 nM SCH 79687 ( $n = 6$ ; data not shown). In addition, SCH 79687 ( $\leq 10 \mu\text{M}$ ) demonstrated no effects on the baseline tone or *l*-norepinephrine ( $pD_2 = 6.2 \pm 0.3$ ;  $n = 3$ )- and KCl (80 mM)-induced contractions of cryopreserved HSVs ( $n = 3$ –4; data not shown).

**Activity and Pharmacokinetic Profile of Intravenous SCH 79687 in the Guinea Pig.** (*R*)- $\alpha$ -Methylhistamine (0.3 mg/kg i.v.) inhibited sympathetic hypertensive responses evoked by stimulation of the medulla oblongata by  $33 \pm 4\%$  ( $n = 12$ ). Figure 2 shows that intravenous SCH 79687 produced a dose-dependent attenuation of the blood pressure effects of (*R*)- $\alpha$ -methylhistamine ( $ED_{50} = 0.28 \text{ mg/kg}$ ;  $n = 5$ –6 animals/group). In a separate study to determine the pharmacokinetics of the compound at this dose, plasma samples were quantified at specified times after i.v. administration of SCH 79687. Mean plasma concentration of SCH 79687 (0.28 mg/kg i.v.) at 1, 5, 15, 20, 30, and 40 min after i.v. dosing are shown in Table 3.

**Nasal Decongestant Effect of SCH 79687 in Combination with Loratadine.** Fig. 3 displays the decongestant actions of combined  $H_1$  and  $H_3$  blockade with loratadine and SCH 79687. Loratadine (3 mg/kg i.v.) administered together with SCH 79687 (3 mg/kg i.v.) significantly blocked the increase in nasal resistance produced by aerosolized compound 48/80 (1%). Loratadine (3 mg/kg i.v.) and SCH 79687 (3 mg/kg i.v.) given alone did not alter nasal responses to compound 48/80. PPA (1 mg/kg i.v.) decreased nasal resistance but also produced a pronounced increase in mean arterial blood pressure (Fig. 3B). Loratadine plus SCH 79687 had no effect on blood pressure compared with control animals. The oral decongestant activity of combined loratadine and SCH

