Pharmacological Characterization of a Novel Long-acting Histamine H₁ Receptor Antagonist, KAA-276

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The pharmacological profile of a newly synthesized histamine H₁ receptor antagonist, KAA-276 (1-[1-(4-fluorophenylmethyl)-1H-benzimidazol-2-yl]-5-[2-[4-(2-carboxyethyl)-phenyl]ethyl]-1,5-diazacyclooctane sulfate), was characterized. In a H₁ receptor binding assay *in vitro*, KAA-276 inhibited [³H]mepyramine binding to guinea pig cerebellar membrane preparations with an IC₅₀ of 0.66 nM. The inhibitory potency of KAA-276 was greater than that of terfenadine, but similar to that of astemizole and ketotifen. KAA-276 antagonized the histamine-induced constriction of ileum and trachea isolated from guinea pigs in a dose-dependent manner with a concomitant reduction in the maximum response. Furthermore, the inhibitory effect of KAA-276 on histamine induced contraction was potentiated depending on the duration of preincubation time and revealed an irreversible property. KAA-276 given orally, intraduodenally, and by inhalation significantly inhibited histamine-induced bronchoconstriction dose-dependently in guinea pigs. Inhalation of KAA-276 exhibited inhibitory activity with a rapid onset and long duration, while intraduodenal administration resulted in action with a slow onset. Therefore, KAA-276, an irreversible and selective histamine H₁ receptor antagonist, was shown to be a useful drug for therapeutic strategies against bronchial asthma when administered by the aerosol route.

Key words histamine; antihistamine; aerosol; bronchoconstriction

Histamine is released by a variety of noxious stimuli, including chemicals and anaphylactic injury.¹⁾ The locally released histamine binds to specific receptors on cells and appears to initiate alteration in bronchial tone, vascular flow, and permeability. For this reason, histamine is thought to be one of the important chemical mediators in several inflammatory and allergic diseases, especially bronchial asthma.²⁾ However, the use of conventional H₁ antihistamines in allergic disorders is still limited by various side effects. The major side effect is sedation or impaired performance, which is considered to be associated with H₁ receptor antagonism in the central nervous system.^{3,4)} Other side effects are related to several other pharmacological properties including anticholinergic, antiadrenergic, and antiserotonergic ones. Recently, new antihistamines, retaining long duration of action and lacking sedative effects, have been developed and are being used orally for the treatment of allergic disorders.^{5–7)} However, these new antihistamines are not completely devoid of anticholinergic, antiadrenergic, and antiserotonergic activities.^{6,8–11)}

For the remedy of bronchial asthma, inhalation of steroids and β_2 -stimulants have long been used; however, inhaled antihistaminic agents have never been used clinically. The superiority of the aerosol route of drug application has been recognized.¹²⁾ Aerosol delivery of drugs avoids the systemic effects, allows dosing of the drug in higher concentrations to the local target area, and exhibits an immediate effect compared with oral administration.¹³⁾

We synthesized KAA-276 (1-[1-(4-fluorophenylmethyl)-1H-benzimidazol-2-yl]-5-[2-[4-(2-carboxyethyl)-phenyl]ethyl]-1,5-diazacyclooctane sulfate), shown in Fig. 1, as a novel, selective, and long-acting antihistaminic agent. In the present study, we confirmed its affinity for histamine receptors and pharmacological antihistaminergic characteristics *in vitro*. In addition, we assessed the inhibitory property of inhaled and oral KAA-276 on the histamine-induced bron-

choconstriction in guinea pigs, compared with that of antihistamines ketotifen and terfenadine given orally.

MATERIALS AND METHODS

Materials KAA-276, astemizole, and terfenadine were synthesized in Kissei Pharmaceutical Co., Ltd. The following compounds were purchased from commercial sources: ketotifen, cetirizine, mepyramine and chlorpheniramine, from Sigma Chemical, St. Louis, MO, U.S.A.; histamine hydrochloride from Wako Chemicals, Japan; and [³H]mepyramine (20–30 Ci/mmol) from New England Nuclear, Boston, MA, U.S.A. Other chemicals used were of analytical grade.

