Comparative Effects of Cimetidine and Famotidine on the Vagally Stimulated Acid Secretion in the Isolated Mouse Whole Stomach

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ABSTRACT—We investigated the effects of cimetidine and famotidine on the acid secretory response to electrical vagal stimulation, bethanechol and histamine in the isolated mouse whole stomach preparation. The acid secretion elicited by electrical vagal stimulation at the position of the esophagus (10 Hz, 0.3 msec, 10 V for 5 min) was reproducible by repeated stimulation in each preparation, and it was abolished by tetrodotoxin, atropine and hexamethonium. This vagally stimulated acid secretion was abolished by cimetidine (3 mM), while it was only partly inhibited by famotidine (10–100 μM). Histamine (100 μM)-induced acid secretion was inhibited by cimetidine and famotidine, and the doses of these drugs required for complete inhibition were 3 mM and 10 μM, respectively. In contrast, bethanechol (10 μM)-induced acid secretion was slightly reduced by famotidine (1–100 μM), but markedly reduced by cimetidine (3 mM). In the guinea pig ileum, millimolar concentrations of cimetidine and famotidine shifted the dose-response curve of the contractile response to acetylcholine rightward. These findings suggest that the inhibitory effect of cimetidine on the vagally stimulated or bethanechol-induced acid secretion is elicited at least partly through mechanisms different from H_2-antagonism.

Keywords: Gastric acid secretion, Histamine, Vagus nerve, Histamine H_2-antagonist, Stomach (mouse isolated)

Physiological roles of endogenous histamine in mediating vagally stimulated acid secretion have been widely studied. In many in vivo (1, 2) and in vitro (3–6) studies, histamine H_2-antagonists such as cimetidine and metiamide have been shown to reduce acid secretion stimulated by gastrin-like peptides and cholinergics as well as histamine. These findings support a possibility that histamine is the final common mediator for the acid secretion elicited by all secretagogues (7). On the other hand, the existence of muscarinic receptors on parietal cells has been demonstrated by ^{3}H-QNB binding (8) and ^{14}C-aminopyrine accumulation studies (9) with secretagogues. By summarizing the above findings, Black and Shankley (10) gave support to the so-called "transmission hypothesis" for acid secretory mechanism. According to this theory, muscarinic receptors exist both on the parietal cells and the histamine-storing cells, but the receptors that contribute to acid secretion are mainly on the histamine-storing cells. Recently, Krommer et al. (11) also supported this theory, demonstrating that the acid

secretion induced by electrical field stimulation in isolated mouse stomach was completely abolished by an H_2-antagonist such as cimetidine and lupitidine, and compound 48/80 also inhibited the secretory response. However, cimetidine has some other effects such as anticholinesterase activity, sympathetic ganglion blocking and neuromuscular blocking activities (12). Furthermore, Ishikawa et al. (13) reported that cimetidine (1–100 μM) inhibited the acid secretion induced by forskolin, which directly activates adenylate cyclase to increase intracellular cyclic AMP. The dosage of cimetidine employed is not so high in comparison with that used for H_2-antagonism (9) or for the inhibitory effect on the acid secretion induced by electrical field stimulation (11). Famotidine is at least 50 times more potent in H_2-receptor antagonism than cimetidine (14), but it remains to be clarified whether or not famotidine inhibits cholinergic or nerve-mediated acid secretion in vitro. Thus, we investigated the effect of cimetidine and famotidine on the vagally mediated acid secretion using an isolated mouse whole stomach prepara-

tion. For this purpose, we devised a novel method to induce acid secretion by electrical vagal stimulation at the position of the esophagus. The electrical vagal stimulation is thought to be more physiological than the electrical field stimulation of the stomach undertaken by previous investigators (3, 11).

