Biochemical Pharmacology, Vol. 55, pp. 151–157, 1998.

ISSN 0006-2952/98/$19.00 + 0.00
PII S0006-2952(97)00416-4

Structure–Activity Characterization of an H_2-Receptor Antagonist, 3-Amino-4-[4-[4-(1-piperidinomethyl)-2-pyridyloxy]-*cis*-2-butenylamino]-3-cyclobutene-1,2-dione Hydrochloride (IT-066), Involved in the Insurmountable Antagonism against Histamine-Induced Positive Chronotropic Action in Guinea Pig Atria

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ABSTRACT. IT-066 (3-amino-4-[4-[4-(1-piperidinomethyl)-2-pyridyloxy]-*cis*-2-butenylamino]-3-cyclobutene-1,2-dione hydrochloride), an H_2-receptor antagonist, shows highly potent, time-dependent, and irreversible antagonism at H_2-receptors. We identified the structurally important parts of IT-066 involved in its interaction with the H_2-receptor, and explored its unique mode of action by investigating the H_2-receptor blocking action of IT-066 and related compounds in guinea pig isolated atria. IT-066 is structurally divided into three different parts: a tertiary amine and hydrophobic group, a connecting carbon chain, and a polar group. Though the replacement of its pyridine ring with a benzene ring maintained the mode of the H_2-receptor blocking action of IT-066, the oxidation of the piperidine ring completely attenuated this blocking action. By replacing the connecting carbon chain of IT-066, *cis*-2-butene, with butane, *trans*-2-butene, or 2-butyne, the irreversible antagonism disappeared and the potency was reduced. On the other hand, BMY25368, whose connecting carbon chain is trimethylene, showed irreversible antagonism comparable to that of IT-066. Hydrolysis of the polar group of IT-066 completely attenuated the H_2-receptor blocking activity. Among the compounds tested, only the compound that had 3,4-diamino-3-cyclobutene-1,2-dione as a polar group showed time-dependent and insurmountable H_2-receptor blocking action. These data suggest the importance of the following structural features of IT-066: the piperidine ring of IT-066 and -NH_2 in its polar group are essential for the interaction with the H_2-receptor; and the 3,4-diamino-3-cyclobutene-1,2-dione group and the connecting carbon chain of IT-066 are crucial for determining the irreversibility of H_2-receptor blocking action, though the connecting carbon chain is replaceable with another chain with appropriate length and configuration. BIOCHEM PHARMACOL **55**;2:151–157, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. IT-066; histamine H_2-receptor antagonist; guinea pig atria; insurmountable antagonism; structure–activity relationship

Since the development of burimamide by Black *et al.* [1], a number of H_2-receptor antagonists have been synthesized. Because of their potent inhibitory action on acid secretion, these receptor antagonists became the cornerstone of peptic ulcer therapy [2, 3].

H_2-receptor antagonists developed in the early stage, such as metiamide or cimetidine [4, 5], had in their structure an imidazole ring, which was thought to be essential for the binding with H_2-receptors. However, this line of thinking was refuted by the development of the more potent and specific H_2-receptor antagonists ranitidine and famotidine, which have, respectively, dimethylaminomethylfuran and diaminomethyleneaminothiazole instead of an imidazole ring [6, 7]. Subsequently, H_2-receptor antagonists that contain a piperidinomethylphenyl moiety in their structure have also been reported to have very potent and long-lasting activity [8–10]. IT-066 (3-amino-4-[4-[4-(1-piperidinomethyl)-2-pyridyloxy]-*cis*-2-butenylamino]-3-cyclobutene-1,2-dione hydrochloride) is one of the compounds that contains a piperidinomethylpyridyl moiety in its structure (Fig. 1) [11]. In our previous

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Received 19 August 1996; accepted 27 June 1997.

