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## A Role for Histamine and Substance P in Immediate Allergic Responses in Guinea Pig Airways: Characterization of MDL 108,207DA, a Dual H<sub>1</sub>/NK-1 Receptor Antagonist

### Key Words

Antihistamine  
Tachykinin antagonist  
Guinea pig  
Allergic rhinitis

### Abstract

**Background:** Histamine is a critical mediator of immediate hypersensitivity reactions. Sensory neuropeptides, such as substance P (SP), may also contribute to acute inflammatory responses. A compound which antagonizes both H<sub>1</sub> and NK-1 receptors, such as MDL 108,207DA, may present a significant therapeutic advantage over pure antihistamines. **Methods:** The binding affinity of MDL 108,207DA for H<sub>1</sub> and NK-1 receptors was evaluated and its potency of antagonism evaluated in vitro. The in vivo antagonism of SP- or histamine-induced microvascular leakage in guinea pig airways was examined. A role for these mediators in antigen-induced microvascular leakage in ovalbumin-sensitized guinea pig airways was examined using MDL 108,207DA as well as the NK-1-selective antagonist FK888 and the H<sub>1</sub>-selective antagonist pyrilamine alone or in combination. **Results:** The affinity of MDL 108,207DA for H<sub>1</sub> and NK-1 receptors is similar to that of receptor-selective antagonists. The compound inhibits both receptors in vitro and in vivo with comparable potencies for each. The efficacy of FK888 in combination with pyrilamine and MDL 108,207DA on antigen-induced microvascular leakage in sensitized guinea pig airways supports a role for both SP and histamine in early allergic responses. **Conclusion:** The contribution of both SP and histamine to immediate hypersensitivity reactions supports the utility of NK-1 and H<sub>1</sub> receptor antagonist therapy. MDL 108,207DA incorporates both activities into the same compound and, as a result, may be useful in the treatment of allergic diseases.

### Introduction

Immediate hypersensitivity reactions occur when allergen molecules cross-link to Fab components of adjacent IgE molecules on the surface of mast cells or basophils thereby precipitating the release of preformed mediators, such as histamine [1], from secretory granules. The efficacy of H<sub>1</sub>

receptor antagonists in attenuating symptoms of human allergic diseases attests to the importance of histamine in IgE-mediated reactions. In the upper airways, histamine can increase vascular permeability, elicit sneezing and pruritus and stimulate reflex-mediated glandular secretions whereas in the lower airways bronchoconstriction also occurs [2–4].

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In the case of allergic rhinitis, other mediators also apparently contribute to the vascular permeability, glandular secretion and congestion occurring upon antigen exposure [4, 5]. For example, the neuropeptide substance P (SP) produces a number of effects in animal models which are consistent with the symptomatology observed in rhinitic patients including increased nasal blood flow, microvascular leakage, mucus secretion and sneezing [6–9]. Likewise, in allergic rhinitis patients, SP elicits nasal obstruction, increased nasal blood flow, microvascular leakage and inflammatory cell recruitment [10–12]. In fact, SP produced a greater increase in nasal resistance in allergic rhinitis patients than in control subjects [13].

Because of potential complementary effects which histamine and SP might have in the acute allergic response, we developed a compound which could effectively antagonize both histamine H<sub>1</sub> and tachykinin NK-1 receptors. In this paper, we describe the pharmacology of the novel compound MDL 108,207DA (DA = dihydrochloride salt) which is capable of binding to and antagonizing the physiological functions of both receptors in vitro and in vivo. Furthermore, we demonstrate that both histamine and SP contribute to antigen-induced plasma protein extravasation in sensitized guinea pig airways and that the dual antagonist MDL 108,207DA is effective in this model.

## Methods

### *Radioligand Binding Studies: Tachykinin Receptors*

Receptor binding assays were performed in crude tissue membranes as has been described previously [14]. The NK-1 receptor binding affinity of potential tachykinin receptor antagonists was evaluated in guinea pig lungs (Harlan Bioproducts, Indianapolis, Ind., USA). Affinity for the NK-2 receptor was evaluated in HSKR-1 cells which are mouse 3T3 fibroblasts expressing the human jejunal NK-2 receptor [15]. The NK-3 receptor source was guinea pig cerebral cortex. Briefly, tissues or cells were homogenized in 50 mM Tris-HCl buffer (pH 7.4, 4°C) containing 120 mM NaCl and 5 mM KCl and centrifuged at 48,000 g for 12 min at 4°C. The pellet was resuspended in the Tris-HCl buffer containing 10 mM dipotassium EDTA and 300 mM KCl and then set on ice for 30 min. The suspension was centrifuged as above and the pellet washed by resuspension and spun twice more. Receptor binding was initiated by addition of 250 µl membrane preparation in duplicate to 0.1 nM of the following radioligands: for NK-1 receptor assays, [<sup>125</sup>I]Bolton-Hunter-labeled Lys<sup>3</sup> SP (New England Nuclear, specific activity, 2,200 Ci/mmol); for NK-2 receptor assays, 2-[<sup>125</sup>I]iodohistidyl-1-NKA (Amersham, specific activity, 2,000 Ci/mmol), and for NK-3 receptor assays [<sup>125</sup>I]Bolton-Hunter-labeled Lys<sup>4</sup> eledoisin (New England Nuclear, specific activity, 2,200 Ci/mmol) in a final volume of 500 µl of incubation buffer containing 50 mM Tris-HCl (pH 7.4 at room temperature), 0.1% bovine serum albumin, 2 mM MnCl<sub>2</sub>, 40 µg/ml bacitracin, 4 µg/ml each of leupeptin and chymostatin, 10 µM thiorphan and various doses of the putative

tachykinin receptor antagonists. Incubations were performed at room temperature for 90 min (NK-1 receptor assays) or 2 h (NK-2 and NK-3 receptor assays). Binding was terminated by addition of Tris-HCl buffer and filtration under vacuum through GF/B filters presoaked with 0.1% polyethylenimine (NK-1 receptor assay) or 0.5% bovine serum albumin (NK-2 and NK-3 receptor assays). Nonspecific binding was defined as binding in the presence of 1 µM SP (Peptides International Inc., Louisville, Ky., USA; NK-1 receptor assay), NKA (Peptides International Inc.; NK-2 receptor assay) or eledoisin (Peninsula Laboratories Inc., Belmont, Calif., USA; NK-3 receptor assay).