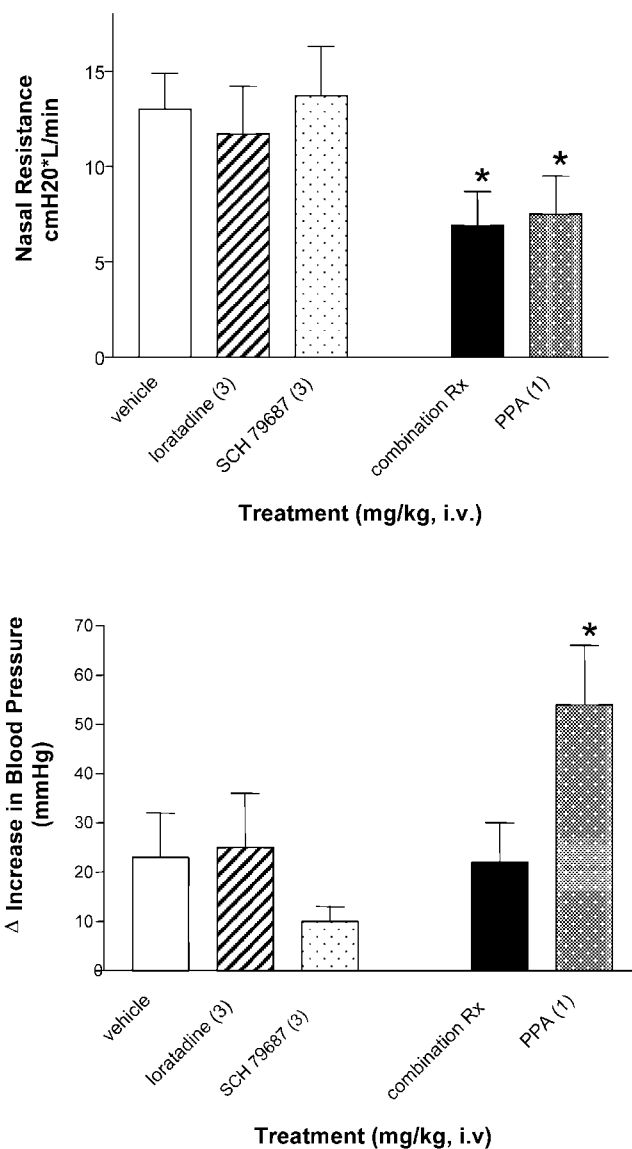


**Fig. 2.** Histamine  $H_3$ -receptor antagonist activity in the guinea pig. Figure illustrates the intravenous antagonist activity of SCH 79687 (0.03–3 mg/kg i.v.) on (*R*)- $\alpha$ -methylhistamine inhibitory effects of electrically induced hypertensive responses. Each value represents the mean  $\pm$  S.E.M. of five to six animals per group (\*,  $p < 0.05$  compared with control).

**TABLE 3**

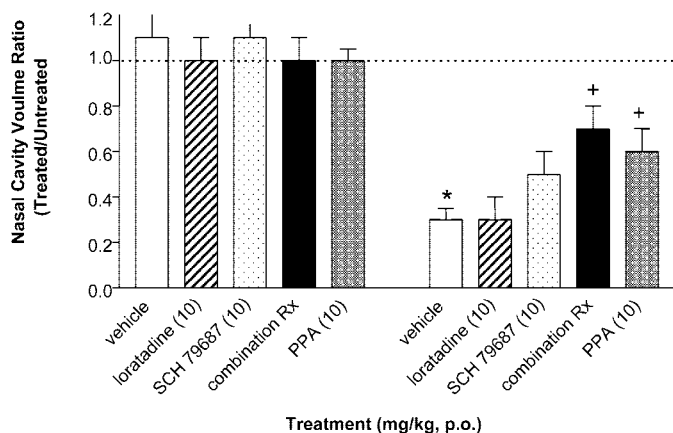
Mean plasma concentration of intravenous SCH 79687 in guinea pigs at the  $ED_{50}$  (0.28 mg/kg)

Time Post Dose	<i>n</i>	Mean Plasma Concentration	Coefficient of Variation
		ng/ml	%
1	5	644	43
5	5	112	42
15	5	39	43
20	4	30	43
30	5	25	61
40	5	18	67



**Fig. 3.** Effect of SCH 79687 in combination with loratadine on nasal resistance and blood pressure in the cat. A, effect of vehicle, SCH 79687 (3 mg/kg i.v.) alone, loratadine (3 mg/kg i.v.) alone, PPA (3 mg/kg), and SCH 79687 plus loratadine on aerosolized compound 48/80 (1%)-induced increases in nasal resistance. B, effect of these treatments on mean arterial blood pressure. Each column represents the mean  $\pm$  S.E.M. of six to seven animals per group (\*,  $p < 0.05$  compared with compound 48/80 alone;  $n = 9$ ).

79687 is shown in Fig. 4. Loratadine (10 mg/kg p.o.) given together with SCH 79687 (10 mg/kg p.o.) inhibited the decrease in nasal cavity volume due to nasal instillation of