MATERIALS AND METHODS

Acid secretion in the isolated mouse whole stomach

Male mice of the ddY strain (16–28 g), given free access to food and water, were used. Under urethane (18 mg/10 g, i.p.) anesthesia, the stomach was exposed, and a small incision was made at the fundic portion; then the lumen was flushed with warm mucosal solution (15 ml), and a dual cannula (internal; silicon $\phi=0.5$ mm, external; polyethylene $\phi=3$ mm) was inserted into the incision. After the ligation of the pylorus and esophagus, the stomach was rapidly dissected out and placed in a 20-ml organ bath containing serosal solution, which was kept at 37°C and gassed with 95% $O_2+5\%$ CO_2. The stomach lumen was perfused through the cannula at the rate of 1 ml/min with oxygenated mucosal solution. The effluent perfusate from the stomach was introduced to a titrating bath at a level of 20 cm above the stomach level to distend the organ. Acid output was measured by titrating hydrogen ion with 1/500 N NaOH to the end point of pH 5.0 (initial pH of mucosal solution) by an automatic titrator (Toa Electronics Co., HM-5ES, HSM-10A, Tokyo). The digital pulse (2 μl/pulse) from the titrator was sent to a personal computer (Fujitsu, FM77, Tokyo) equipped with a pulse counter (developed by our laboratory). The composition of the nutrient solution was: serosal: 128 mM NaCl, 4.8 mM KCl, 1.2 mM $MgSO_4$, 1.3 mM $CaCl_2$, 30 mM glucose and 10 mM HEPES (pH 7.0 adjusted with 10 N NaOH (0.8 ml/l) and gassed with 95% O_2 + 5% CO_2); mucosal: 137 mM NaCl, 4.8 mM KCl, 1.2 mM $MgSO_4$, 1.3 mM $CaCl_2$, 30 mM glucose (pH 5.0 adjusted with 0.1 N HCl and gassed with 100% O_2).

Vagal stimulation and experimental designs

Vagal stimulation was applied via a pair of platinum electrodes (wire diam., 0.25 mm; ring diam., 1.2 mm; and a distance of 1.5 mm) at the position of the lower esophagus. Square-wave pulses were delivered from an electronic stimulator (Nihon Kohden, SEN-7203, Tokyo). After equilibration for 20 min, the 1st vagal stimulation was applied (10 Hz, 0.1 msec, 10 V, for 5 min). When acid secretion was initiated within 10 min after the onset of stimulation, the preparation was thought to be suitable for the experiment. At 30 min after the onset of the 1st stimulation, the 2nd vagal stimulation was applied (10 Hz, 0.3 msec, 10 V, for 5 min). The response to the 2nd stimulation was

used as a control response for the following treatments. At 30 min after the 2nd stimulation, the 3rd vagal stimulation was applied. Test drugs were applied at 10 min before the onset of the 3rd stimulation.

To examine the effects of H_2-antagonists on acid stimulation elicited by histamine (100 μM) and bethanechol (10 μM), the secretagogues were applied after 30 min of the 2nd vagal stimulation. H_2-antagonists were applied at 10 min before the secretagogue treatment. The total volume of drug solution added to the 20-ml organ bath did not exceed 0.2 ml.

Evaluation of acid secretory response

The acid secretory response to vagal stimulation or secretagogues was expressed as a net increase in acid output over the control acid output for 10 min before the start of stimulation. The effect of various drugs on vagally stimulated acid secretion was calculated as the ratio of the 3rd to the 2nd stimulation and expressed as a percentage. Histamine or bethanechol induced acid secretion (for 30 min) was expressed as a percentage of the response to the 2nd vagal stimulation, since the correlation of acid secretion between the 2nd vagal stimulation and the following histamine- or bethanechol-induced response in each preparation was statistically significant (see Results). All data were expressed as the mean±S.E.M. Statistical analysis was performed by Student's t-test, and statistical significance was evaluated at the P value not more than 0.05.

Contractile response in the guinea pig longitudinal muscle of the ileum

Male Hartley strain guinea pigs (300–390 g) were used. After these animals were killed by a blow on the head, the ileum was isolated. The longitudinal muscle was separated from the circular muscle and the ileal mucosa. A piece of strip, about 1.5 cm in length, was suspended in a 5-ml organ bath containing modified Tyrode solution at 32°C and continuously aerated. The contractile responses were isotonically recorded (under a resting tension of 0.5 g) using a displacement transducer (San-ei, 45347, Tokyo), a DC-strain amplifier (San-ei, 6M96) and a DC-recorder (Hitachi, 561-1003, Tokyo). The composition of modified Tyrode solution was: 137 mM NaCl, 2.7 mM KCl, 1.0 mM $MgSO_4$, 1.8 mM $CaCl_2$, 5.6 mM glucose and 10 mM HEPES (pH 7.4 adjusted with 10 N NaOH). After an equilibration period of 30 min, acetylcholine (10^{-8} to 3×10^{-6} M) was cumulatively added into the organ bath (control response). After the determination of control responses, the strips were treated with an H_2-antagonist (cimetidine or famotidine); and 10 min later, the dose-response curves of acetylcholine were obtained in the presence of the antagonist. These procedures were repeat-