Animals Male Hartley guinea pigs (Japan SLC Inc., Hamamatsu, Japan), weighing about 350–450 g, were used for histamine H₁ receptor binding assays, and those weighing about 510–550 g for histamine H₂ receptor binding assay, histamine induced contraction of isolated organs, *in vitro* experiments, and histamine induced bronchoconstriction *in vivo* experiments. Male Sprague-Dawley rats (Charles River Japan Inc., Kanagawa, Japan) weighing 280–510 g were used for radioligand assays. The animals were housed in groups of five *per* cage with free access to commercial food pellets and tap water in a room with a 12-h light/12-h dark cycle.

Radioligand Binding Assay for Histamine H₁ Receptor The cerebellum was rapidly removed from exsanguinated

Fig. 1. Chemical Structure of KAA-276

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Table 1. Membrane Preparations Including Receptor Type and Radioligand for Binding Assays

Receptor		Tissue	Species	Radioligand	Conc. (nM)
Histamine	H ₂	Cerebral cortex	Guinea pig	Tiotidine	2
Adrenergic	α_1	Forebrain	Rat	Prazosin	0.2
	α_2	Forebrain cortex	Rat	Rauwolsine	0.5
	β_1	Forebrain cortex	Rat	Dihydroalprenolol	1
	β_2	Cerebellum	Rat	Dihydroalprenolol	1
	5-HT ₁	Forebrain cortex	Rat	Serotonin	5
Serotonergic	5-HT ₂	Forebrain cortex	Rat	Ketanserin	0.5
Muscarinic	M ₁	Corpus striatum	Rat	Pirenzepine	0.2
	M ₂	Heart	Rat	AF-DX384	0.5
Dopaminergic	D ₁	Corpus striatum	Rat	SCH23390	0.5
	D ₂	Corpus striatum	Rat	Sulpiride	1
GABAergic	A	Cerebellum	Rat	GABA	10
	B	Cerebellum	Rat	GABA	10
Nicotinic		Forebrain cortex	Rat	Methylcarbamil choline iodine	5

guinea pigs ($n=10$), rinsed in ice-cold 50 mM phosphate buffer (pH 7.8), and homogenized in 10 ml of ice-cold 50 mM phosphate buffer. The homogenate was centrifuged at $2000\times g$ for 10 min at 4 °C. The pellet obtained was resuspended twice with 50 mM phosphate buffer. The binding assay was performed in a final volume of 1 ml consisting of the cerebellar membrane preparation (0.3 mg protein) with various concentrations of KAA-276. After incubation for 60 min at 32 °C, [³H]mepyramine (70 nM) was added to the assay medium and the incubation was then continued for a further 60 min. The reaction was terminated by centrifugation ($2000\times g$ for 10 min) and sediment was washed twice with 50 mM phosphate buffer (pH 7.8). The radioactivity was counted after addition of 0.5 ml of aquazole 2 (New England Nuclear) with a liquid scintillation counter (1900CA, Packard). Nonspecific binding was determined in the presence of 100 μ M astemizole, and specific binding was estimated by the difference between total and nonspecific binding. Protein content of the tissue suspension was determined using of a bicinchoninic acid protein assay (Pierce, Rockford, IL, U.S.A.). The assay was performed in duplicate. Scatchard plots were analyzed by linear least squares regression analysis. IC₅₀ values were determined by computer fitting with a Graph Pad PRISM™ software package (Graph Pad Software Inc., San Diego, CA, U.S.A.).

Radioligand Binding Assay for Other Receptors
Brain, heart, and submandibular gland tissues obtained from male Sprague-Dawley rats or from male Hartley guinea pigs were used for radioligand binding assays. The best suited species tissue membrane preparations including receptor type and radioligands for each assay are summarized in Table 1. KAA-276, respective tritiated ligand and tissue homogenate were incubated under each condition. For all assays, the incubation was terminated by filtration through a glass fiber filter (Whatman GF/B) and washing with ice-cold buffer. The radioactivities of the filters were measured in a liquid scintillation counter. The specific binding was defined as the difference between the total counts in the absence of unlabeled ligand and the counts obtained in the presence of a specific inhibiting compound. Protein content of tissue suspension was determined using the a Bradford method. These binding assays were performed in duplicate.