### *Radioligand Binding Studies: Histamine Receptors*

The histamine receptor source was a Chinese hamster ovary (CHO) cell line which had been transfected with human H<sub>1</sub> receptor gene by the method of De Backer et al. [16]. Cells were homogenized with a Polytron in 50 mM potassium phosphate buffer (pH 7.4, 4°C), and the homogenate was centrifuged at 48,000 g for 12 min at 4°C. The pellet was resuspended in phosphate buffer at a final protein concentration of 40 mg/ml. Receptor binding was initiated by addition of 250 µl of cell suspension in duplicate to 2 nM [<sup>3</sup>H]pyrilamine (Amersham, specific activity, 28.0 Ci/mmol) to test compound or vehicle in a final volume of 500 µl incubation buffer which consisted of 50 mM phosphate buffer (pH 7.4 at room temperature) containing 0.1% bovine serum albumin. Incubation was carried out at room temperature for 30 min. Binding was terminated by addition of 3.5 ml 0.9% NaCl (4°C) and filtration under vacuum through GF/B filters presoaked with 0.1% polyethylenimine. Nonspecific binding was defined as binding in the presence of 10 µM promethazine. During preliminary characterization of the H<sub>1</sub>/CHO cell line, it was determined that compounds with histamine H<sub>2</sub> (cimetidine: IC<sub>50</sub> = 10,000 nM) or H<sub>3</sub> (thioperamide: IC<sub>50</sub> = 5,275–816 nM) antagonist properties had very low affinity for receptors from this source. Histamine H<sub>2</sub> and H<sub>3</sub> receptor binding studies were performed by NovaScreen (Hanover, Md., USA) using [<sup>3</sup>H]tiotidine binding to guinea pig striatal membranes (H<sub>2</sub> assay) or [<sup>3</sup>H]N<sup>2</sup>-methylhistamine binding to rat forebrain membranes (H<sub>3</sub> assay).

Specific binding was calculated by subtracting nonspecific binding from total binding and corresponded to 70–95% of total binding. Data were analyzed and IC<sub>50</sub> values generated by nonlinear regression using an iterative curve fitting program (Prism, San Diego, Calif., USA). The IC<sub>50</sub> was assumed to be equal to the K<sub>i</sub> since the radioligand concentrations used were approximately tenfold less than K<sub>d</sub> values. The protein content was determined by the method of Lowry et al. [17].

### *SP-Induced [<sup>3</sup>H]Inositol Phosphate Accumulation in vitro*

The ability of putative tachykinin receptor antagonists to inhibit SP-induced increases in phosphatidylinositol turnover in a dose-dependent manner was evaluated in the human astrocytoma NK-1 receptor cell line UC11 [18] as has been described previously [14]. Briefly, cultured cells were loaded with 0.5 ml of 0.2 µM myo[2-<sup>3</sup>H(N)] inositol (American Radiolabeled Chemicals Inc., specific activity, 20 Ci/mmol) 20–24 h prior to the assay and maintained at 37°C in a 5% CO<sub>2</sub> environment. On the day of the assay, cells were incubated in RPMI-1640 medium containing 40 µg/ml bacitracin, 4 µg/ml each of leupeptin and chymostatin, 0.1% bovine serum albumin, 10 µM thiorphan and 10 mM LiCl and tachykinin antagonist or vehicle. SP or incubation buffer for control was added 15 min later at various concentrations (from 0.1 nM to 10 µM in a volume of 0.1 ml) and incubated for 60 min in a final volume of 1 ml. To terminate the reaction, the medium

was aspirated and methanol added to all wells. Chloroform (1 ml) was added to each tube containing the methanol/cell suspension followed by 0.5 ml distilled H<sub>2</sub>O. The tubes were then vortexed and centrifuged for 10 min at 1,700 g. An aliquot of 0.9 ml of the aqueous (top) phase was removed and placed in chloroform-resistant tubes containing 2 ml ddH<sub>2</sub>O. Samples were loaded onto a 1 ml 50% Bio-Rad AG 1-X8 (formate form, 100- to 200-mesh) exchange column (Bio-Rad Laboratories, Hercules, Calif., USA). The columns were then washed, in order, with: (1) 10 ml ddH<sub>2</sub>O, (2) 5 ml of 5 mM disodium tetraborate/60 mM sodium formate, and (3) 2 ml of 1 M ammonium formate/0.1 M formic acid. The third elution was collected and counted in 9 ml scintillation fluid. A 50- $\mu$ l aliquot of the organic (bottom) phase was removed, dried in a scintillation vial and counted in 7 ml scintillation fluid.

The ratio of disintegrations per minute in the aqueous-phase aliquot (total inositol phosphates) to the disintegrations per minute in the 50- $\mu$ l organic-phase aliquot (total [<sup>3</sup>H]inositol incorporated) was calculated for each sample. Data are expressed as a percent of agonist-induced accumulation of [<sup>3</sup>H]inositol phosphates over basal levels. Receptor antagonism was deduced from the rightward shift of the concentration-response curves to the tachykinins. Schild regression analysis using three or more antagonist concentrations was used to derive pA<sub>2</sub> (negative logarithm of the molar concentration of antagonist that produces a twofold parallel shift to the right of the agonist concentration curve) and slope values [19].

#### *Histamine-Induced Ileum Contraction*

Compounds were evaluated for histamine H<sub>1</sub> receptor antagonism by measuring their ability to inhibit contraction of guinea pig ileum produced in response to the mediator. Guinea pigs fasted overnight were sacrificed using a pentobarbital overdose; the abdomen was opened and a section of ileum removed. After flushing with Tyrode's buffer, sections of ileum of approximately 2.5 cm in length were cut and transferred to an organ bath containing Tyrode's buffer (37°C) and 95% O<sub>2</sub>/5% CO<sub>2</sub>. Measurements of ileum contractions were made using an isometric transducer. Tissues were hung under 1 g of tension and equilibrated for approximately 1–2 h prior to use with intermittent bath changes. A maximally effective dose of histamine (10  $\mu$ M) was administered and tissues were repeatedly washed. When baseline tension had resumed, MDL 108,207DA ( $3 \times 10^{-9}$  to  $3 \times 10^{-8}$  M) or vehicle were added. Following a 15-min incubation period, a cumulative dose-response curve to histamine ( $1 \times 10^{-8}$  to  $3 \times 10^{-4}$  M) was generated. Contraction was expressed as a percent of each tissue's maximum response to histamine. Data were analyzed using the method of Arunlakshana and Schild [19].

#### *Histamine- or SP-Induced Plasma Protein Extravasation in Guinea Pig Airways*

Plasma protein leakage through postcapillary venules was assessed by measuring Evans blue dye accumulation in guinea pig airways as has been described previously [14]. Animals were anesthetized with pentobarbital (40 mg/kg, i.p.) then injected with Evans blue dye (20 mg/kg, i.v., prepared in 0.9% NaCl). Two minutes later, the test compound or vehicle was injected intravenously followed in 2 min by SP ( $1 \times 10^{-10}$  mol/kg, i.v.) or histamine ( $3 \times 10^{-7}$  mol/kg, i.v.). In preliminary experiments, these mediator concentrations were found to produce significant but submaximal amounts of Evans blue dye accumulation in guinea pig airways (fig. 4). After 5 min, excess dye was removed from the circulation by transcardiac perfusion with 50 ml 0.9% NaCl solution. The trachea and primary bronchi were removed, blotted dry and weighed. Dye quantitation was performed spectrophotometri-

cally (620 nm) after extracting tissues in formamide overnight at 50°C. Background dye accumulation was determined in the airways of animals receiving Evans blue dye only. In those experiments in which oral efficacy was examined, animals fasted overnight received the test compound by oral gavage 1 h prior to mediator administration.