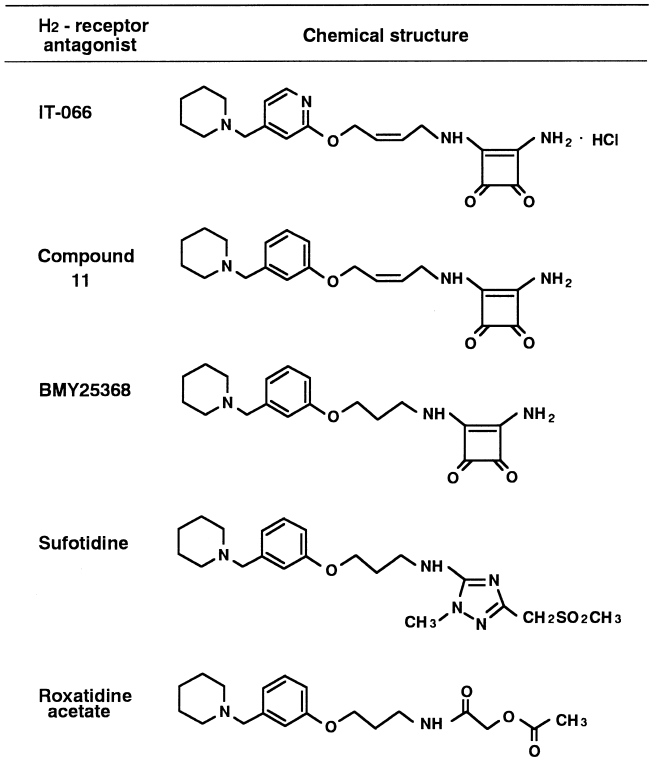


FIG. 1. Chemical structures of IT-066 and other H_2 receptor antagonists.

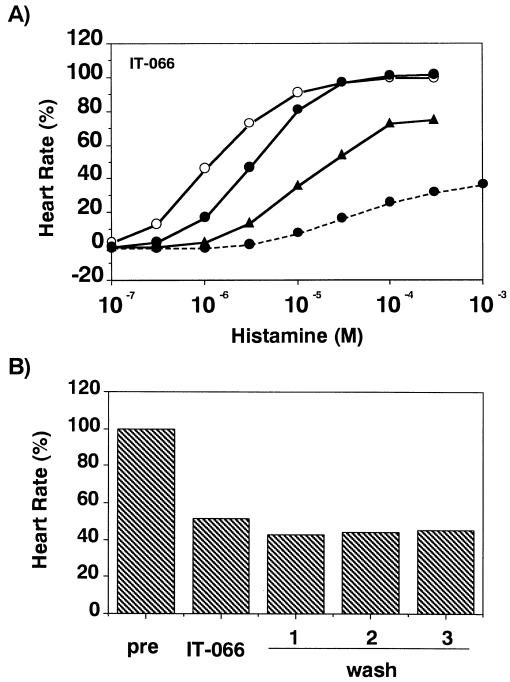


FIG. 2. Effect of IT-066 on the positive chronotropic action induced by histamine. (A) Potency of IT-066 and the time-dependency. IT-066 was incubated for 5 min at 3×10^{-7} M (●, solid line) and 10^{-6} M (▲, solid line) and for 60 min at 3×10^{-7} M (●, dashed line). The open circles (○) indicate the control response induced by a cumulative dose of histamine. (B) Reversibility of the inhibitory effect of IT-066. IT-066 (3×10^{-8} M) was incubated for 60 min. Reversibility was examined after washout of IT-066 from the bath. Each value is the mean obtained from 4 experiments.

investigation on the kinetics of the H_2-receptor blocking action of IT-066, it was found to have a highly potent and long-lasting H_2-receptor blocking action that is characterized by time-dependent and irreversible interaction with H_2-receptors in the guinea pig atria [12]. The time-dependent and irreversible antagonism was also observed in isolated rabbit parietal cells [13]. Moreover in an *in vivo* experiment, IT-066 demonstrated potent and long lasting anti-secretory and anti-ulcer action, which is considered to be due to the insurmountable H_2-receptor blocking action [14].

The molecular structure of IT-066 is divided into three different parts: a tertiary amine and hydrophobic group, a polar group, and a connecting carbon chain. In the present study, we investigated the effects of IT-066 and related compounds on histamine-induced positive chronotropic action in guinea pig isolated atria, to elucidate which part of the IT-066 structure contributes to the unique mode of H_2-receptor blocking action.