Histamine-Induced Contraction of Isolated Guinea Pig

Ileum Male Hartley guinea pigs were sacrificed by a blow to the neck, and the ileum was removed immediately. Segments of ileum about 1.0 cm in length were suspended in an organ bath containing 10 ml of Tyrode's solution aerated with a mixture of 95% O₂ and 5% CO₂ at 30 °C. The response to a given histamine solution was isometrically recorded under a resting tension of 1 g for all preparations. Cumulative concentration-response curves for histamine were then obtained by increasing the concentration of the histamine. At least 3 contractions of about equal magnitude were produced before testing KAA-276. The drug was added to the organ bath 5 min before the addition of histamine. Contractile responses were expressed as a percentage of the maximal response.

In separate experiments, we studied the effects of preincubation time of KAA-276 on contraction by histamine (1 μ M). The response to a given histamine was isometrically recorded under a resting tension of 1 g. KAA-276 was added to the organ bath 5, 30, 60 and 120 min before the addition of histamine. In the recovery experiment, KAA-276 at dose of 10 μ M was added to the organ bath and then histamine (1 μ M) was added at intervals of 10 min. The preparation was washed out following each histamine addition.

Histamine Induced Contraction of Isolated Guinea Pig Trachea Trachea chain preparations were prepared from isolated guinea pig trachea and suspended in an organ bath containing 10 ml of Locke-Ringer's solution aerated with a mixture of 95% O₂ and 5% CO₂ at 37 °C. The response to a given histamine solution was isometrically recorded under a resting tension of 1 g. At least 3 contractions of about equal magnitude were produced by the cumulative addition of histamine before testing KAA-276, which was added to the organ bath 30 min before histamine treatment. Contractile responses were expressed as a percentage of the response to 1 μ M histamine.

Histamine Induced Bronchoconstriction in Guinea Pigs Male Hartley guinea pigs were anesthetized with intraperitoneal administration of urethane (500 mg/kg) and α -chloralose (20 mg/kg). An endotracheal cannula was inserted into the guinea pig and connected to a respirator (SN-860, Shinano Seisakusyo, Tokyo, Japan), and then the animal was ventilated at a tidal volume of 10 ml/kg and at a frequency of 60 breaths/min. A side-hole catheter through the endotracheal cannula was linked to a pressure transducer (P-231D,

Table 2. Effect of KAA-276 and other Histamine H_1 Antagonists on [3H]Mepyramine Binding to Guinea Pig Cerebellum Membranes

Compound	IC ₅₀ (nM)
KAA-276	0.66
Astemizol	0.20
Terfenadine	41.70
Ketotifen	0.10
Cetirizine	19.50
Mepyramine	2.04
Chlorpheniramine	2.63

Data show the mean of 2 separate experiments.

Gould, U.S.A.) to measure endotracheal pressure. Histamine (1 mg/ml) was atomized with an ultrasonic nebulizer (NE-U10B, OMRON, Kyoto, Japan) for 30 s and delivered to the anesthetized guinea pigs by inhalation.

KAA-276 (0.3–3 mg/kg), terfenadine (0.3–3 mg/kg) and ketotifen (0.01, 0.1 mg/kg) were given intraduodenally (i.d.) to anesthetized guinea pigs at 0.5, 2 and 4 h before histamine exposure and orally to conscious at 4, 8 and 24 h before. Furthermore, KAA-276 (0.0001–0.1%) was inhaled by the anesthetized guinea pigs for 1 min at 0.5, 2 and 4 h before histamine treatment. In the other experiment, KAA-276 (0.0001–0.1%) was inhaled by conscious guinea pigs for 15 min in a glass desiccator (inside diameter 30 cm) and histamine was delivered 4, 8 and 24 h thereafter. Inhalation time by conscious animals was determined by inhibition rate with KAA-276.

Statistical Analysis Dunnet's multiple comparison test was used after ANOVA. *p* values of less than 0.05 were considered significant. ED₈₀ was calculated by Probit method.