The ability of at least 3 doses of the test compound to inhibit SP- or histamine-mediated plasma protein extravasation was evaluated, expressed as percent inhibition relative to that obtained from vehicle-treated animals and the ED<sub>50</sub> (mg/kg) value calculated from linear regression analysis.

#### *Histamine-Induced Skin Wheal in Guinea Pigs*

Guinea pigs were anesthetized with pentobarbital (40 mg/kg, i.p.) and their dorsal surface shaved. Animals were pretreated with test compound or vehicle, and 5 min (for intravenous administration) or 1 h (for oral administration) later histamine was injected intradermally at two dorsal sites at doses of  $3 \times 10^{-6}$  and  $3 \times 10^{-7}$   $\mu$ g/site in a volume of 100  $\mu$ l. Immediately afterwards Evans blue dye (20 mg/kg, i.v.) was injected. Twenty minutes later, the animals were sacrificed by CO<sub>2</sub> asphyxiation. The dorsal skin was removed and the diameter of the blue skin wheals measured. The diameters for the two spots were added and the percent inhibition produced in response to the test compound figured based on the sum of spots for treated versus control animals. ED<sub>50</sub> values were calculated as described above.

#### *Antigen-Induced Plasma Protein Extravasation in Guinea Pig Airways*

Guinea pigs were sensitized to ovalbumin by exposure to ovalbumin aerosol (1% in phosphate-buffered saline) for 15 min on 2 occasions separated by 1 week. Seven days following the second exposure, animals were anesthetized with pentobarbital, then injected intravenously with vehicle or antagonist. In those experiments in which compounds were administered orally, a 1-hour pretreatment time was used. Two minutes later, Evans blue dye (30 mg/kg, i.v.) was administered followed in 2 min by ovalbumin administration (0.025 mg/kg, i.v.). Animals were perfused 10 min later with 0.9% NaCl, and tissues were removed and treated as described above. Background dye accumulation was determined in the airways of sensitized animals receiving Evans blue dye only.

#### *Materials*

All reagents were obtained from Sigma Chemical Co. (St. Louis, Mo., USA), unless otherwise noted. The vehicle for compounds in all in vivo studies was 40% hydroxypropyl- $\beta$ -cyclodextrin (Aldrich) in water. FK888 was a kind gift from Fujisawa Pharmaceutical Co. (Osaka, Japan).

Cetirizine was synthesized at Dow Chemical Company (Midland, Mich., USA). Terfenadine, emedastine, MDL (106,848KF, i.e. (*R,S*)-(+/-)-1-(3,4,5-trimethoxybenzoyl)-3-{2-[4-(1-(2-ethoxyethyl)-1H-benzimidazol-2-yl)]1,4-diazepan-1-yl}ethyl]-3-phenylpyrrolidine tris-methanesulfonate), MDL 108,207DA (fig. 1) or MDL 108,207DV (*R*-enantiomer, DV = difumarate salt) and MDL 106,687DA (*S*-enantiomer, DA = dihydrochloride salt) were synthesized at Hoechst Marion Roussel, Cincinnati, Ohio, USA.

#### *Data*

Unless otherwise specified, results are expressed as mean  $\pm$  SEM. Student's *t* test was used for comparison between two groups such as the effects of pyrilamine on SP-induced plasma protein extravasation. One-way analysis of variance (ANOVA) was used to compare the ef-

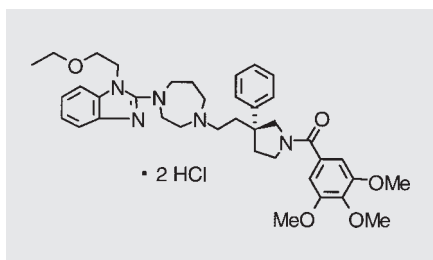


**Fig. 1.** Chemical structure of MDL 108,207DA, (*R*)-(+)-1-(3,4,5-trimethoxybenzoyl)-3-[2-[4-(1-(2-ethoxyethyl)-1H-benzimidazol-2-yl)[1,4]diazepan-1-yl]ethyl]-3-phenylpyrrolidine dihydrochloride.

ffects of more than two groups, such as a comparison between the efficacy of FK888 and pyrilamine, alone and in combination, and multiple comparison procedures made using the Student-Newman-Keuls method. In the absence of equal variances, one-way ANOVA was performed on ranks with Dunn's method used for multiple comparison procedures (SigmaStat). Significance was assigned at  $p < 0.05$ .

## Results

### Receptor Binding Affinities

MDL 108,207DA and MDL 108,207DV bound with good affinity to both  $H_1$  and NK-1 receptors (table 1). Its affinity for NK-1 receptors was slightly improved over the racemic compound MDL 106,848KF whereas its  $H_1$  receptor binding affinity was slightly less. The NK-1 receptor binding affinity of the compound appears to be stereoselective as MDL 106,687DA exhibited a significantly lower affinity for this receptor than did MDL 108,207DA. The  $H_1$  receptor binding affinity of MDL 108,207DA was similar to that of terfenadine although emedastine and pyrilamine had approximately a 25 $\times$  higher affinity. The  $H_1$  receptor binding affinities obtained in the human  $H_1$ /CHO cell line were consistent with those obtained by Laduron et al. [20] for astemizole (40 nM) and pyrilamine (7 nM) which were generated using guinea pig cerebellum as the receptor source. None of the reference antihistamines examined had significant NK-1 receptor binding affinity. FK888 [14, 21] bound with affinity to guinea pig NK-1 receptors which was 2–5 $\times$  higher than that of MDL 108,207DA but did not exhibit significant affinity for  $H_1$  receptors.

MDL 108,207DA binds selectively to the  $H_1$  receptor since the compound did not demonstrate appreciable affinity ( $IC_{50} > 1 \mu M$ ) for histamine  $H_2$  or  $H_3$  receptors (data not shown). Similarly, MDL 108,207DA binding to tachykinin receptors appears to be selective for NK-1 receptors since the compound had no significant affinity ( $IC_{50} > 1 \mu M$ ) for

**Table 1.** NK-1 and  $H_1$  receptor binding affinity ( $IC_{50}$  nM)

| Compound                                | $H_1$           | NK-1            |
|-----------------------------------------|-----------------|-----------------|
| Histamine                               | $> 10,000$      | –               |
| SP                                      | –               | $0.24 \pm 0.02$ |
| FK888                                   | $> 10,000$      | $3.13 \pm 0.34$ |
| Pyrilamine                              | $8.70 \pm 1.70$ | $> 10,000$      |
| Emedastine                              | $9.56 \pm 1.98$ | $> 10,000$      |
| Terfenadine                             | $563 \pm 109$   | $> 10,000$      |
| Astemizole                              | $46.6 \pm 8.32$ | $1,386 \pm 194$ |
| MDL 106,848KF ( <i>R,S</i> -enantiomer) | $121 \pm 28$    | $32.6 \pm 7.96$ |
| MDL 108,207DV ( <i>R</i> -enantiomer)   | $242 \pm 67.5$  | $12.5 \pm 2.12$ |
| MDL 108,207DA ( <i>R</i> -enantiomer)   | $233 \pm 13$    | $17.0 \pm 3.48$ |
| MDL 106,687DA ( <i>S</i> -enantiomer)   | $163 \pm 38$    | $241 \pm 62$    |

Data are means  $\pm$  SEM of the molar concentration of compound required to produce 50% inhibition of binding of [ $^{125}$ I]Bolton-Hunter-labeled SP to NK-1 receptors from guinea pig lung and [ $^3$ H]pyrilamine to  $H_1$  receptors from the transfected human  $H_1$  receptor CHO cell line. Values were obtained from at least 2 independent experiments performed in duplicate.

