Differences in the Antisecretory Actions of the Proton Pump Inhibitor AG-1749 (Lansoprazole) and the Histamine H₂-Receptor Antagonist Famotidine in Rats and Dogs

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ABSTRACT—Antisecretory effects of a substituted benzimidazole, ($\pm$)-2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl]sulfinyl]-1H-benzimidazole (AG-1749) were compared with those of a histamine H₂-receptor antagonist, famotidine. AG-1749 inhibited acid formation regardless of the stimulant in isolated canine parietal cells, while famotidine inhibited the histamine-stimulated acid formation selectively. In pylorus-ligated rats, AG-1749 suppressed basal acid secretion, histamine-, bethanechol-, pentagastrin-, 2-deoxy-D-glucose- and stress (restraint and water-immersion)-induced acid secretion; ID₅₀ values were 1.0–6.0 mg/kg. On the other hand, famotidine only partially inhibited the acid secretion induced by 2-deoxy-D-glucose or stress, although it suppressed the acid secretion stimulated by other secretagogues several times more potently than AG-1749. The antisecretory effect of AG-1749 lasted longer than that of famotidine, especially in the case of bethanechol-stimulated acid secretion. In Heidenhain pouch dogs, both AG-1749 and famotidine potently inhibited histamine-, bethanechol-, pentagastrin- and peptone meal-stimulated acid secretion, but the inhibitory effect of famotidine was short-lived in the case of bethanechol- and pentagastrin-stimulated acid secretion. These results suggest that AG-1749 persistently inhibits acid secretion induced by both peripheral and central stimuli and suggest that the antisecretory effect of famotidine depends on the nature of the stimuli.

Gastric acid secretion is stimulated by histamine, acetylcholine or gastrin via their respective receptors on the parietal cells (1). The stimulation causes an increase in second messengers such as cAMP and Ca²⁺ in the cells (2, 3) which is followed by the activation of ion channels (4) and the transformation of the intracellular membrane (5–7) which are required for the activation of the proton pump. Substituted benzimidazoles such as AG-1749 and omeprazole suppress acid secretion by in-

hibiting (H⁺ + K⁺)-ATPase (8–11). On the other hand, histamine H₂-receptor antagonists such as cimetidine and ranitidine specifically inhibit histamine-stimulated acid secretion in isolated parietal cells but inhibit acid secretion stimulated by various secretagogues in vivo (12). These results suggest that endogenous histamine is actively involved in acid secretion stimulated by other secretagogues (1). In this study, to elucidate the differences in the antisecretory actions of proton pump inhibitors

and histamine H_2 -receptor antagonists, the effects of a potent histamine H_2 -receptor antagonist, famotidine, on the acid secretion induced by peripherally or centrally acting gastric stimulants were compared with those of AG-1749.

MATERIALS AND METHODS

Isolation of parietal cells

Mongrel dogs of either sex were exsanguinated under pentobarbital anesthesia and their stomachs were removed. The fundic mucosa was bluntly dissected free from the underlying muscle layer. The tissue was minced with scissors and then exposed to collagenase (0.25 mg/ml) and EDTA to isolate the cells, according to the method of Soll (1). A crude cell suspension was layered at the top of a preformed 50% Percoll gradient, and the preparation was then centrifuged at 1,000 rpm for 25 min according to the method of Fryklund et al. (13) with slight modifications. The cell fraction with a density below 1.05 was collected and washed 2 or 3 times to remove Percoll and then suspended in Ca^{2+} -free Hanks' balanced salt solution (BSS) containing 1 mM EDTA, 10 mM HEPES and 0.2% bovine serum albumin. After being stained with Turk solution, parietal cells were identified by their large size and concentric nuclei as observed under a light microscope ($\times 200$). Cell viability was tested by the trypan blue exclusion technique. The concentration of parietal cells was adjusted to $\sim 2 \times 10^6$ cells/ml, and the cell suspension was used as the parietal cell-rich fraction (purity, 50–70%; viability, $\sim 70\%$).

Measurement of acid formation

Acid formation was measured as the accumulation of the weak base [^{14}C]aminopyrine (14). The parietal cell-rich fraction ($\sim 4 \times 10^5$ cells/200 μ l) was suspended in 2 ml of Earl's BSS containing 0.2 μ Ci [^{14}C]aminopyrine, 25 mM HEPES (pH 7.4), 0.2% bovine serum albumin (w/v), and various concentrations of each test compound, and then various secreta-