ed with high concentrations (2- to 10-fold) of the antagonist in the same preparation. Contractile responses to acetylcholine were assessed as a percentage of the control response $(3 \times 10^{-6}$ M), and the data were expressed as the mean $\pm$S.E.M. Since the both H_2-antagonists shifted the dose-response curves of acetylcholine rightward, Schild plots were made, and pA_2 values and slopes of the linear regression were calculated.

Drugs

Drugs used were as follows: tetrodotoxin (Sankyo, Tokyo); atropine sulfate and histamine dihydrochloride (Nacalai Tesque, Inc., Kyoto); hexamethonium dichloride (Wako Pure Chemical Industries, Osaka), bethanechol chloride and cimetidine (Sigma Chemical Co., St. Louis, MO, USA); acetylcholine chloride (Ovisot[®], Daiichi Seiyaku Co., Ltd., Tokyo); and famotidine (Yamanouchi Pharmaceutical Co., Ltd., Tokyo). Cimetidine and famotidine were prepared freshly in nutrient solution after being dissolved with a small volume of 0.1 N HCl. All other drugs were dissolved in saline.

RESULTS

Acid secretion in the isolated mouse whole stomach

Stable basal acid output $(50-100$ nEq/min) was established 10 min after setting up the preparation, and this lasted at least for 3 hr. This basal secretion was completely abolished by 30 mM sodium thiocyanate. A typical pattern of vagally stimulated acid secretion is shown in Fig. 1A. A measurable change in acid secretion began in 4 min after the onset of the 1st vagal stimulation; there was a dead volume of about 3 ml within the collecting tubing from the stomach to the pH electrode. The peak acid secretion $(250-400$ nEq/min) took place after $8-10$ min (i.e., $4-6$ min after the measurable change), then gradually decreased, and disappeared in about 15 min after the stop of vagal stimulation. The response to the 2nd stimulation was greater than the 1st response, and the 3rd response was almost the same as the 2nd response. The response to the 3rd vagal stimulation was not significantly different from the 2nd response (the total increase in acid output by the 2nd or the 3rd vagal stimulation was 1640 ± 196 or 1522 ± 209 ΔnEq/stim., respectively, and the 3rd response was $91.9 \pm 2.9\%$ of the 2nd response; $n = 5$, Fig. 2). The acid secretion by vagal stimulation was completely abolished in the presence of tetrodotoxin (0.3 μM), and it was recovered by washing (Figs. 1B and 2). Furthermore, atropine and hexamethonium also completely inhibited the vagally stimulated acid secretion (Fig. 2).

The effects of histamine H_2-antagonists on vagally stimulated acid secretion are shown in Fig. 3. Cimetidine
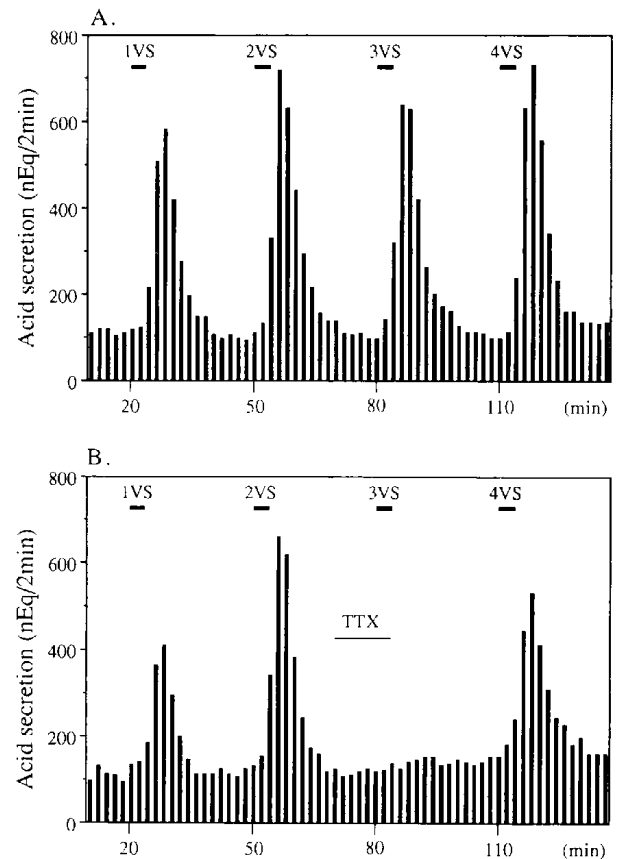