NK-2 or NK-3 receptors. MDL 108,207DA and MDL 108,207DV also had no significant affinity ( $IC_{50} > 1 \mu M$ ) for calcium channels (nitrendipine or verapamil binding sites), adrenergic ( $\alpha_1$  or  $\alpha_2$  and  $\beta$ ) receptors, muscarinic ( $m_2$  and  $m_3$ ) receptors or adenosine  $A_1$  receptors (data not shown).

### *In vitro* Potency: NK-1 and $H_1$ Receptor Antagonism

MDL 108,207DA dose-dependently inhibited histamine-induced contraction of guinea pig ileum in vitro  $pA_2 = 8.02 \pm 0.025$ ) in a manner consistent with competitive antagonism (fig. 2). The potency of the compound was similar to that of astemizole ( $pA_2 = 8.20 \pm 0.06$ ) and reported values for terfenadine (8.23) although less than pyrilamine (9.03) [22]. MDL 108,207DV also dose-dependently inhibited SP-induced accumulation of [ $^3$ H]inositol phosphates in UC11 cells ( $pA_2 = 8.01 \pm 0.13$ ) in a manner consistent with competitive antagonism (fig. 3). The potency of the compound was significantly less than that of FK888 ( $pA_2 = 8.83$ ) [14]. MDL 108,207 produced no agonist activity in either assay.

### *In vivo* Activity: NK-1 and $H_1$ Receptor antagonism

SP produced microvascular leakage in guinea pig airways, as measured by Evans blue dye accumulation, in a concentration-dependent manner (fig. 4). MDL 108,207DA inhibited the effects of SP ( $1 \times 10^{-10}$  mol/kg, i.v.) following intravenous and oral administration in a dose-dependent manner (table 2). FK888 has previously been shown to produce significant NK-1 receptor antagonism in this assay [14] (table 2).

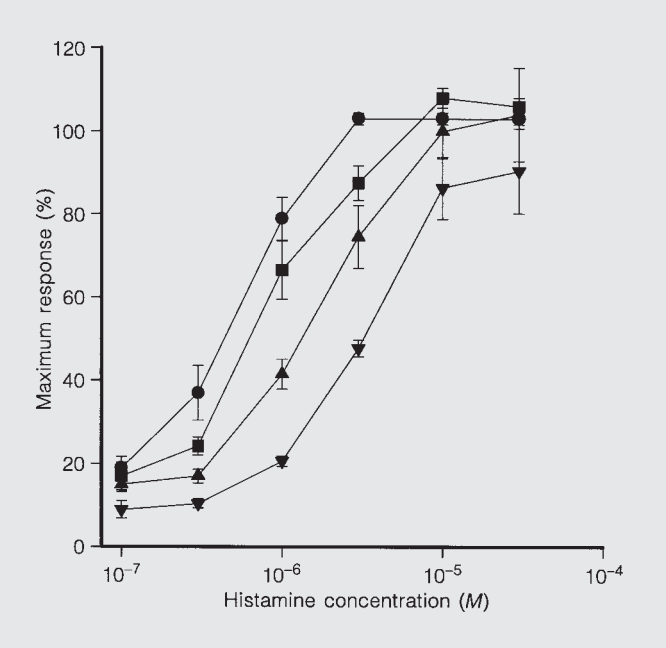


**Fig. 2.** Effects of MDL 108,207DA on histamine-induced ileum contractions in vitro. Data are means  $\pm$  SEM of values expressed as percent of maximum contraction from at least 5 tissues pretreated with vehicle ( $\bullet$ ), MDL 108,207DA  $3 \times 10^{-9}$  M ( $\blacksquare$ ),  $10^{-8}$  M ( $\blacktriangle$ ) or  $3 \times 10^{-8}$  M ( $\blacktriangledown$ ) prior to generation of a cumulative dose response to histamine.

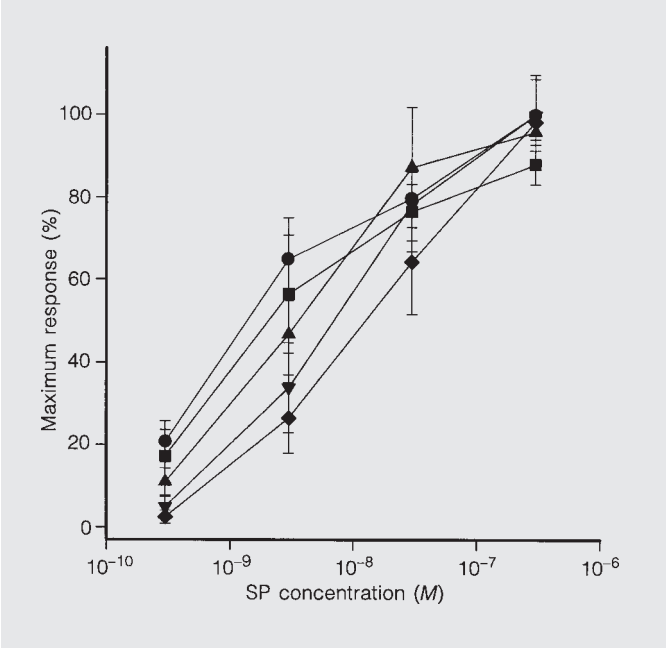


**Fig. 3.** Effects of MDL 108,207DV on SP-induced incorporation of [ $^3$ H]inositol into UC11 cells. Data are means  $\pm$  SEM of values expressed as percent of maximum response to SP from 3 separate experiments performed in duplicate. Cells were pretreated with vehicle ( $\bullet$ ), MDL 108,207DV  $10^{-8}$  M ( $\blacksquare$ ),  $3 \times 10^{-8}$  M ( $\blacktriangle$ ),  $10^{-7}$  M ( $\blacktriangledown$ ) or  $3 \times 10^{-7}$  M ( $\blacklozenge$ ), then exposed to SP at various concentrations.