gogues were added. The reaction mixture was incubated at 37°C for 25 min under an atmosphere of 95% O_2 and 5% CO_2 . Three aliquots (0.5 ml) of cell suspension were layered over 1 ml of Hanks' BSS in 1.5 ml-microfuge tubes and centrifuged at $9,000 \times g$ for 1 min. The supernatant was discarded, and the tip of each microfuge tubes was cut off. The cell pellets were then digested with 0.5 ml of tissue solubilizer (NCS[®]). After a liquid scintillator was added, radioactivity was counted using a scintillation counter. The radioactivity in the cell pellet in the presence of 0.1 mM dinitrophenol was subtracted from all data to correct for trapped [^{14}C]aminopyrine. The ratio of the concentration of [^{14}C]aminopyrine accumulated in the parietal cells to that in the medium was used to evaluate the acid forming activity and was designated as the aminopyrine ratio (AP ratio). The AP ratio was determined by the equation:

$$AP \text{ ratio} = R_p / \text{parietal cell volume} / R_m$$

where R_p is the radioactivity accumulated in the cell pellet, R_m is the radioactivity per milliliter of incubation medium, and the parietal cell volume is the product of the number of parietal cells and the cell volume. The cell volume used was $3.1 \times 10^{-9} \text{ cm}^3$, according to Soll (15).

Gastric secretion in pylorus ligated rats

Seven week-old male Jcl: Sprague-Dawley rats weighing 190 to 230 g were used. The animals were fasted for 24 hr but had free access to water.

Basal secretion: The pylorus was ligated under light ether anesthesia, and the abdomen was then closed by suturing. A drug or the vehicle was given i.d. just after the pylorus was ligated. Three hours later, an overdose of ether was given, the stomach was removed, and the gastric contents were collected and centrifuged at 3,000 rpm for 10 min. The volume of each sample was measured, and the acid concentration was determined by automatic titration to pH 7.0 with 0.1 N NaOH. The total acid output during the 3-hr period was then calculated.

Stress-induced acid secretion: Pylorus-ligated rats were given a drug or the vehicle i.d. Thirty minutes later, the animals were placed in a stress cage and were immersed vertically to the level of the xiphoid process in a water bath maintained at 23°C. The animals were subjected to this stress for 150 min. The gastric contents were then collected and the total acid output was determined. The acid secretion was increased by the restraint and water-immersion stress to more than twice that observed in the group without stress. The acid secretion in response to the stress was suppressed by vagotomy and was antagonized by atropine.

Gastric secretion in anesthetized rats

The pylorus was ligated under urethane (1.2 g/kg, i.p.) anesthesia, and the abdomen was then closed by suturing. A drug or the vehicle was given i.d. just after the pylorus was ligated. Thirty minutes later, histamine (30 mg/kg), bethanechol (3 mg/kg), pentagastrin (1 mg/kg) or 2-deoxy-D-glucose (200 mg/kg) was injected s.c. Three hours later, the stomach was removed, and the gastric contents were collected. Total acid secretion was then determined as described above.

In another experiment, the time course for the inhibitory effects of AG-1749 and famotidine on histamine- or bethanechol-stimulated acid secretion were studied. The acid secretory response to histamine (30 mg/kg, s.c.) or bethanechol (3 mg/kg, s.c.) was examined 0.5, 2, 4, 8 or 24 hr after the test drug had been given p.o. The pylorus was ligated under urethane anesthesia, and each secretagogue was administered just after the operation. Three hours later, the stomach was removed, and the total acid output was determined.

Gastric surface pH in conscious rats

Rats were given a drug or the vehicle p.o. One hour later, they were killed by CO₂ asphyxiation. The stomach was then removed, opened along the great curvature and mounted on a cork board. The pH of the gastric surface was measured using pH paper

(Merck).

In another experiment, the time course of the changes in surface pH after the administration of each drug at 10 mg/kg, p.o., were studied. The surface pH was determined 0.5, 1, 2, 4 or 8 hr after dosing.

Gastric secretion in Heidenhain pouch dogs

Male Beagle dogs prepared with Heidenhain pouches were fasted but had free access to water for 16 to 24 hr before each experiment. Histamine (30 µg/kg), pentagastrin (3 µg/kg) or bethanechol (100 µg/kg) was injected s.c. 0.5, 2, 4, 8, 24 or 48 hr after AG-1749, famotidine or vehicle was given p.o. The gastric juice was collected from the pouch continuously for 90 min after the administration of a secretagogue. The volume of gastric juice was measured, the acidity was determined by automatic titration (Radiometer TTA81) to pH 7.0 with 0.1 N NaOH, and the total acid output during the 90-min period was calculated. This experiment was done once a week. The secretory response after the administration of a drug was expressed as a percentage of the mean of the control acid output, which was measured 3 times by administering a secretagogue 1 week and 1 day before and 1 week after the drug was administered.