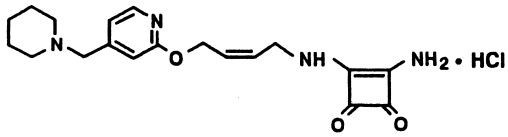
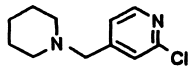
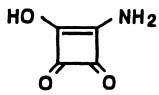
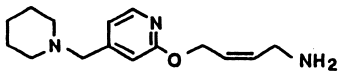
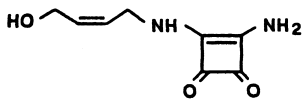
MATERIALS AND METHODS

Materials

IT-066 and other test compounds were synthesized in our laboratory according to the methods of Fujinawa and Nohara [11]. The melting points (m.p.) and boiling points (b.p.) of the synthesized compounds were as follows: IT-066 (m.p. = 211–214°), compound **1** (b.p. = 140–145°/2 mm Hg), **2** (b.p. = 110–115°/15 mm Hg), **3** (m.p. = 240–243°), **4** (yellow oil), **5** (m.p. = 228–233°), **6** (m.p. = 174–177°), **7** (brown oil), **8** (m.p. = 227–231°), **9** (m.p. = 213–216°), **10** (m.p. = 177–179°), and **11** (m.p. = 214–218°). BMY25368 (m.p. = 201–204°) and sufotidine (m.p. = 95–96°) were synthesized in our laboratory. Elemental analysis data were as follows: IT-066 (Anal. calcd. for $C_{19}H_{24}N_4O_3 \cdot HCl$: C, 58.08; H, 6.41; N, 14.26. Found: C, 57.97; H, 6.45; N, 14.18), BMY25368 (Anal. calcd. for $C_{19}H_{25}N_3O_3 \cdot HCl \cdot 0.2H_2O$: C, 59.50; H, 6.94; N, 10.96. Found: C, 59.66; H, 6.98; N, 11.02), and sufotidine (Anal. calcd. for $C_{20}H_{31}N_5O_3S$: C, 56.98; H, 7.41; N, 16.61. Found: C, 56.83; H, 7.42; N, 16.51), compound **8** (Anal. calcd. for $C_{19}H_{26}N_4O_3$: C, 63.67; H, 7.31; N, 15.63. Found: C, 63.37; H, 7.27; N, 15.58), **9** (Anal. calcd. for $C_{19}H_{24}N_4O_3 \cdot HCl$: C, 58.08; H, 6.41; N, 14.26. Found: C, 58.05; H, 6.46; N, 14.04), **10** (Anal. calcd. for $C_{19}H_{22}N_4O_3 \cdot 0.2H_2O$: C, 63.74; H, 6.31; N, 15.64. Found: C, 63.86; H, 6.23; N, 15.52), and **11** (Anal. calcd. for $C_{20}H_{25}N_3O_3 \cdot HCl$: C, 61.30; H, 6.69; N, 10.72. Found: C, 61.47; H, 6.80; N, 10.72).

Roxatidine acetate was obtained from commercially available pharmaceuticals. Histamine dihydrochloride was purchased from Wako Pure Chemical Industries (Osaka, Japan). All other reagents were obtained commercially and were of the highest purity available.

TABLE 1. Effects of IT-066 and its fragment structures on the positive chronotropic action induced by histamine

Compound	Chemical structure	IC_{50} (μM)
IT-066		0.28
1		>10
2		>10
3		>10
4		>10
5		>10

The IC_{50} values were determined from the response against a single concentration (3×10^{-6} M) of histamine. The test compounds were incubated for 5 min. Each value is expressed as the mean of 3–4 experiments.

Male Hartley strain guinea pigs were purchased from Hamuri (Ibaraki, Japan).

Preparation of Guinea Pig Atria

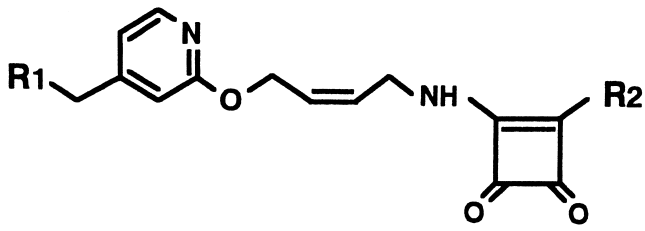
Male Hartley strain guinea pigs weighing 300–450 *g* were killed under anesthesia. The spontaneously beating right atrium was dissected from the surrounding tissue and suspended in a glass chamber containing 20 mL of Krebs–Hensleit solution at 35°, which was aerated with a mixture of 95% O_2 and 5% CO_2. Tissue was attached under 0.5 g of tension to an isometric force displacement transducer for rate recording, and measurement was allowed to stabilize for at least 60 min before the experiment commenced.