**Table 2.** Efficacy of  $H_1$  and NK-1 receptor antagonists in mediator-induced microvascular leakage in guinea pigs

| Compound     | Route of administration | SP-PPE airways ED <sub>50</sub> mg/kg | HIS-PPE airways ED <sub>50</sub> mg/kg | HIS-PPE dermis ED <sub>50</sub> mg/kg |
|--------------|-------------------------|---------------------------------------|----------------------------------------|---------------------------------------|
| MDL 108207DA | i.v.                    | 0.33 (0.11–0.65)                      | –                                      | 0.75 (0.52–1.35)                      |
|              | p.o.                    | 1.89 (0.18–3.99)                      | 0.8 (0.22–1.49)                        | 1.95 (1.7–2.25)                       |
| Pyrilamine   | i.v.                    | >>>0.5                                | 0.018 (0.001–0.025)                    | –                                     |
| Terfenadine  | p.o.                    | –                                     | –                                      | 1.67 (1.50–1.87)                      |
| Astemizole   | p.o.                    | –                                     | –                                      | 0.56 (0.44–0.73)                      |
| FK888        | i.v.                    | 0.08 <sup>1</sup>                     | >>>0.5                                 | –                                     |

Data are means (with 95% confidence limits in parentheses) of values from at least 3 guinea pigs per dose. At least 3 doses were used in linear regression analysis for the determination of the ED<sub>50</sub>. Animals receiving compounds intravenously were pretreated for 5 min whereas those treated by oral gavage received the compound 1 h prior to analysis of plasma protein extravasation (PPE) as measured by Evans blue dye accumulation in the trachea and primary bronchi of guinea pigs.

<sup>1</sup> Data obtained from Kudlacz et al. [14].

Histamine also produces microvascular leakage in guinea pig airways (fig. 4) although this mediator was markedly less potent than SP as nearly 1,000 $\times$  more histamine was required to produce a degree of Evans blue dye accumulation equivalent to that of the sensory neuropeptide. Pyri-

lamine potentially inhibited the vascular effects produced by histamine ( $3 \times 10^{-7}$  mol/kg, i.v.) as did MDL 108,207DA (table 2). In the dermis, histamine-induced microvascular leakage can be identified by the formation of skin wheals. MDL 108,207DA also inhibited this effect following intra-

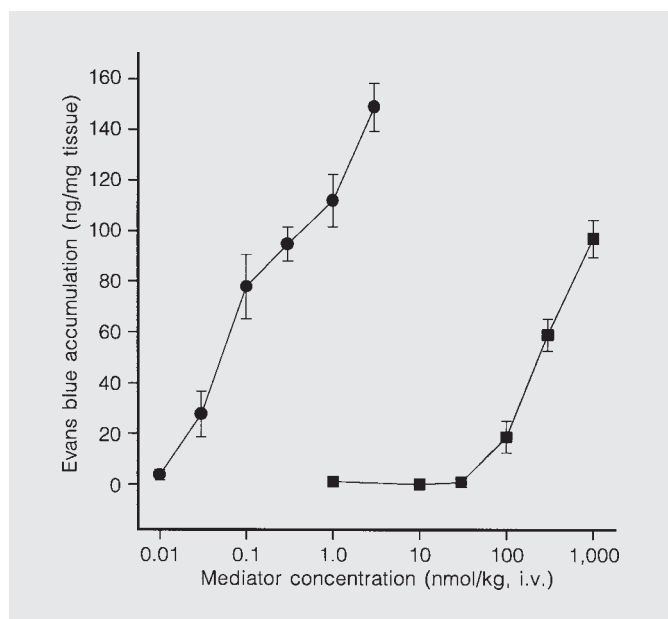


**Fig. 4.** Histamine- and SP-induced plasma protein extravasation in guinea pig airways. Data are means  $\pm$  SEM of values from 3–4 animals per concentration per mediator. Evans blue dye accumulation in the trachea and primary bronchi was measured following intravenous administration of various concentrations of histamine (■) or SP (●). Values are expressed above background levels.

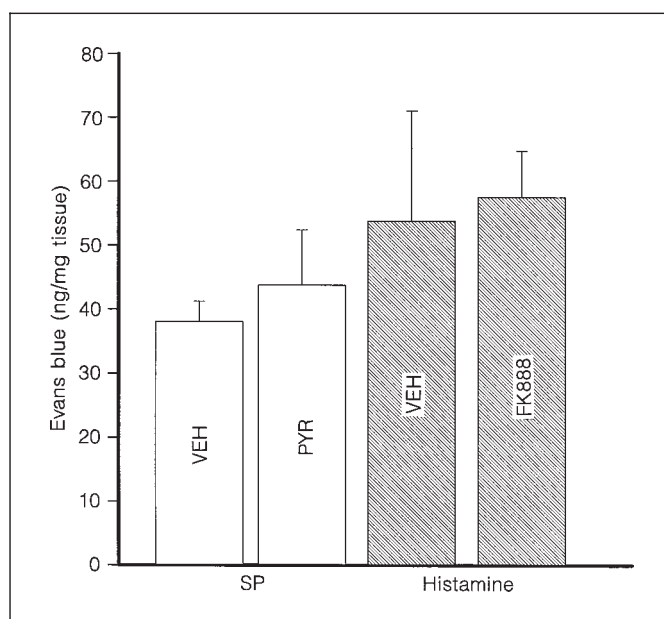


**Fig. 5.** Relationship between histamine- and SP-mediated plasma protein extravasation in guinea pig airways. Data are means  $\pm$  SEM of values from at least 3 animals per treatment group. To examine the role of histamine in the SP-mediated response, animals were pretreated with pyrilamine (PYR; 0.5 mg/kg, i.v.) prior to administration of the sensory neuropeptide ( $1 \times 10^{-10}$  mol/kg, i.v.). To examine the role of SP in histamine-mediated plasma protein extravasation, pyrilamine (0.5 mg/kg, i.v.) was administered to animals prior to administration of histamine ( $3 \times 10^{-7}$  mol/kg, i.v.). No significant differences between the groups were observed. VEH = Vehicle.

venous and oral administration at doses which were comparable to reference antihistamines, i.e. terfenadine and astemizole.

Pyrilamine administered at a dose which maximally inhibits histamine-induced microvascular leakage in guinea pig airways (0.5 mg/kg, i.v.), did not affect the plasma protein extravasation produced in response to SP (fig. 5). FK888 administered at a dose which maximally inhibits SP-induced microvascular leakage in guinea pig airways (0.5 mg/kg, i.v.) did not affect the plasma protein extravasation produced in response to histamine (fig. 5).

#### *Antigen-Induced Microvascular Leakage: NK-1 and $H_1$ Receptor Antagonism*

Guinea pigs sensitized by aerosol to ovalbumin exhibited increased airway microvascular leakage in the trachea and primary bronchi upon subsequent intravenous antigen exposure ( $186 \pm 9.31$  ng Evans blue/mg tissue above background dye accumulation;  $n = 24$ ). Pretreatment with the NK-1 receptor antagonist FK888 (0.5 mg/kg, i.v.) markedly

inhibited antigen-induced microvascular leakage as did the  $H_1$  receptor antagonist pyrilamine (0.5 mg/kg, i.v.; table 3). When a combination of the two agents was used, the inhibitory response obtained was greater ( $\sim 90\%$ ) than that obtained upon administration of either receptor antagonist alone. The same additive effect was observed when submaximal doses of both compounds were administered (table 3). These data suggest that the microvascular leakage produced by SP and histamine occurs via separate receptors and is consistent with the absence of effect of pyrilamine on SP-induced plasma protein extravasation and the lack of inhibition of FK888 on the same effect produced by histamine (fig. 5).