RESULTS

Affinity of KAA-276 for Histamine H_1 -Receptor and Other Receptors KAA-276 and other histamine H_1 receptor antagonists were compared by their IC₅₀ values in blocking [3H] mepyramine binding to histamine H_1 receptor sites in guinea pig cerebellum (Table 2). The IC₅₀ value of KAA-276 was 0.66 nM. The affinity of KAA-276 for histamine H_1 receptor was similar to that of astemizole and ketotifen, whereas it was more potent than that of terfenadine and cetirizine. KAA-276 had hardly any affinity for receptors (IC₅₀>10000 nM), histamine H_2 , adrenergic (α_1 , α_2 , β_1 and β_2), serotonergic (5-HT₁ and 5-HT₂), muscarinic (M₁ and M₂), dopaminergic (D₁ and D₂), nicotinic, or GABAergic (A and B). In the *in vitro* studies, KAA-276 had a higher affinity for histamine H_1 receptors than for other receptors.

Effect of KAA-276 on Histamine Induced Contraction in Guinea Pig Ileum As shown in Fig. 2, KAA-276 (0.1–3 μ M), terfenadine (0.1–30 μ M), and ketotifen (0.001–0.03 μ M) dose-dependently caused a rightward shift in the histamine concentration–response curve with a reduction in the maximal response. Furthermore, the inhibitory potency of KAA-276 on histamine-induced contraction depended on the preincubation time (Fig. 3A). That is to say, the IC₅₀ value of KAA-276 was 3.3, 0.56, 0.07 and 0.05 μ M at 5, 30, 60 and 120 min of incubation with the drug, respectively. The time course for the recovery of the response to histamine following treatment with KAA-276 (10 μ M) is shown in Fig. 3B. In spite of continual washing of the tissue with Tyrode's solu-

Fig. 2. Effects of KAA-276 (KAA: A), Terfenadine (T: B), and Ketotifen (Ke: C) on Histamine-Induced Contractile Response in the Isolated Guinea Pig Ileum

Cumulative concentration–response curves for histamine were obtained by increasing its concentration of the histamine. The drug was added to an organ bath 5 min before the addition of histamine. Contractile responses were expressed as a percentage of the maximal response. Each point indicates the mean of 4 experiments.

tion every 10 min, little of the antihistaminic effect of KAA-276 had disappeared even after 3 h of additional incubation.

Effect of KAA-276 on Histamine Induced Contraction in Guinea Pig Trachea As shown in Fig. 4, KAA-276 inhibited the histamine-induced contraction of isolated guinea pig trachea dose-dependently with a concomitant reduction in the maximum response.

Effect of KAA-276 on Histamine Induced Bronchoconstriction in Guinea Pig Figures 5A and B show that KAA-276 given intraduodenally, orally and by inhalation significantly inhibited inhaled histamine-induced bronchoconstriction. In the treatment of asthma, it is desirable to inhibit bronchoconstriction completely, so we used the ED₈₀ (the concentration of antihistamines causing 80% inhibition of

Fig. 3. Time Course of the Onset (A) of and Recovery (B) from the Inhibitory Effect of KAA-276 as Reflected by the Contractile Response to Histamine ($1 \mu\text{M}$) in the Isolated Guinea Pig Ileum

In the onset study, KAA-276 was added to the organ bath 5, 30, 60, or 120 min before the addition of histamine. In the recovery experiment, KAA-276 at a dose of $10 \mu\text{M}$ was added to the organ bath and then histamine ($1 \mu\text{M}$) was added at intervals of 10 min. Contractile responses were expressed as a percentage of the response to $1 \mu\text{M}$ histamine. Each point indicates the mean of 4 experiments. Vertical bars show standard error of the mean.