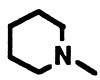
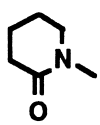
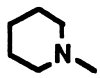
Experiment of H_2-Receptor Antagonism

The response of guinea pig atria to a cumulative concentration (10^{-7}–10^{-3} M) of histamine was obtained before and after the incubation with a test compound. The increase of heart rate was expressed as the percentage of maximal increase induced by histamine obtained before the administration of a test compound.

The response to a single concentration (3×10^{-6} M) of histamine was obtained before each compound addition, after incubation with a test compound, and then at three time intervals after washout of the compound from the bath. Twenty-minute periods, each with three buffer washes, were allowed between each trial with histamine after the initial removal of the compound. Test compounds were incubated 5 or 60 min before histamine administration. The increase of heart rate was expressed as the percentage of increase induced by histamine obtained before the administration of a test compound.

TABLE 2. Effects of IT-066 and its derivatives on the positive chronotropic action induced by histamine



Compound	R_1	R_2	IC_{50} (μM)
IT-066		NH_2	0.28
6		NH_2	>10
7		OH	>10

The IC_{50} values were determined from the response against a single concentration (3×10^{-6} M) of histamine. The test compounds were incubated for 5 min. Each value is expressed as the mean of 3–4 experiments.

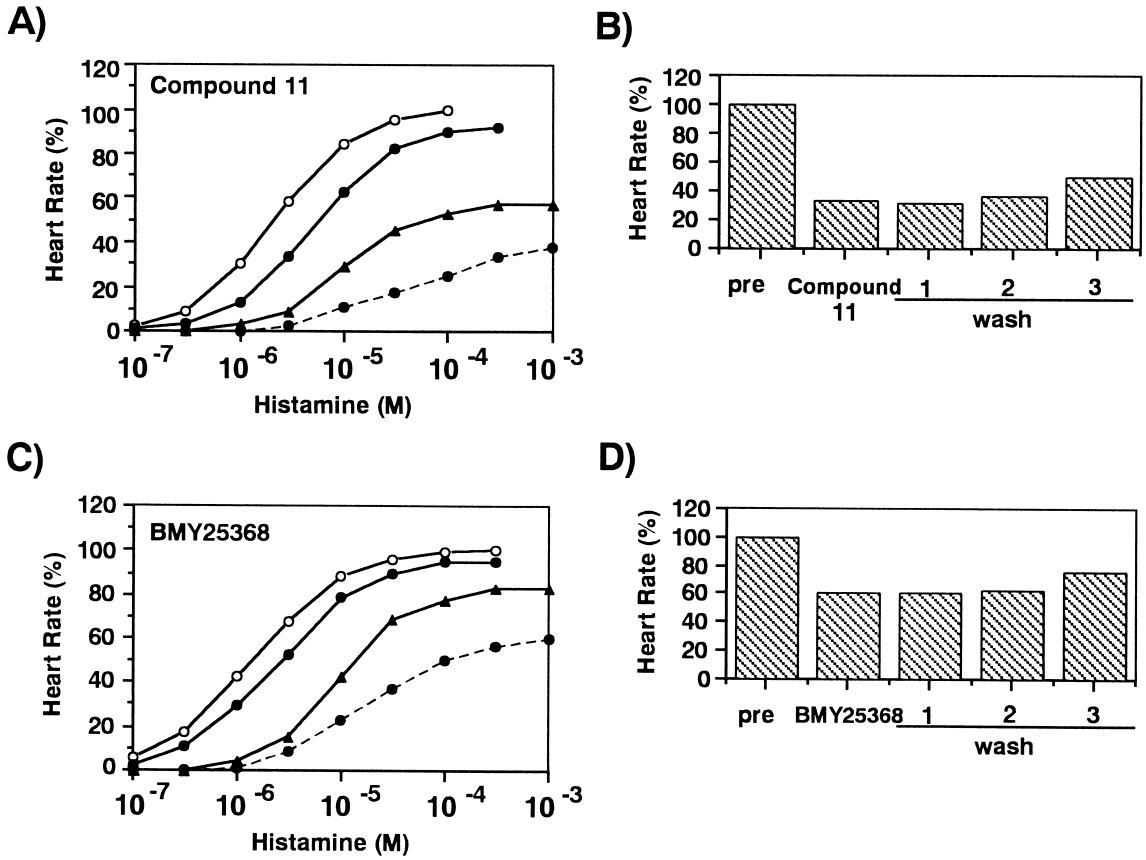


FIG. 3. Effects of compound 11 and BMY25368 on the positive chronotropic action induced by histamine. (A) Potency of compound 11 and the time-dependency. Compound 11 was incubated for 5 min at 10^{-7} M (●, solid line) and 3×10^{-7} M (▲, solid line) and for 60 min at 10^{-7} M (●, dashed line). The open circles (○) indicate the control response induced by a cumulative dose of histamine. (B) Reversibility of the inhibitory effect of compound 11. Compound 11 (3×10^{-8} M) was incubated for 60 min. Reversibility was examined after washout of compound 11 from the bath. (C) Potency of BMY25368 and the time-dependency. BMY25368 was incubated for 5 min at 10^{-7} M (●, solid line) and 3×10^{-7} M (▲, solid line) and for 60 min at 10^{-7} M (●, dashed line). The open circles (○) indicate the control response induced by a cumulative dose of histamine. (D) Reversibility of the inhibitory effect of BMY25368. BMY25368 (3×10^{-8} M) was incubated for 60 min. Reversibility was examined after washout of BMY25368 from the bath. Each value is the mean obtained from 4 experiments.