Fig. 1. Typical patterns of acid secretory response to electrical vagus nerve stimulation in the isolated mouse whole stomach. Each column represents the acid output for 2 min. (A) The electrical stimulation was performed at the position of the lower esophagus using a pair of platinum electrodes, and the conditions were as follows: 10 Hz, 0.3 msec (only the 1st vagal stimulation (1VS): 0.1 msec), 10 V for 5 min, and 25 min of the stimulation interval. (B) Effect of tetrodotoxin (TTX) and recovery of acid secretion by washing. TTX (0.3 μM) was applied in serosal solution 10 min before the 3rd vagal stimulation (3VS) (as indicated by the horizontal bar). After the end of 3VS, the preparation was immediately washed by normal serosal solution, and then the 4th vagal stimulation (4VS) was started.

$(30-3000$ μM) inhibited the vagally stimulated acid secretion in a concentration-dependent manner, and at a dose of 3000 μM, complete inhibition was observed. Famotidine $(10-100$ μM) inhibited the acid secretory response by about 30%; the inhibition seemed to be maximal.

The peak acid secretion induced by 100 μM histamine was developed at $10-15$ min after histamine addition. In most cases, the peak level was maintained for $10-20$ min, and then it reduced gradually. In some cases, the maximal level abruptly fell in 30 to 40 min after histamine addition. Based on these findings, we assessed the total acid secretion for 30 min after histamine application; the acid secretion induced by histamine was 6766 ± 512 ΔnEq/30 min $(n = 20)$. The acid secretory response to 10 μM