histamine-induced bronchoconstriction) to evaluate the efficacy of antihistamines *in vivo*. KAA-276 inhibited histamine-induced contraction by intraduodenal administration with ED_{80} of 1.38 and 1.9 mg/kg at 2 and 4 h in anesthetized guinea pigs; however, this antihistaminic effect was not expressed at 0.5 h. In oral administration, KAA-276 inhibited this contraction with an ED_{80} of 1.74, 2.31, and 2.21 mg/kg at 4, 8, and 24 h, respectively, in conscious guinea pigs (Table 3). However, the antihistaminic effect of KAA-276 was not evident at 0.5 h after oral administration. In the inhalation study, KAA-276 inhibited histamine-induced constriction in the anesthetized animals with an ED_{80} of 0.09% (0.27 mg/kg), 0.03% (0.09 mg/kg), and 0.03% (0.09 mg/kg) at 0.5, 2, and 4 h, and that in conscious animals with a value of 0.06% (0.18 mg/kg) and 0.08% (0.24 mg/kg) at 4 and 8 h, respectively, following inhalation (Table 3). The antihistaminic effect of inhaled KAA-276 appeared as easily as 0.5 h ($\text{ED}_{80}=0.09\%$) and diminished at 24 h ($\text{ED}_{80}\geq 0.1\%$) following the administration (Fig. 5B). Ketotifen and terfenadine also significantly inhibited histamine-induced bronchoconstriction (Fig. 5C, D and Table 3). The inhibitory effect of ketotifen on this bronchoconstriction was maximal at 0.5 h following the intraduodenal administration and diminished gradually. Although terfenadine, like KAA-276, caused no significant inhibition at 0.5 h, it showed inhibitory activity with an ED_{80} of 24.3 mg/kg (i.d.) 2 h after the administration

Fig. 4. Effect of KAA-276 on Histamine-Induced Contractile Response in Isolated Trachea of Guinea Pig

The response to a given histamine solution was isometrically recorded under a resting tension of 1 g. KAA-276 was added to the organ bath 30 min before the cumulative addition of histamine. Contractile responses were expressed as a percentage of the response to $1 \mu\text{M}$ histamine. Each point indicates the mean of 4 experiments. Vertical bars show standard error of the mean.

and one of 11.0 mg/kg (*p.o.*) 4 h following the administration (Table 3).

DISCUSSION

Histamine H_1 antagonists have been widely used in the treatment of allergic disorders, particularly allergic rhinitis.^{14–19} However, the use of conventional antihistamines is limited because they have various side effects, such as sedation, local anesthetic action, and dryness of mouth. Recently, the new antihistamines, terfenadine,²⁰ astemizole²¹ and cetirizine,²² the so called “second-generation” H_1 antagonists, have been developed and are being used in the treatment of allergic diseases. These new antihistamines do not induce sedation. With the goal of avoiding the antihistamine-induced adverse effects described above, we synthesized KAA-276, a novel histamine H_1 receptor antagonist, and evaluated its pharmacological efficacy *in vitro* and *in vivo*.

In this study, we showed that KAA-276 specifically inhibited the binding of [^3H]mepyramine to the histamine H_1 receptor in guinea pig cerebellar membrane. The affinity of KAA-276 for histamine H_1 receptor ($\text{IC}_{50}=0.66 \text{ nM}$) was similar to that of astemizole and ketotifen and higher than that of other antihistamines, terfenadine, cetirizine, mepyramine and chlorpheniramine. KAA-276 had no affinity ($\text{IC}_{50}>10000 \text{ nM}$) for adrenergic (α_1 , α_2 , β_1 , and β_2), serotonergic (5-HT $_1$ and 5-HT $_2$), muscarinic (M_1 and M_2), or histamine H_2 receptors. Although second-generation H_1 antihistamines have more selectivity for the histamine H_1 receptor than the conventional antihistamines, these new antihistamines are not completely devoid of antiadrenergic and anti-serotonergic activity.²¹ In contrast to those antihistamines, KAA-276 displayed a remarkable selectivity for the histamine H_1 receptor.

In *in vitro* experiments, KAA-276 exerted an inhibitory effect on the histamine-induced contraction of the ileum and trachea of guinea pigs. KAA-276 inhibited the histamine-induced contractile response in a dose-dependent and noncompetitive manner. Terfenadine and ketotifen exhibited an inhibitory profile essentially similar to that of KAA-276 for the histamine-induced ileal contraction. In the isolated guinea

Fig. 5. Effects of KAA-276 (KAA: A, B), Terfenadine (T: C), and Ketotifen (Ke: D) on Aerosol Histamine-Induced Bronchoconstriction *in Vivo* in Guinea Pigs

Bronchoconstriction was induced by inhaled histamine (1 mg/ml for 30 s). KAA-276 was given intraduodenally (i.d.) or orally (A) and by inhalation (B) to anesthetized (0.5–4 h pre-treatment time) and conscious (4–24 h pre-treatment time) guinea pigs. Like KAA-276, ketotifen and terfenadine were also administered intraduodenally or orally. Each point indicates the percent inhibition of the contraction induced by aerosol histamine (1 mg/ml for 30 s) and is the mean of 5–10 animals. *: $p < 0.05$ and **: $p < 0.01$ vs. control.