RESULTS

Effect of IT-066 on the Positive Chronotropic Action Induced by Histamine

The maximum increase of heart rate induced by a cumulative concentration of histamine (10^{-7}–3×10^{-4} M) was 120.5 ± 6.6 beats/min (mean ± SEM of 4 experiments). IT-066 concentration dependently inhibited the positive chronotropic action induced by histamine (Fig. 2A). When IT-066 (3×10^{-7} M) was incubated with guinea pig atria for 5 min, the concentration–response curve of histamine shifted to the right in parallel fashion. IT-066, at a concentration of 10^{-6} M, further shifted the curve to the right, accompanied by inhibition of the maximal response. With prolongation of the incubation time to 60 min, IT-066 (3×10^{-7} M) further shifted the concentration–response curve to the right and markedly inhibited the maximal response induced by histamine. After a 60-min incubation of the tissue with IT-066 (3×10^{-8} M), repeated washing of the tissue did not restore the sensitivity to a single concentration of histamine for at least 60 min (Fig. 2B).

Effect of each Fragment of the IT-066 Molecule

The increase of heart rate induced by a single concentration of histamine (3×10^{-6} M) was 85.3 ± 13.0 beats/min (mean ± SEM of 3 experiments). Incubation of IT-066 (10^{-6} M) for 5 min significantly inhibited the response induced by a single concentration of histamine (90.5%), and the inhibitory action was sustained after washing the tissue at least three times (data not shown). However, three individual fragments of IT-066, compounds **1**, **2**, and **3**, did not antagonize the positive chronotropic action induced by histamine even at a higher concentration of 10^{-5} M. Neither of the two compounds composed of two fragments, compounds **4** and **5**, showed H_2-receptor blocking action (Table 1).

Conversion of a Tertiary Amine and Hydrophobic Group

Oxidization of the piperidine ring of IT-066 (compound **6**) resulted in the complete loss of the H_2-blocking action (Table 2). On the other hand, compound **11** (Fig. 1),