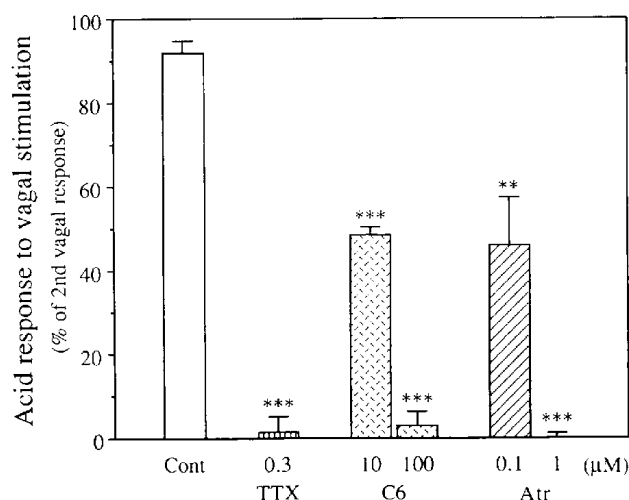
Fig. 2. Effect of autonomic drugs on the vagally stimulated acid secretion in the isolated mouse whole stomach. Each column represents the acid secretion elicited by the 3rd vagal stimulation (3VS) and is assessed as a percent of the 2nd vagal stimulation (2VS). Tetrodotoxin (TTX, 0.3 μM), hexamethonium (C6, 10-100 μM) or atropine (Atr, 0.1-1 μM) was applied 10 min before 3VS. Control (Cont) means the group without drug treatment. Results represent the mean±S.E.M. of 4-5 experiments. **P<0.01, ***P<0.001, significantly different from the respective 2VS response. The averaged increases in acid secretion by 2VS in all groups were 1640-2585 ΔnEq/stimulation.
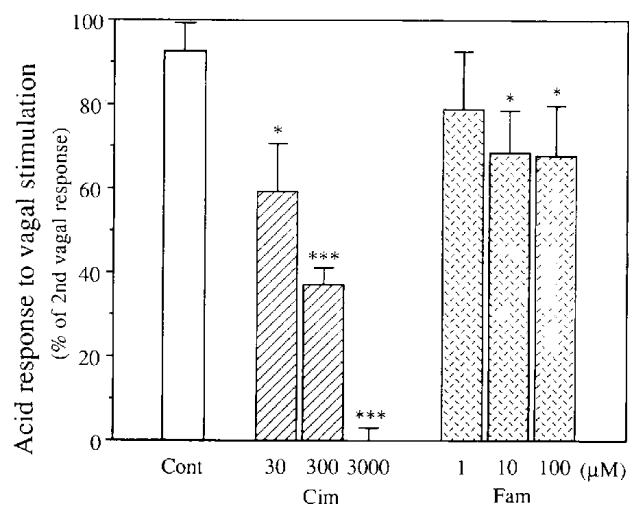
Fig. 3. Effect of H₂-antagonists on the vagally stimulated acid secretion in the isolated mouse whole stomach. Each column represents the acid secretion elicited by the 3rd vagal stimulation (3VS) and is assessed as a percent of the 2nd vagal stimulation (2VS). Cimetidine (Cim, 30-3000 μM) or famotidine (Fam, 1-100 μM) was applied 10 min before 3VS. Control (Cont) means the group without any antagonist. Results represent the mean±S.E.M. of 4-5 experiments. *P<0.05, ***P<0.001, significantly different from the respective 2VS response. The averaged increases in acid secretion by 2VS in all groups were 1245-2086 ΔnEq/stimulation.
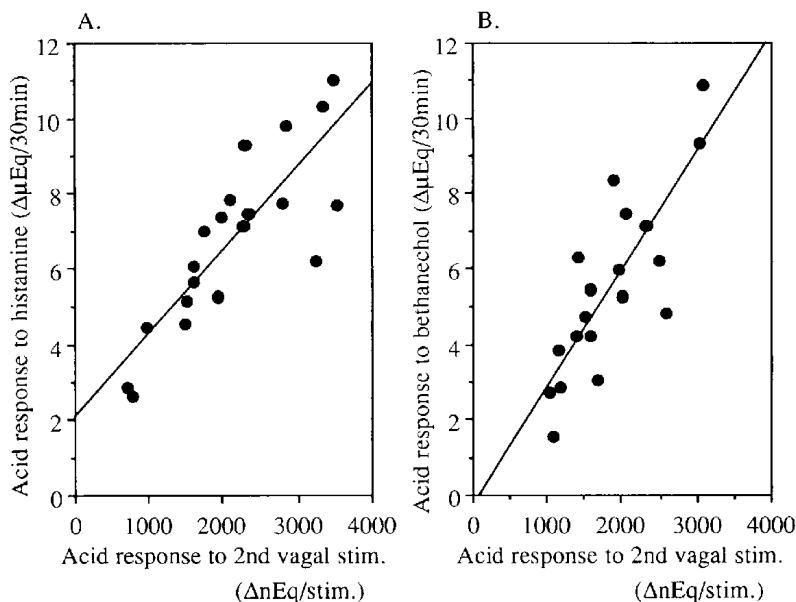