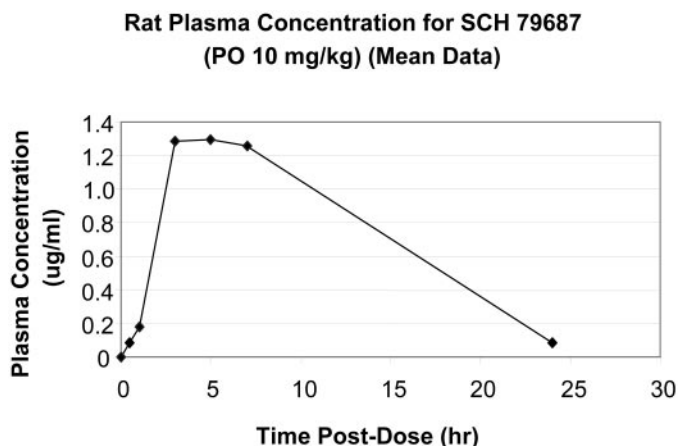


**Fig. 4.** Effect of SCH 79687 in combination with loratadine on feline nasal cavity volume. Figure displays the oral effects of vehicle, SCH 79687 (10 mg/kg) alone, loratadine (10 mg/kg) alone, PPA (10 mg/kg), and SCH 79687 plus loratadine on topical compound 48/80 (1%) induced decreases in nasal cavity volume. Shown are the effects of these treatment regimens at 2 h (immediately before compound 48/80 exposure) and 3 h after oral administration. Each column represents the mean  $\pm$  S.E.M. of four to five animals per group (\*,  $p < 0.05$  compared with compound 48/80 alone group at 2 h; +,  $p < 0.05$  compared with compound 48/80 alone group at 3 h).

compound 48/80. Similarly, PPA (10 mg/kg p.o.) inhibited the nasal effects of compound 48/80. Loratadine and SCH 79687 given alone had no effect on compound 48/80-induced changes in nasal geometry. However, PPA significantly increased systolic blood pressure ( $157 \pm 5$ ;  $n = 4$ ) compared with control animals ( $111 \pm 11$  mm Hg;  $n = 5$ ). The blood pressure in cats treated with a combination of loratadine (10 mg/kg p.o.) and SCH 79687 (10 mg/kg p.o.) was  $114 \pm 12$  mm Hg ( $n = 5$ ) and was not different from vehicle-treated animals.

**Pharmacokinetic Profile of SCH 79687 in the Rat.** The four rats showed similar (PK) profiles. The mean  $C_{max}$ ,  $T_{max}$ , and AUC values were  $1.5 \mu\text{g/ml}$ , 4.5 h, and  $18.1 \mu\text{g} \cdot \text{h/ml}$ , respectively. The mean PK profile for the four rats is shown in Fig. 5. After a single p.o. dose of 10 mg/kg, the rats still showed measurable levels of SCH 79687 at 24 h postadministration.

**Pharmacokinetic Profile of SCH 79687 Administered to Monkeys.** The four monkeys dosed p.o. showed similar



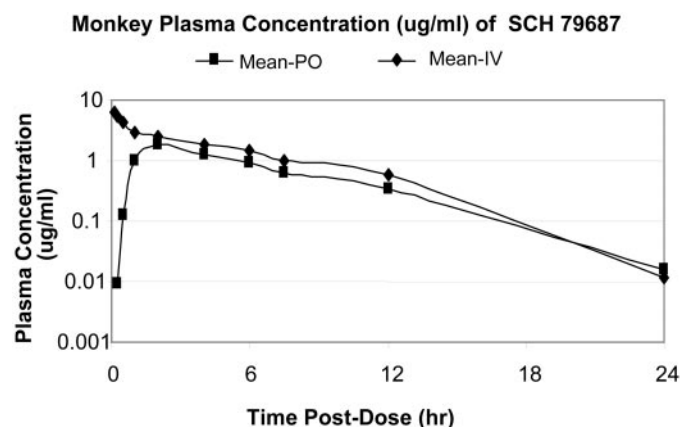
**Fig. 5.** Pharmacokinetic profile of SCH 79687 in the rat. Figure illustrates the mean plasma concentration of SCH 79687 (10 mg/kg;  $n = 4$ ) after oral administration.