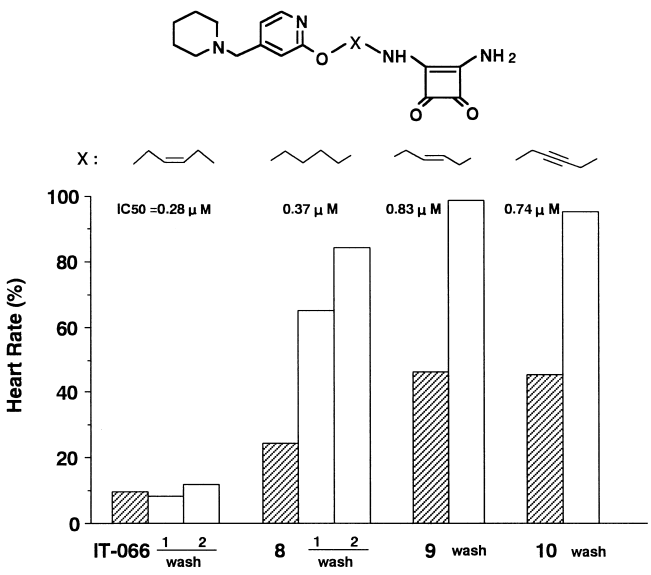


FIG. 4. Potency and reversibility of the H_2 receptor antagonizing effect of IT-066 and related compounds. The test compounds were incubated for 5 min. The IC_{50} values were determined from the response against a single concentration (3×10^{-6} M) of histamine. In the reversibility experiments, the inhibitory actions obtained after a 5-min incubation of the test compounds (10^{-6} M) were expressed as a percentage of the response to 3×10^{-6} M histamine (hatched columns). After washout of the test compound from the bath, reversibility was examined in 1 or 2 series of experiments (white columns). Each value is the mean obtained from 3–4 experiments.

which has a benzene ring instead of a pyridine ring, exhibited a potent H_2-receptor blocking action, and the antagonism was time-dependent and irreversible, similar to that of IT-066 (Fig. 3, A and B).

Conversion of a Connecting Carbon Chain

The connecting carbon chain between the tertiary amine and hydrophobic group and the polar group of IT-066 is 1,4-disubstituted *cis*-2-butene; and those of compounds **8**, **9**, and **10** are butane, *trans*-2-butene, and 2-butyne, respectively. Although these three compounds inhibited the histamine-induced increase of heart rate, the potency was slightly weaker than that of IT-066 (Fig. 4). Furthermore, the inhibitory effect was reversed completely by the removal of the compound from the organ bath. The distance between the nitrogen in the piperidine ring and -NH_2 in the polar group of IT-066 was 12.3 nm. The distances of compounds **8** and **9** were 13.6 and 13.3 nm, respectively (slightly longer than that of IT-066). On the other hand, the distance between the two nitrogens of compound **10** was 8.8 nm, which was shorter than that of IT-066 owing to the triple bond in its connecting carbon chain. Though the estimated length between the two nitrogens of BMY25368, which has trimethylene as a connecting carbon chain, was slightly shorter than that of IT-066 (10.9 nm), BMY25368 had a potent H_2-receptor blocking action comparable to that of IT-066, and the antagonism was time dependent and irreversible (Fig. 3, C and D).

Conversion of a Polar Group

IT-066 has 3,4-diamino-3-cyclobutene-1,2-dione as a polar group. Compound **11** and BMY25368 have the same polar group as IT-066, while their connecting carbon chains are different (*cis*-2-butene and trimethylene, respectively) (Fig. 1). Their H_2-receptor blocking action is time dependent and irreversible as observed for IT-066 (Fig. 4). Hydrolysis of the polar group of IT-066 (compound **7**) completely abolished its H_2-blocking action (Table 2). Sufotidine and roxatidine acetate have a different polar group from IT-066, and their connecting carbon chains are trimethylene (Fig. 1). Sufotidine and roxatidine acetate only shifted the concentration–response curve of histamine to the right with a short incubation (Fig. 5, A and C). Prolongation of the incubation time further shifted the concentration–response curve, but the maximum response did not change. The inhibitory action of sufotidine and roxatidine acetate was gradually reversed by the washout of the drug (Fig. 5, B and D).

DISCUSSION

IT-066 is a potent H_2-receptor antagonist, characterized by a time-dependent and irreversible antagonism with the histamine H_2-receptor in guinea pig isolated atria and rabbit parietal cells [12, 13]. This insurmountable interaction seems to be involved in its potent and long-lasting anti-secretory and anti-ulcer action [14]. In the present study, the structural importance of IT-066 in the potent, irreversible, and time-dependent H_2-receptor blocking action was investigated in guinea pig isolated atria.

The IT-066 molecule is composed of three structurally different parts: a tertiary amine and hydrophobic group, a polar group, and a connecting carbon chain. The compounds making up each part of the IT-066 molecule (Table 1) did not by themselves inhibit the positive chronotropic action induced by histamine.