Fig. 4. Correlation of acid secretion between electrical vagal stimulation and secretagogue treatments in the isolated mouse whole stomach. Each point represents the acid secretion elicited by the 2nd vagal stimulation (2VS), by 100 μM histamine (A) or by 10 μM bethanechol (B) for 30 min in the same preparation. A statistically significant relationship was obtained between 2VS and the histamine response (r=0.834, P<0.001 with 20 experiments) or the bethanechol response (r=0.815, P<0.001 with 19 experiments).
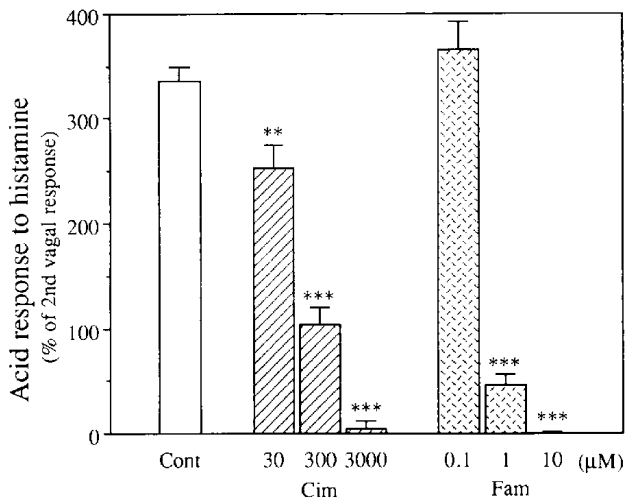
Fig. 5. Effect of H_2-antagonists on the histamine induced acid secretion in the isolated mouse whole stomach. Each column represents the acid secretion elicited by histamine for 30 min and is assessed as a percent of the 2nd vagal stimulation (2VS). Cimetidine (Cim, 30-3000 μM) or famotidine (Fam, 0.1-10 μM) was applied 10 min before 100 μM histamine addition. Control (Cont) means the group without any antagonist. Results represent the mean±S.E.M. of 4-8 experiments. **P<0.01, ***P<0.001, significantly different from the control. The averaged increases in acid secretion by 2VS in all groups were 1355-2403 ΔnEq/stimulation.
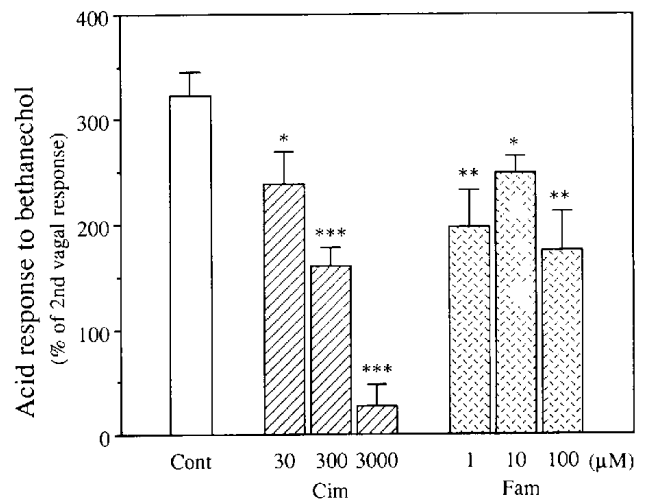
Fig. 6. Effect of H_2-antagonists on the bethanechol induced acid secretion in the isolated mouse whole stomach. Each column represents the acid secretion elicited by bethanechol for 30 min and is assessed as a percent of the 2nd vagal stimulation (2VS). Cimetidine (Cim, 30-3000 μM) or famotidine (Fam, 1-100 μM) was applied 10 min before 10 μM bethanechol addition. Control (Cont) means the group without any antagonist. Results represent the mean±S.E.M. of 5-8 experiments. *P<0.05, **P<0.01, ***P<0.001, significantly different from the control. The averaged increases in acid secretion by 2VS in all groups were 1166-2117 ΔnEq/stimulation.

bethanechol for 30 min resembled that to histamine; the acid secretion was 5486±549 ΔnEq/30 min (n=19). On the other hand, it was found that there existed a marked difference in the secretory response to secretagogues among mouse preparations. For this reason, each acid secretory response was relatively evaluated on the basis of the vagal response as a standard, instead of averaging the acid output. In brief, the correlation of acid secretion between the 2nd vagal stimulation and the following histamine or bethanechol response in each preparation was statistically significant (Fig. 4). Accordingly, acid secretory responses to secretagogues were expressed as a percentage of the 2nd vagal response; the secretory rates of histamine and bethanechol were 333.9±14.3% and 295.2±18.0%, respectively.

The effects of H_2-antagonists on histamine induced acid secretion are shown in Fig. 5. Cimetidine (30-3000 μM) and famotidine (1-10 μM) inhibited the 100 μM histamine-induced acid secretion in a concentration-dependent manner, and the doses required for the complete inhibition were 3000 μM and 10 μM, respectively. On the other hand, both cimetidine and famotidine significantly reduced the 10 μM bethanechol-induced acid secretion (Fig. 6). The inhibitory pattern of cimetidine was concentration-dependent and the maximal inhibition by 3000

μM cimetidine was approximately 90%, while that of famotidine (1-100 μM) was not concentration-dependent, and the maximal inhibition by 1-100 μM famotidine ranged between 30% and 50%.