MDL 108,207DA (0.1–1 mg/kg, i.v.) produced a dose-dependent inhibition of antigen-induced microvascular leakage in sensitized guinea pig airways ( $ED_{50} = 0.14$  mg/kg, 0.07–0.19; fig. 6). The compound also significantly inhibited the effect following oral administration; doses as low as 0.5 mg/kg, p.o., produced approximately 70% inhibition (fig. 6).

**Table 3.** Effects of H<sub>1</sub> and NK-1 receptor antagonism on ovalbumin-induced microvascular leakage in sensitized guinea pig airways

|                              | Inhibition, % |
|------------------------------|---------------|
| <b>Protocol 1</b>            |               |
| Pyrilamine 0.5 mg/kg, i.v.   | 50.4±9.06     |
| FK888 0.5 mg/kg, i.v.        | 55.8±8.64     |
| Pyrilamine 0.5 + FK888 0.5   | 88.3±8.20*    |
| <b>Protocol 2</b>            |               |
| Pyrilamine 0.25 mg/kg, i.v.  | 50.7±9.5      |
| FK888 0.25 mg/kg, i.v.       | 12.3±7.0      |
| Pyrilamine 0.25 + FK888 0.25 | 78.7±10.2*    |
| <b>Protocol 3</b>            |               |
| Pyrilamine 0.01 mg/kg, i.v.  | 16.0±4.08     |
| FK888 0.25 mg/kg, i.v.       | 33.4±12.8     |
| Pyrilamine 0.01 + FK888 0.25 | 42.0±13.8     |

Data are means ± SEM of values from 3–5 animals per treatment group expressed as a percent inhibition of plasma protein extravasation produced in ovalbumin-sensitized guinea pigs in response to antigen (0.025 mg/kg, i.v.) pretreated with vehicle. Groups within a given protocol were compared using one-way ANOVA. In protocols 1 and 2, the combined effects of pyrilamine and FK888 were significantly different from both antagonists administered alone (protocol 1) or different only from FK888 (protocol 2). Significance was assigned to \* $p < 0.05$ .

## Discussion

MDL 108,207DA is a novel compound which combines histamine H<sub>1</sub> with neurokinin NK-1 receptor antagonism in the same molecule. It is unique in its ability to inhibit both SP- as well as histamine-mediated effects in vitro and in vivo with a potency similar to both effects. Other first-generation antihistamines, such as pyrilamine, and second-generation antihistamines, such as terfenadine and astemizole, do not demonstrate significant affinity for NK-1 receptors nor do NK-1-receptor-selective antagonists, such as FK888, exhibit affinity for H<sub>1</sub> receptors. Hence, this property of dual antagonism is unique to MDL 108,207DA which was specifically designed to incorporate both properties. Interestingly, like terfenadine [23], binding affinity for the H<sub>1</sub> receptor was not markedly different between the *R*-enantiomer MDL 108,207DA and the *S*-enantiomer MDL 106,687DA. In contrast, the NK-1 receptor binding affinities between the two were approximately tenfold different as is consistent with the stereoselectivity exhibited by other non-peptide NK-1 receptor antagonists [24].

Although histamine is clearly a major mediator of acute hypersensitivity reactions, it is not the sole mediator. For

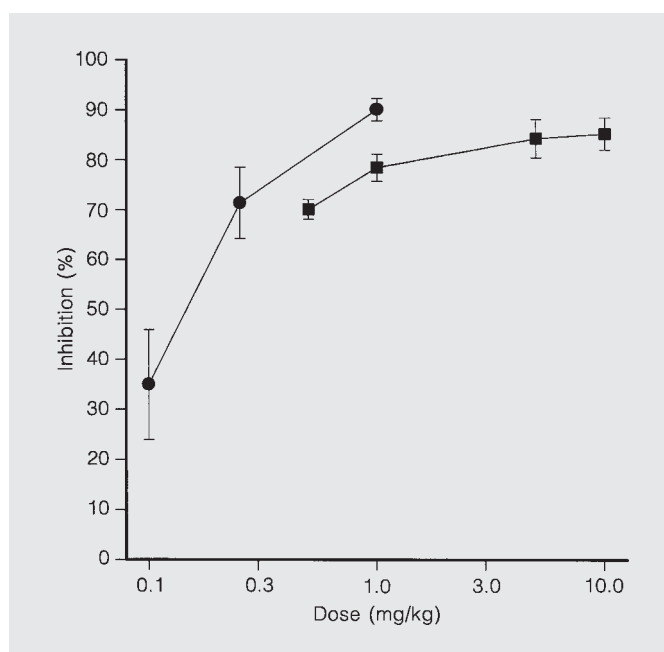


**Fig. 6.** Effects of MDL 108,207DA on ovalbumin-induced plasma protein extravasation in sensitized guinea pig airways. Data are means ± SEM of values from 3–8 sensitized animals per dose per route of administration and are expressed as a percent of response of those receiving vehicle treatment. Animals received MDL 108,207DA by either the intravenous (●) or oral (■) route of administration.

example, in one allergic guinea pig model, histamine and leukotrienes (but not platelet-activating factor) were found to mediate antigen-induced increases in vascular permeability [25]. In contrast, Misawa and Iwamura [26] demonstrated the efficacy of platelet-activating factor antagonism in another guinea pig model of allergic rhinitis. Observations such as these have led to the coupling of antihistaminic activity to inhibition of other inflammatory mediators. For example, 5-lipoxygenase inhibition [27], thromboxane A<sub>2</sub> and leukotriene D<sub>4</sub> antagonism [28, 29] have been reported in addition to antihistaminic activity for various compounds. In addition, combined platelet-activating factor/antihistamine antagonists such as SCH 37370 [30] and rupatadine [31] have also been under development for the treatment of allergic and inflammatory conditions.

Inflammatory mediators may produce their direct effects via receptors localized on target tissues or indirectly via activation of sensory nerves. In the nasal mucosa, SP-immunoreactive fibers are found within and under the epithelium, around blood vessels and exocrine glands whereas in the trachea and bronchi these nerve fibers are also located in smooth muscle and around ganglion cells [32]. A role for sensory nerves in allergic airway disease is suggested by

clinical and experimental evidence. Capsaicin-desensitized allergic guinea pigs exhibited reduced sneezing reflexes in response to various stimuli including histamine and antigen challenge [33] as well as diminished allergen-induced alterations in nasal resistance (which results from increased vascular leakage and dilatation of capacitance vessels) and microvascular leakage [34]. When applied topically to the nasal mucosa, capsaicin produces rhinorrhea and nasal obstruction which is particularly pronounced in patients with chronic nonallergic rhinitis [35]. Repeated topical administration of capsaicin has resulted in symptomatic relief in chronic rhinitic patients [35, 36].