PK profiles. The mean  $C_{max}$ ,  $T_{max}$ , and AUC values were  $1.7 \mu\text{g/ml}$ , 4.4 h, and  $12.6 \mu\text{g} \cdot \text{h/ml}$ , respectively. The two monkeys dosed i.v. were very well matched in their PK profile. The mean AUC and  $t_{1/2}$  values were  $24.1 \mu\text{g} \cdot \text{h/ml}$  and 2.5 h, respectively. The mean PK profiles for the monkeys are shown in Fig. 6. After a single p.o. dose of 3 mg/kg, the monkeys still showed measurable levels of SCH 79687 at 24 h post dose.

## Discussion

SCH 79687 is a novel, selective, orally active histamine  $H_3$  antagonist. SCH 79687 binds to  $H_3$  receptors with high affinity. We found that SCH 79687 displayed a weaker binding in the guinea pig ( $K_i = 13$  nM) compared with the rat ( $K_i = 1.9$  nM). Differences in  $H_3$  receptor binding affinities among difference species, including humans, are not unexpected (Lovenberg et al., 2000). In point of fact, previous studies have shown that imidazole-containing  $H_3$  receptor antagonists, including thioperamide, GT-2016, and iodophenpropit typically display less potency against human  $H_3$  receptors compared with  $H_3$  receptors from other species (Ireland-Denny et al., 2001). Nonetheless, SCH 79687 has very weak or no affinity for other receptors, including histamine  $H_1$ ,  $H_2$ , adrenergic, and muscarinic receptors. We also found that SCH 79687 showed dose-dependent antagonist activity in vitro against (*R*)- $\alpha$ -methylhistamine in the isolated fresh GPI and isolated fresh and cryopreserved HSV functional  $H_3$  bioassays. The selective histamine  $H_3$  receptor antagonist thioperamide demonstrated similar  $H_3$  receptor antagonist potency to SCH 79687 in HSVs. In the GPI thioperamide but was approximately 6-fold weaker than SCH 79687. Also in the GPI, the Schild analysis was consistent with competitive blockade of (*R*)- $\alpha$ -methylhistamine by SCH 79687 and thioperamide at the concentrations tested. The estimated  $pA_2$  for thioperamide against (*R*)- $\alpha$ -methylhistamine is similar to previous  $H_3$  receptor  $pA_2$  estimates reported for this compound in GPI (Menkveld and Timmerman 1990; Rizzo et al., 1995). Together, these findings confirm that SCH 79687 is a functional histamine  $H_3$  receptor antagonist in vitro.

In guinea pig studies, SCH 79687 inhibited (*R*)- $\alpha$ -methylhistamine-induced attenuation of electrically provoked hypertensive responses in a dose-dependent manner. Inhibition of (*R*)- $\alpha$ -methylhistamine-induced depression of sympathetic



**Fig. 6.** Pharmacokinetic profile of SCH 79687 in the monkey. Shown are the mean plasma concentration of SCH 79687 after oral ( $n = 4$ ) and i.v. ( $n = 2$ ) administration.