Effects of cimetidine and famotidine on the contractile responses to acetylcholine in the guinea pig ileum

Cimetidine (<300 μM) did not affect any contractile response to acetylcholine (data not shown). In the presence of higher concentrations of cimetidine (1-6 mM), the dose-response curves of acetylcholine were shifted rightward, and the maximal responses were rather augmented (Fig. 7A). The pA_2 value was 3.20±0.05 (n=5), but the slope factor (1.51±0.08) was significantly different from unity. In the case of famotidine at concentrations of 10-100 μM, there was almost no effect on the contractile responses to acetylcholine. At 1 mM famotidine, however, the dose-response curve of acetylcholine was shifted rightward without any change in the maximal response (Fig. 7B). The pA_2 value was 4.00±0.09 (n=5), and the slope factor (0.89±0.08) was not significantly different from unity.
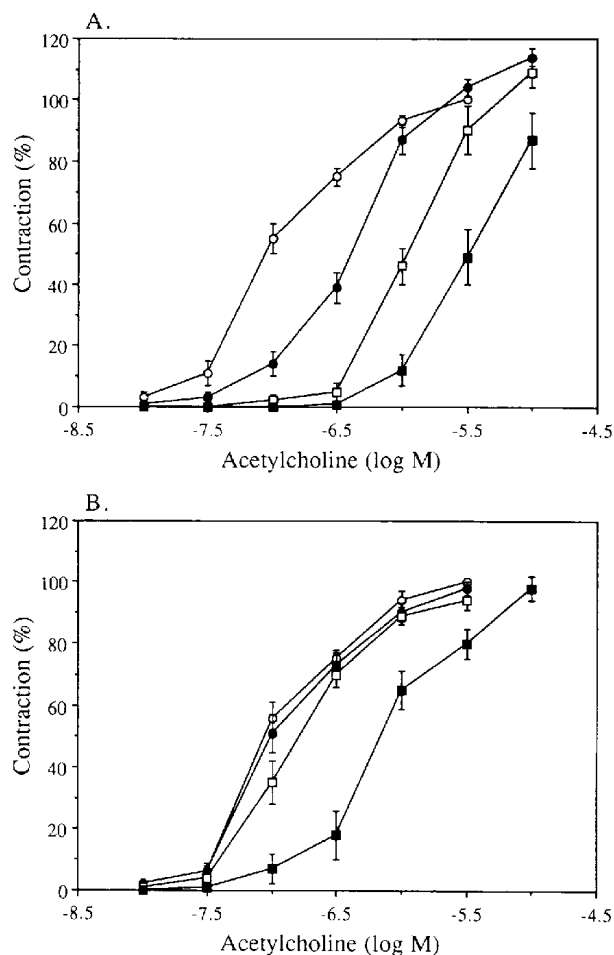
Fig. 7. Effects of cimetidine and famotidine on the cumulative dose-response curve for acetylcholine induced contraction in the guinea pig longitudinal ileum. Contraction (%) is assessed as a percent of the contractile response to acetylcholine (3 μM) alone. A: Cimetidine was treated 10 min before acetylcholine application. Control response (○); cimetidine, 1 mM (●), 3 mM (□) and 6 mM (■). B: Famotidine was treated 10 min before acetylcholine application. Control response (○); famotidine, 10 μM (●), 100 μM (□) and 1 mM (■). Results represent the mean±S.E.M. of 5 experiments.

DISCUSSION

Electrical vagal stimulation was conducted at the position of the lower esophagus. The acid secretory response was reproducible to repeated stimulation in each preparation. The conditions of vagal stimulation (10 Hz, 0.3 msec, 10 V, for 5 min) were relatively similar to those reported in electrical field stimulation (3). The electrical stimulation described here was considered to selectively activate the parasympathetic preganglionic fibers, because of the complete blockade by hexamethonium, atropine or tetrodotoxin. In contrast, the secretory response to electrical field stimulation was not completely blocked

by hexamethonium at 100 μM (3, 11); the reduction was approximately 80%. It is supposed that the acid secretory response to electrical field stimulation occurs through more complicated neuronal mediation, since the stimulation is capable of acting on all kinds of neurons including the vagus nerves and sympathetic nerves, regardless of the pre- or post-synaptic fibers.