Concerning the tertiary amine and hydrophobic group, compound **6** (Table 2), a metabolite of IT-066, had no effect on the positive chronotropic action induced by histamine. The H_2-receptor blocking activity of IT-066 was attenuated by the oxidation of nitrogen in the piperidine ring. The inability of compound **6** to antagonize against histamine-induced responses in guinea pig atria suggests that the reactivity of the nitrogen in the piperidine ring, the nucleophilicity of the lone pair, is necessary for the H_2-receptor blocking action of IT-066. However, the nitrogen in the pyridine ring of the IT-066 molecule may not be directly involved in the binding with the H_2-receptor, because compound **11** (Fig. 1) in which a pyridine ring is converted into a benzene ring, has a potent H_2-receptor blocking action, comparable to that of IT-066. These data suggest that the nitrogen in the piperidine ring plays a key

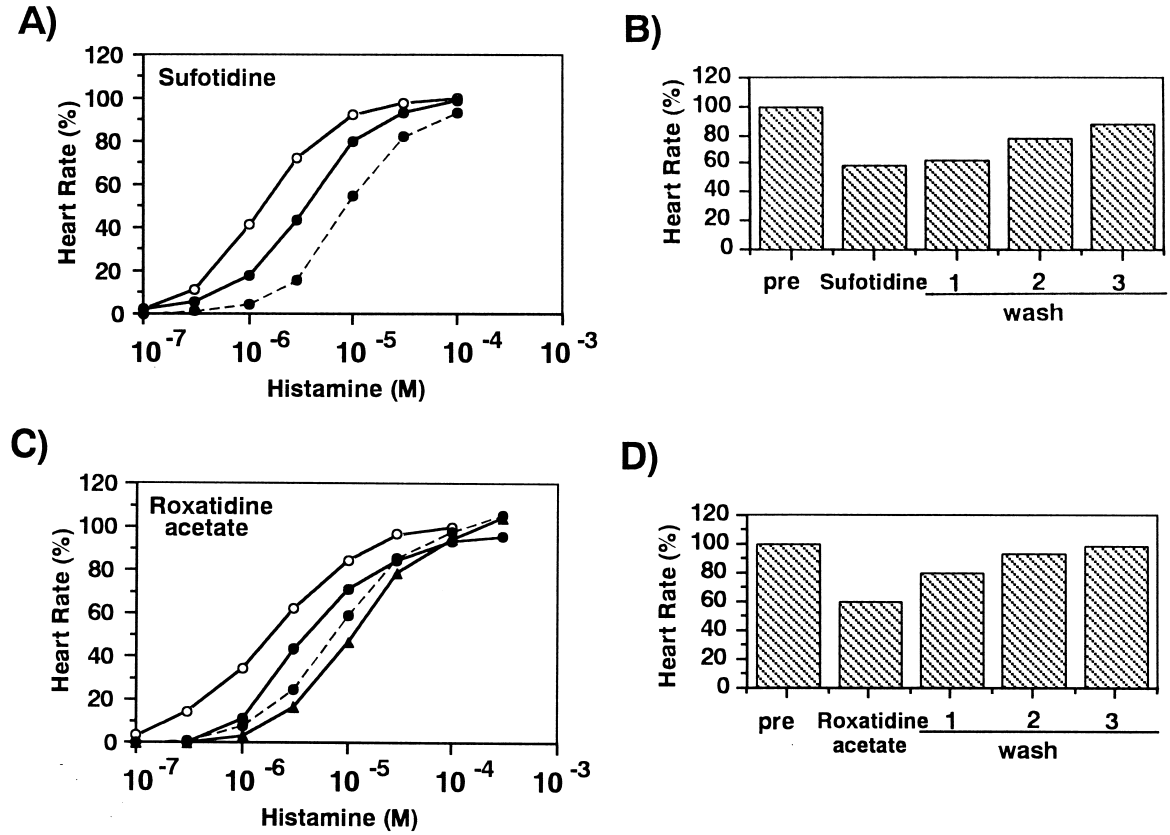


FIG. 5. Effects of sufotidine and roxatidine acetate on the positive chronotropic action induced by histamine. (A) Time-dependency of the inhibitory effect of sufotidine. Sufotidine (10^{-6} M) was incubated for 5 min (●, solid line) and 60 min (●, dashed line). The open circles (○) indicate the control response induced by a cumulative dose of histamine. (B) Reversibility of the inhibitory effect of sufotidine. Sufotidine (3×10^{-6} M) was incubated for 60 min. Reversibility was examined after washout of sufotidine from the bath. (C) Potency and time-dependent alteration of the inhibitory effect of roxatidine acetate. Roxatidine acetate was incubated for 5 min at 10^{-7} M (●, solid line) and 3×10^{-7} M (▲, solid line) and for 60 min at 10^{-7} M (●, dashed line). The open circles (○) indicate the control response induced by a cumulative dose of histamine. (D) Reversibility of the inhibitory effect of roxatidine acetate. Roxatidine acetate (10^{-7} M) was incubated for 60 min. Reversibility was examined after washout of roxatidine acetate from the bath. Each value is the mean obtained from 4 experiments.

role in the interaction of IT-066 with the H_2-receptor, although the pyridine ring of IT-066 could be replaced with a benzene ring to maintain the time-dependent and insurmountable blockade of the H_2-receptor.