hypertensive responses in the guinea pig is a method used to characterize the H<sub>3</sub> antagonist activity in vivo (Hey et al., 1992). In these studies, we found that SCH 79687 (ED<sub>50</sub> = 0.28 mg/kg i.v.) displays potency comparable with the standard H<sub>3</sub> antagonist thioperamide (ED<sub>50</sub> = 0.4 mg/kg i.v.). Additional evidence for the in vivo H<sub>3</sub> antagonist activity of SCH 79687 is provided by the feline congestion studies. We have previously demonstrated that the combination of a histamine H<sub>3</sub> antagonist with an H<sub>1</sub> antagonist produces nasal decongestant activity (McLeod et al., 1999, 2001a). This study demonstrates that combination blockade of H<sub>1</sub> and H<sub>3</sub> receptors with oral or intravenous loratadine and SCH 79687 produces decongestant activity in the cat equivalent to the  $\alpha$ -agonist decongestant phenylpropanolamine. On the other hand, in contrast to phenylpropanolamine, which produces a significant hypertensive effect after oral administration, SCH 79687 plus loratadine did not alter systemic blood pressure. It is important to note that the nasal decongestant effect of combined SCH 79687 and loratadine are not likely due to either central blockade of either histamine H<sub>1</sub> and/or H<sub>3</sub> receptors. Loratadine is a second-generation antihistamine that does not cross the blood-brain barrier. Similarly, based on our plasma/brain pharmacokinetic analysis, SCH 79687 does not enter the CNS to a significant extent. After a 10-mg/kg oral dose in rats, the ratio of the drug concentration (AUC over 6 h) in the brain relative to the concentration in the plasma was 0.02, indicating that SCH 79687 does not penetrate the blood-brain barrier to a significant extent. Moreover, at the oral dose tested in the cat (10 mg/kg), SCH 79687 did not elicit behavior effects indicative of central histamine H<sub>3</sub> receptor blockade (i.e., excitation and heightened arousal). Together, our studies suggest that the decongestant activity of SCH 79687 plus loratadine is likely due to peripheral activity at the level of the nasal mucosal blood vessels and the sympathetic nerves that innervate them. We proposed that during a nasal allergic reaction, mast cell-derived histamine stimulates prejunctional H<sub>3</sub> receptors to produce a dilation of blood vessels in the nose contributing to nasal congestion (McLeod et al., 2001a). This histamine H<sub>3</sub> receptor mediated activity is in addition to the stimulation of postsynaptic H<sub>1</sub> receptors that elicit plasma extravasation, vasodilation, and mucus secretion. In support of this hypothesis, a recent study by Varty and Hey (2002) demonstrated that activation of histamine H<sub>3</sub> receptors inhibited neurogenic sympathetic vasoconstrictor responses in isolated pig nasal turbinates.

The mean plasma concentration of SCH 79687 (Table 3) in the guinea pig efficacy model at the ED<sub>50</sub> (0.28 mg/kg i.v.) was found to be 25 ng/ml at 30 min post dose when activity was still observed. These data provide a minimum plasma level for SCH 79687 that will be needed for activity. The pharmacokinetics in both the rat and the monkey showed a good plasma profile for SCH 79687 after oral dosing with levels above 25 ng/ml at 12 h (Figs. 5 and 6) and measurable levels at 24 h. These data demonstrate that SCH 79687 has favorable PK properties that would allow for b.i.d. oral dosing at moderate dose levels.

Interestingly, SCH 79687 demonstrated moderate binding to rat imidazoline I<sub>2</sub> receptors ( $K_i$  = 155 nM) and human  $\alpha_{2a}$ -adrenoceptors receptors ( $K_i$  = 78 nM). A role for imidazoline I<sub>2</sub>-receptors in the modulation of nasal patency has not been established. Presynaptic imidazoline receptors that

modulate release of norepinephrine from postganglionic sympathetic nerves innervating the cardiovascular system have been identified (Molderings et al., 1997). However, these receptors are distinct from the rat I<sub>2</sub>-receptor to which SCH 79687 binds. We have previously demonstrated that activation of  $\alpha_2$ -adrenergic receptors with drugs such as BHT-920 produced nasal decongestion in the cat (McLeod et al., 2001b). However, the observation that SCH 79687 did not alter the inhibitory actions of clonidine on EFS-induced HSV contractions or affect baseline HSV tone suggest that  $\alpha_2$ -adrenoceptors are not activated in vitro at the concentration presently studied. Moreover, in the feline congestion model, SCH 79687 administered alone did not significantly increase nasal patency or produce changes in blood pressure as would be expected with a functionally active  $\alpha$ -adrenergic receptor ligand. Consequently, we have demonstrated that the combination of the selective histamine H<sub>3</sub> antagonist SCH 79687 and loratadine produces decongestant activity in the cat by a mechanism that eliminates the untoward side effects associated with sympathomimetic decongestants.

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