In the present study, there was quite a large difference in the histamine- or bethanechol-stimulated acid secretion among individual mouse preparations. At present, we do not know the cause of this difference; it was not correlated to the body weight of mice, the level of basal acid output or the season of the experiments. However, the acid secretory response to histamine or bethanechol could be normalized by setting the 2nd vagal stimulation as an internal standard in each preparation. The normalized data indicate a similarity in relative sensitivity to secretagogues between preparations, and thus validate that our experimental procedures are suitable for evaluating the effects of drugs on acid secretion in the isolated whole stomach.

Of the most interest was our demonstration that inhibition of vagally stimulated or bethanechol-induced acid secretion by famotidine was slight to moderate even at a dose of 100 μM. In contrast, the histamine induced acid secretion was completely blocked by famotidine at a dose of 10 μM. From the present results with famotidine, it is deduced that histamine H_2-receptor mediation is not primarily responsible for cholinergic stimulation of acid secretion. On the other hand, cimetidine concentration-dependently blocked the vagally stimulated, bethanechol-induced or histamine-induced gastric acid secretion. In previous studies by other investigators (3, 11), cimetidine and metiamide completely inhibited the acid secretion elicited by electrical field stimulation as well as that induced by histamine. Furthermore, metiamide also inhibited the acid secretion stimulated by carbachol (3). These findings on cimetidine and metiamide seemingly support the histamine final common mediator theory (7) and transmission hypothesis (10). As a result, the deduction from the results using cimetidine and metiamide is contradictory to that from the present results using famotidine.

The two contradictory deductions were considered to result from some mechanisms of the cimetidine effect that differ from H_2-antagonism at high concentrations. In fact, the blocking effect of cimetidine on the carbachol induced contraction of the guinea pig ileum was reported (15). According to our results with the guinea pig longitudinal ileum preparation, cimetidine and famotidine, at millimolar concentrations, shifted the dose-response curve of the acetylcholine induced contraction rightward without any decrease in the maximal response. In the case of the effect of cimetidine, the Schild slope was significant-

ly different from unity, and thus the antagonizing effect of cimetidine on muscarinic receptors was not completely competitive in nature, suggesting some non-competitive mechanisms were also involved (12). On the other hand, the acid response to vagal stimulation or bethanechol was completely inhibited by cimetidine at a concentration of 3 mM.

Black and Shankley (10) reported that tiotidine inhibited the acid secretion induced by electrical field stimulation and that the inhibition was attenuated in the presence of eserine, because acetylcholine released from nerve endings readily diffuses close to the parietal cells when cholinesterase is blocked. If famotidine has anticholinesterase activity, the acid secretion by vagal stimulation might be unchanged by the drug. According to our experiments with guinea pig ileum, famotidine at doses of $10-100$ μM did not shift acetylcholine induced contraction leftward, suggesting a lack of anticholinesterase activity, like eserine.

Several previous findings lead us to consider the possibility of vagus-mediated gastrin release which in turn elicits histamine release. Håkanson and Liedberg (16) reported that insulin could not reduce the gastric histamine content in antrectomized rats, probably because of the lack of gastrin, but stimulated acid secretion. In the totally isolated, vascularly perfused rat stomach, Sandvik et al. (17) showed that the electrical vagal stimulation did not significantly change the histamine output in the vascular effluent in spite of marked stimulation of acid secretion. In contrast, gastrin was found to increase the histamine output, and the acid secretion induced by gastrin was significantly correlated to the histamine release (18, 19). These reports suggested that histamine may be released by vagal excitation via the release of gastrin. In the isolated mouse whole stomach, Schubert et al. (20) reported that the CCK_B/gastrin antagonist L365,260 (1 μM) had no significant effect on either basal or electrically field-stimulated acid secretion. Different from in vivo experiments, there is little or no possibility in our preparations that by vagal stimulation, blood-borne gastrin moves from the antrum to the oxyntic area where the histamine-storing cells are adjacently located.

In conclusion, the present findings on the effects of famotidine suggest that vagally stimulated acid secretion is mainly due to mediation of the muscarinic receptors on parietal cells. In addition, the inhibitory effect of cimetidine on the acid secretion induced by cholinergic stimulation may be, at least partly, due to some mechanism (i.e., anticholinergic) different from H_2-antagonism.

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