Table 3. Inhibitory Effects of KAA-276, Ketotifen and Terfenadine against Histamine-Induced Bronchoconstriction in Anesthetized (A) or Conscious (B) Guinea Pigs

(A) Anesthetized guinea pigs				
Drug	Route	ED ₈₀		
		Time after drug administration (h)		
		0.5	2	4
KAA-276	i.d. (mg/kg)	>3	1.38	1.9
KAA-276	inhalation (%)	0.09	0.03	0.03
Ketotifen	i.d. (mg/kg)	0.07	0.07	>0.1
Terfenadine	i.d. (mg/kg)	>30	24.3	8.3

(B) Conscious guinea pigs				
Drug	Route	ED ₈₀		
		Time after drug administration (h)		
		4	8	24
KAA-276	p.o. (mg/kg)	1.74	2.31	2.21
KAA-276	inhalation (%)	0.06	0.08	>0.1
Ketotifen	p.o. (mg/kg)	>0.1	>0.1	>0.1
Terfenadine	p.o. (mg/kg)	11.0	>30	>30

ED₈₀: the concentration of drug causing 80% inhibition of aerosol histamine-induced bronchoconstriction.

pig ileum, the antihistaminergic activity of KAA-276 reached maximum after a 60 min preincubation with the drug. In addition, the antihistaminergic activity of KAA-276 was irreversible and lasted for more than 3 h. These results indicated that KAA-276 has a long-acting antihistaminergic effect with a slow onset.

KAA-276 thus proved to be an irreversible and selective histamine H₁ receptor antagonist based on the results of the *in vitro* experiment. Then we examined the ability to inhibit the bronchospastic response caused by histamine *in vivo*. KAA-276 strongly inhibited the histamine-induced bronchoconstriction in guinea pigs when given intraduodenally, orally, or by inhalation. By intraduodenal administration, the antagonist showed the antihistaminergic effect with a slow onset. At 3 mg/kg KAA-276 completely inhibited the histamine-induced bronchoconstriction 2 h after administration. However, even at the same dose it did not significantly inhibit bronchoconstriction 0.5 h after administration. This phenomenon is in accordance with the *in vitro* effects of KAA-276 on histamine-induced contraction of the guinea pig ileum, where one hour was required to reach maximal antihistaminergic activity *in vitro*. In contrast, the onset of action of KAA-276 was notably rapid when given by inhalation. It has long been recognized that aerosol delivery of drugs allows the airway to be exposed to a drug at a high concentration and results in more immediate and effective inhibition than

oral administration. Moreover, in our inhalation study, suppression of bronchoconstriction by KAA-276 continued for more than 8 h. This long period of action is also reflected by the results obtained from its *in vitro* evaluation. On the other hand, ketotifen administered orally or intraduodenally exhibited its antihistaminergic effect with a rapid onset but for a short period. Conversely, the antihistaminergic effect of terfenadine was of long duration but of slow onset. This result is consistent with the findings of Cheng and Woodward²⁰⁾ who demonstrated that the antihistaminergic effect of terfenadine was slow in onset and long-lasting.

The second-generation H₁ antagonists are largely excluded from the brain when given at therapeutic doses, because they did not cross the blood-brain barrier appreciably, and thus they antagonize preferentially peripheral histamine H₁ receptors rather than central nervous system histamine H₁ receptors.²⁰⁾ In another of our experiments, KAA-276 was detected only slightly in the rat brain when administered *via* the intratracheal route (data not shown). Moreover, KAA-276 showed no significant prolongation in a hexobarbital-induced sleeping time test (<1000 mg/kg, *p.o.* data not shown). Therefore, KAA-276 is assumed to have no sedating action. In this present study, we showed topical application of KAA-276 to be beneficial in inhibiting histamine-induced bronchoconstriction in guinea pigs. Its topical application is also valuable to avoid not only the side effects related to the central nervous system but also the systemic effects.