Stimulation of nasal and airway sensory nerves results in the local release of neuropeptides including SP. This tachykinin has been shown to elicit many of the symptoms, i.e. increased nasal blood flow, microvascular leakage, mucus secretion and sneezing, of allergic airway disease, such as rhinitis [6–9]. In addition to the effects of the neuropeptide which have been shown to be consistent with the rhinitic phenotype, SP has also been reported to enhance antigen-evoked mediator release from human nasal mucosa [10]. In further support of the hypothesized role of the mediator in the disease, SP was found in nasal lavages following antigen challenge in allergic individuals [37–39]. Data from allergic animal models also support a role for SP in antigen-induced vascular effects. Allergen-induced plasma protein extravasation in sensitized guinea pig airways was significantly reduced by pretreatment with the NK-1 receptor-selective antagonist CP-96,345 [40]. Similar to our own results, allergen-induced microvascular leakage was significantly reduced by pretreatment with FK888 in guinea pig nasal mucosa [41].

NKA and calcitonin gene-related peptide are also localized to sensory nerves in the upper and lower airways and can be released in conjunction with SP upon nerve stimulation [9]. The predominant lower airway effect of NKA appears to be bronchoconstriction mediated via NK-2 receptors [42], whereas its nasal effects, including fluid secretion and vasodilatation, appear to be mediated via NK-1 receptors [11, 43]. Calcitonin gene-related peptide does not produce glandular secretion or albumin exudation upon intranasal administration [44]; however, it does cause persistent increases in blood flow [9]. Hence, in our allergic guinea pig model, it is not expected that the plasma protein extravasation produced in sensitized guinea pig trachea and primary bronchi in response to ovalbumin challenge results from sensory neuropeptides other than SP.

SP and histamine may work in conjunction to promote vascular changes occurring in the airways in response to noxious stimuli. There is anatomical support for this con-

tention since sensory nerves have been found near mast cells in various tissues including the airways [45]. SP, or stable analogs thereof, have been shown to elicit histamine release from guinea pig lungs in vivo [46] and human pulmonary mast cells [47] and nasal mucosa in vitro [48]. In our own studies SP-induced plasma protein extravasation in guinea pig airways was apparently not mediated by histamine (which may have been released from mast cells in response to the neuropeptide) since a maximally effective dose of pyrilamine did not modify the effect. Conversely, histamine has been shown to stimulate tachykinin release from guinea pig lungs [49]. Again, the increased microvascular permeability induced by histamine in our studies appears not to be mediated via SP since a maximally effective dose of the NK-1 receptor antagonist FK888 had no effect on this response. Therefore, it is likely that the effects of MDL 108,207DA on antigen-induced plasma protein extravasation occur as a result of antagonism of  $H_1$  and NK-1 receptors at vascular sites.

In conclusion, our data support a role for SP and histamine in the production of antigen-induced microvascular leakage in sensitized guinea pig airways. The dual  $H_1$ /NK-1 receptor antagonist MDL 108,207DA also inhibits the immediate hypersensitivity response following intravenous or oral administration. Based upon its pharmacological profile, the compound may represent a novel therapy for the treatment of allergic airway disease, particularly rhinitis.