The polar group of IT-066 also plays an important role in the binding with the H_2-receptor, because the H_2-receptor blocking action of IT-066 was abolished completely by hydrolysis of the polar group (Table 2). The compounds whose polar group is 3,4-diamino-3-cyclobutene-1,2-dione, IT-066 and BMY25368, showed an irreversible and time-dependent mode of H_2-receptor blocking action. On the other hand, sufotidine and roxatidine acetate, whose polar group differs from that of IT-066 and BMY25368, exhibited a reversible H_2-receptor antagonizing effect, and the effect was not time dependent. These data suggest that, in the IT-066 molecule, the nitrogen in the polar group and the nitrogen in the piperidine ring play an important role in the binding with H_2-receptors, and that the polar group determines the irreversible and time-dependent mode of action. Some variation of a polar group may be permitted in the interaction with the H_2-receptor, but specifically the potency and degree of time-dependency and reversibility depend on the structure of the polar group.

The connecting carbon chain of IT-066 is 1,4-disubstituted *cis*-2-butene. Compounds that have a 4-membered carbon chain, butane (compound **8**), *trans*-2-butene (compound **9**), and 2-butyne (compound **10**), clearly demonstrated H_2-receptor blocking action, but the effect was reversible. Alternatively, the antagonism of BMY25368, which had trimethylene as a connecting carbon chain, was similar to that of IT-066, though the potency and the time-dependency were slightly reduced. Differences in the conformation of a connecting carbon chain will produce differences in the shape of the molecule. For example, *cis* and *trans*, or a double bond and a triple bond give different conformations to a whole molecule. These conformational differences may possibly be involved in the difference in interaction with the receptor. And then it is supposed that the distance between the nitrogen in the piperidine ring and the nitrogen in the polar group has some bearing on the interaction with H_2-receptors and the mode of action, since the two nitrogens are necessary for the H_2-receptor block-

ing action of IT-066. These results suggest that the connecting carbon chain, probably the conformation due to the connecting carbon chain, is important for determining the potency and mode of the H_2-receptor blocking action of IT-066.

Recently, Gantz *et al.* [15, 16] succeeded in cloning the gene encoding the H_2-receptor and studied the interaction of histamine with the H_2-receptor at a molecular level. They proposed a model of interaction between histamine and the H_2-receptor. The H_2-receptor belongs to a broad class of seven transmembrane G-protein-linked receptors. An aspartic acid residue (Asp^{98}) of the third transmembrane domain and an aspartic acid residue (Asp^{186}) and a threonine residue (Thr^{190}) of the fifth transmembrane domain are key amino acids in histamine binding, and are essential for histamine binding and action, the H_2 selectivity, and the kinetics. In the case of IT-066, the piperidine ring and the polar group are involved in the H_2-receptor blocking action. Additionally, the molecular conformation determined by the connecting carbon chain relates to the kinetics of IT-066, and to the time-dependent and insurmountable H_2-receptor antagonism. It is conjectured that two nitrogens in the piperidine ring and the polar group would bind to amino acid residues of the H_2-receptor. To further clarify the interaction, we are now investigating [^{3}H]IT-066 binding using the expressed H_2-receptor.

Considering the data of the present study, it is suggested that the nitrogen in the piperidine ring and the nitrogen in the polar group, 3,4-diamino-3-cyclobutene-1,2-dione, of IT-066 are essential to the interaction of IT-066 with the H_2-receptor. In addition, the polar group of IT-066, 3,4-diamino-3-cyclobutene-1,2-dione, is crucial for determining the irreversibility and time-dependency of the H_2-receptor blocking action of IT-066. Furthermore, the study suggested that both the distance between the two nitrogens that are essential for the interaction with the H_2-receptor and the molecular configuration determined by the structure of a connecting carbon chain were important in the insurmountable interaction of IT-066 with the H_2-receptor.

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