In conclusion, KAA-276 is an irreversible and highly selective histamine H₁ antagonist. Although it is effective when given either orally or by inhalation, its inhalation is more useful because of the more rapid onset of effect and the longer lasting inhibition. Based on the pharmacological profiles shown in our studies, inhaled KAA-276 would be very useful in the treatment of bronchial asthma.

Acknowledgment The authors are grateful to Professor Kazutaka Momose (School of Pharmaceutical Science, Showa University, Japan) and Dr. Yohsuke Imai (Kissei Pharmaceutical Co. Ltd., Japan) for his kind advice and criticisms.

REFERENCE

- 1) Schild H. O., Hawkins D. F., Monger J. L., Herxheimer H., *Lancet*, **i**, 376—382 (1951).
- 2) Casale T. B., Wood D., Richerson H. B., Trapp S., Metzger W. J., Zavala D., Hunninghake G. W., *J. Clin. Invest.*, **79**, 1197—1203 (1987).
- 3) Roehrs T. A., Tietz E. I., Zorick F. J., Roth T., *Sleep*, **7**, 137—141 (1984).
- 4) Nicholson A. N., Stone B. M., *Eur. J. Clin. Pharmacol.*, **30**, 27—32 (1986).
- 5) Martz B. L., Mumford J. P., Burke J. T., Barlow J. L. R., *Arzneim. Forsch. Drug Res.*, **32**, 1153—1217 (1982).
- 6) Richards D. M., Brogden R. N., Heel R. C., Speight T. M., Avery G. S., *Drugs*, **28**, 38—61 (1984).
- 7) Nicholson A., *Trends. Pharmacol. Sci.*, **8**, 247—249 (1987).
- 8) Tozzi S., Roth F., Tabachnick I., *Agents. Actions.*, **4**, 264—270 (1974).
- 9) VanNeuten J., Xhonneux R., Janssen P., *Arch. Int. Pharmacodyn. Ther.*, **232**, 217—220 (1978).
- 10) Barnett A., Iorio L., Kreutner W., Tozzi S., Ahn H., Gulbenkian A., *Agents. Actions.*, **14**, 590—597 (1984).
- 11) Kreutner W., Chapman R., Gulbenkian A., Siegel M., *Allergy*, **42**, 57—63 (1987).
- 12) Anderson S. D., Seal J. P., Rozea P., Bandler L., Theobald G., Lindsay D. A., *Am. Rev. Respir. Dis.*, **114**, 493—500 (1976).
- 13) Norman P. S., *J. Allergy Clin. Immunol.*, **76**, 366—368 (1985).
- 14) Empey D. W., Bay C., Hoddler M., Hughes D. T. D., *Ann. Allergy*, **34**, 41—46 (1975).
- 15) Schaaf L., Hendeles L., Weinberger M., *J. Allergy Clin. Immunol.*, **63**, 129—133 (1979).
- 16) Diamond L., Gerson K., Cato A., Peace K., Perkins J. G., *Ann. Allergy*, **47**, 87—91 (1981).
- 17) Wong L., Hendeles L., Weinberger M., *J. Allergy Clin. Immunol.*, **67**, 233—238 (1981).
- 18) Simons F. E. R., Simons K. J., *Pediatr. Clin. North. Am.*, **30**, 899—914 (1983).
- 19) Connell J. T., Howard J. C., Dressler W., Perhach J. L., *Am. J. Rhinol.*, **1**, 3—16 (1987).
- 20) Cheng H. C., Woodward J. K., *Arzneim. Forsch. Drug Res.*, **32**, 1160—1166 (1982).
- 21) Laduron P. M., Janssen P. F. M., Gommeren W., Leysen J. E., *Mol. Pharmacol.*, **21**, 294—300 (1982).
- 22) Deborah M. C. R., Micaela M. T. B., Andrew F., *Drugs*, **40**, 762—781 (1990).