## References

- Kaliner M, Lemanske R: Rhinitis and asthma. *JAMA* 1992;268:2807–2829.
- Du Buske LM: Clinical comparison of histamine H<sub>1</sub>-receptor antagonist drugs. *J Allergy Clin Immunol* 1996;98:S307–S318.
- Simons FER, Simons KJ: The pharmacology and use of H<sub>1</sub>-receptor-antagonist drugs. *N Engl J Med* 1994;330:1663–1670.
- White MV, Kaliner MA: Mediators of allergic rhinitis. *J Allergy Clin Immunol* 1992;90:699–704.
- Bousquet J, Vignola AM, Campbell AM, Michel F-B: Pathophysiology of allergic rhinitis. *Int Arch Allergy Immunol* 1996;110:207–218.
- Gawin A, Baraniuk J, Kaliner M: Effects of substance P and calcitonin gene related peptide (CGRP) on guinea pig nasal mucosal secretion in vivo. *Acta Otolaryngol (Stockh)* 1993;113:533–539.
- Imamura T, Kambara T: Substance P as a potent stimulator of sneeze responses in experimental allergic rhinitis of guinea pigs. *Agents Actions* 1992;37:245–249.
- Runer T, Lindberg S, Mercke U, Olsson P: Effects of substance P and methacholine on mucosal blood flow in the upper airways. *Am J Rhinol* 1995;9:335–345.
- Stjarne P, Lundblad L, Anggard A, Hokfelt T, Lundberg JM: Tachykinins and calcitonin gene-related peptide: Co-existence in sensory nerves of the nasal mucosa and effects on blood flow. *Cell Tissue Res* 1989;256:439–446.
- Baumgarten CR, Witzel A, Kleine-Tebbe J, Kunkel G: Substance P enhances antigen-evoked mediator release from human nasal mucosa. *Peptides* 1996;17:25–30.
- Braunstein G, Fajac I, Lacroix J, Frossard N: Clinical and inflammatory responses to exogenous tachykinins in allergic rhinitis. *Am Rev Respir Dis* 1991;144:630–635.
- Konno A, Nagata H, Nomoto M, Motosugi H, Terada N: Role of capsaicin-sensitive trigeminal nerves in development of hyperreactive nasal symptoms in guinea pig model of nasal allergy. *Ann Otol Rhinol Laryngol* 1995;104:730–735.
- Deviller P, Dessanges JF, Rakotosihanaka F, Ghaem A, Boushey HA, Lockhart A, Marsac J: Nasal response to substance P and methacholine in subjects with and without allergic rhinitis. *Eur Respir J* 1988;1:356–361.
- Kudlacz EM, Shatzer SA, Knippenberg RW, Logan DE, Poirot M, van Giersbergen PLM, Burkholder TP: In vitro and in vivo characterization of MDL 105,212A, a nonpeptide NK-1/NK-2 tachykinin receptor antagonist. *J Pharmacol Exp Ther* 1996;277:840–851.
- Kris RM, South V, Saltzman A, Felder S, Ricca GA, Jaye M, Huebner K, Kagan J, Corce CM, Schlessinger J: Cloning and expression of the human substance K receptors and analysis of its role in mitogenesis. *Cell Growth Differ* 1991;2:15–23.
- De Backer MD, Gommeren W, Moereels H, Nobels G, Van Gompel P, Laysen JE, Luyten WH: Genomic cloning, heterologous expression and pharmacological characterization of a human histamine H<sub>1</sub> receptor. *Biochem Biophys Res Commun* 1993;197:1601–1608.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;197:265–278.
- Johnson CL, Johnson CG: Characterization of receptors for substance P in human astrocytoma cells: Radioligand binding and inositol phosphate formation. *J Neurochem* 1992;58:471–477.
- Arunlakshana O, Schild HO: Some quantitative uses of drug antagonists. *Br J Pharmacol Chemother* 1959;14:48–58.
- Laduron PM, Janssen PFM, Gommeren W, Laysen JE: In vitro and in vivo binding characteristics of a new long-acting histamine H<sub>1</sub> antagonist, astemizole. *Mol Pharmacol* 1982;21:294–300.
- Fujii T, Murai M, Morimoto H, Maeda Y, Yamaoka M, Hagiwara D, Miyake H, Ikari N, Matsuo M: Pharmacological profile of a high affinity dipeptide NK-1 receptor antagonist, FK888. *Br J Pharmacol* 1992;107:785–789.
- Cheng HC, Woodward JK: Antihistaminic effect of terfenadine: A new piperidine-type antihistamine. *Drug Dev Res* 1982;2:181–196.
- Zhang M-Q, Ter Laak AM, Timmerman H: Optical isomers of the H<sub>1</sub> antihistamine terfenadine: Synthesis and activity. *Biorg Med Chem Lett* 1991;1:387–390.
- Snider RM, Constantine JW, Lowe JA, Longo KP, Lebel WS, Woody HA, Drozda SE, Desai MC, Vinick FJ, Spencer RW, Hess H-J: A potent nonpeptide antagonist of the substance P (NK-1) receptor. *Science* 1991;251:435–439.
- Evans TW, Rogers DF, Aursudkij B, Chung KF, Barnes PJ: Inflammatory mediators involved in antigen-induced airway microvascular leakage in guinea pigs. *Am Rev Respir Dis* 1988;138:395–399.
- Misawa M, Iwamura S: Platelet-activating factor (PAF)-induced rhinitis and involvement of PAF in allergic rhinitis in guinea pigs. *Jpn J Pharmacol* 1990;54:217–226.
- Tohda Y, Nakajima S, Shizawa T, Maeda K, Ohmori S, Satoh H, Ishii T, Kamitani T: The inhibitory effect of TMK688, a novel anti-allergic drug having both 5-lipoxygenase inhibitory activity and anti-histamine activity, against bronchoconstriction, leukotriene production and inflammatory cell infiltration in sensitized guinea pigs. *Clin Exp Allergy* 1997;27:110–118.
- Ohshima E, Takami H, Harakawa H, Sato H, Obase H, Miki I, Ishii A, Ishii H, Sasaki Y, Ohmori K, Karasawa A, Kubo K: Dibenz[b,e]oxepin derivatives: Novel antiallergic agents possessing thromboxane A<sub>2</sub> and histamine H<sub>1</sub> dual antagonizing activity. *J Med Chem* 1993;36:417–420.
- Zhang MQ, van de Stolpe A, Zuiderveld OP, Timmerman H: Synthesis and pharmacological evaluation of some amino-acid-containing cycloheptadine derivatives as dual antagonists of histamine H<sub>1</sub>- and leukotriene D<sub>4</sub>-receptors. *Eur J Med Chem* 1997;32:95–102.
- Billah MM, Chapman RW, Watnick AS, Egan RW, Siegel MI, Kreutner W: SCH 37370: A new drug combining antagonism of platelet-activating factor (PAF) with antagonism of histamine. *J Pharmacol Exp Ther* 1990;252:1090–1096.
- Merlos M, Giral M, Balsa D, Ferrando R, Queralt M, Puigdemont A, Garcia-Rafanell J, Forn J: Rupatadine, a new potent, orally active dual antagonist of histamine and platelet-activating factor (PAF). *J Pharmacol Exp Ther* 1997;280:114–121.
- Otsuka M, Yoshioka K: Neurotransmitter functions of mammalian tachykinins. *Physiol Rev* 1993;73:230–308.
- Konno A, Numata T, Terada N, Hanazawa T, Nagata H, Motosugi H: Role of substance P in the vascular response of nasal mucosa in nasal allergy. *Ann Otol Rhinol Laryngol* 1996;105:648–653.
- Lundblad L, Brodin E, Lundberg JM, Anggard A: Effects of nasal capsaicin pretreatment and cryosurgery on sneezing reflexes, neurogenic extravasation, sensory and sympathetic neurons. *Acta Otolaryngol (Stockh)* 1985;100:117–127.
- Saria A, Wolf G: Beneficial effect of topically applied capsaicin in the treatment of hyperreactive rhinopathy. *Regul Peptides* 1988;22:167–173.
- Lacroix JC, Buvelot JM, Polla BS, Lundberg JM: Improvement of symptoms of non-allergic chronic rhinitis by local treatment with capsaicin. *Clin Exp Allergy* 1991;21:595–600.
- Mosimann B, White M, Hohman R, Goldrich M, Kaulbach H, Kaliner M: Substance P, calcitonin gene-related peptide and vasoactive intestinal peptide increase in nasal secretions after allergen challenge in atopic patients. *J Allergy Clin Immunol* 1993;92:95–104.
- Nieber K, Baumgarten CR, Rathack R, Furkert J, Oehme P, Kunkel G: Substance P and  $\beta$ -endorphin-like immunoreactivity in lavage fluids of subjects with and without allergic asthma. *J Allergy Clin Immunol* 1992;90:646–652.
- Nieber K, Baumgarten C, Rathack R, Furkert J, Laake E, Muller S, Kunkel G: Effect of azelastine on substance P content in bronchoalveolar and nasal lavage fluids of patients with allergic asthma. *Clin Exp Allergy* 1993;23:69–71.
- Bertrand C, Geppetti P, Baker J, Yamawaki I, Nadel JA: Role of neurogenic inflammation in antigen-induced vascular extravasation in guinea pig trachea. *J Immunol* 1993;150:1479–1485.

- 41 Shirasaki H, Asakura K, Narita A-I, Watanabe M, Kataura A: The effects of NK<sub>1</sub> receptor antagonists (FK224 and FK888) on agonist- and antigen-induced nasal microvascular leakage in guinea pigs. *Inflamm Res* 1997;46:28–31.
- 42 Maggi CA: Tachykinin receptors and airway pathophysiology. *Eur Respir J* 1993;6:735–742.
- 43 Petersson G, Lindell E, Andersson-Larsson K, Wang Z, Hakanson R: Nasal fluid secretion is mediated by NK<sub>1</sub>-receptors in the rat. *J Allergy Clin Immunol* 1994;93:273.
- 44 Guarnaccia S, Baraniuk JN, Bellanti J, Duina M: Calcitonin gene-related peptide nasal provocation in humans. *Ann Allergy* 1994;72:515–519.
- 45 Lundberg JM, Hokfelt T, Martling CR, Saria A, Cuello C: Substance P immunoreactive sensory nerves in the lower respiratory tract of various mammals including man. *Cell Tissue Res* 1984;235:251–261.
- 46 Lilly CM, Hall AE, Rodger IW, Kobzik L, Haley KJ, Drazen JM: Substance P-induced histamine release in tracheally perfused guinea pig lungs. *J Appl Physiol* 1995;78:1234–1241.
- 47 Heaney LG, Cross LJM, Stanford CF, Ennis M: Substance P induces histamine release from human pulmonary mast cells. *Clin Exp Allergy* 1995;25:179–186.
- 48 Schierhorn K, Brunnee T, Schultz K-D, Jahnke V, Kunkel G: Substance-P-induced histamine release from human nasal mucosa in vitro. *Int Arch Allergy Immunol* 1995;107:109–114.
- 49 Martins MA, Shore SA, Drazen JM: Release of tachykinins by histamine, methacholine, PAF, LTD<sub>4</sub>, and substance P from guinea pig lungs. *Am J Physiol* 1991;261 (Lung Cell Mol Physiol 5):L449–L455.

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