In another experiment, 100 ml of 20% peptone meal (Difco Laboratories) was given p.o. by gavage 30 min after dosing with the test agents. Gastric juice from the pouch was collected continuously for 180 min, and the total acid output was determined.

Drugs

The following drugs were used: Histamine·2HCl (Wako), bethanechol (Nihon Zenyaku), pentagastrin (ICI, Sumitomo), carbachol (Tokyo Kasei), dibutyl cyclic AMP (db-cAMP) (Boehringer), 2-deoxy-D-glucose (Wako), [¹⁴C]aminopyrine (New England Nuclear), 2,4-dinitrophenol (Wako), and collagenase (Sigma). AG-1749 and omeprazole were synthesized, and famotidine was extracted from Gaster[®] (Yamanouchi) and purified in the Chemistry Research Laboratory.

rics of our division. All other reagents were the best grade available and were used without further purification.

AG-1749, omeprazole and famotidine were dissolved in absolute methanol for the experiments *in vitro* and suspended in a 5% gum arabic solution for *p.o.* or *i.d.* administration.

Statistics

Data are expressed as means $\pm$ S.E. The statistical significance of differences among groups was determined by Dunnett's test or by Student's *t*-test. ID₅₀ (IC₅₀) values were calculated from the dose (concentration)-inhibition relationships by the method of least squares. Fiducial limits of the ID₅₀ (IC₅₀) values were calculated according to Fieller's theorem (16).

RESULTS

Effects on the acid formation in isolated canine parietal cells

Acid formation in isolated parietal cells was stimulated markedly by adding histamine (10 μ M), carbachol (0.1 mM) or db-cAMP (1

mM) to the incubation mixture. AG-1749 (0.01–1 μ M) concentration-dependently inhibited the acid formation with IC₅₀ values of 0.09, 0.08 and 0.09 μ M, respectively (Fig. 1). On the other hand, famotidine (0.1–10 μ M) inhibited the histamine-stimulated acid secretion selectively with an IC₅₀ value of 0.6 μ M and did not inhibit carbachol- or db-cAMP-stimulated acid formation even at 100 μ M (Fig. 1).

Effects on acid secretion stimulated by various secretagogues in anesthetized rats

Histamine-stimulated acid secretion: Without stimulation, very little gastric secretion was observed in the urethane anesthetized rats during the 3-hr period. Injection of histamine (30 mg/kg, *s.c.*) stimulated acid secretion, and the total acid output was $181.3 \pm 28.5 \mu\text{Eq H}^+ / 3 \text{ hr}$ ($n = 12$). Both AG-1749 (0.3–10 mg/kg) and famotidine (0.1–3 mg/kg) dose-dependently suppressed the acid secretion stimulated by histamine. The ID₅₀ values were 1.6 and 0.4 mg/kg, *i.d.*, respectively (Table 1).

Fig. 1. Effects of AG-1749 and famotidine on the acid formation stimulated by histamine, carbachol and db-cAMP in isolated canine parietal cells. The results are expressed as a percentage of the acid formation in the control group. Data are mean values with S.E. for 3–5 experiments. Histamine (○), carbachol (△), db-cAMP (□).

Table 1. ID₅₀ values of AG-1749 and famotidine for gastric acid secretion in pylorus-ligated rats

Stimulant	ID ₅₀ values (mg/kg, i.d.)	
	AG-1749	Famotidine
Unstimulated	3.6 (2.4–5.9)	0.3 (0.2–0.5)
Histamine	1.6 (1.0–2.6)	0.5 (0.3–1.0)
Bethanechol	6.0 (3.5–10.4)	2.6 (1.3–7.2)
Pentagastrin	1.6 (0.8–3.3)	0.8 (0.5–575.6)
2-Deoxy-D-glucose	2.7 (1.7–4.2)	~ 30 ^{a)}
Stress	1.0 (0.4–1.5)	75.7 (44.7–531.0)

The numbers in parentheses represent the 95% fiducial limits of the ID₅₀ values. ^{a)}ID₅₀ value was not calculated due to the lack of linearity

In another experiment, the time course of the inhibitory effect of AG-1749 (10 mg/kg, p.o.) and famotidine (10 mg/kg, p.o.) on histamine-stimulated acid secretion was studied. Maximal inhibitory effects of AG-1749 and famotidine were observed when histamine was injected 30 min after drug administration. However, the inhibitory effect of AG-1749 lasted longer than that of famotidine; signifi-

cant inhibition associated with AG-1749 was still observed 24 hr after dosing, while the effect of famotidine was only barely observable (Fig. 2).

Bethanechol-stimulated acid secretion: Total acid output stimulated by bethanechol in the control group was $167.5 \pm 27.1 \mu\text{Eq H}^+ / 3 \text{ hr}$ ($n = 11$). AG-1749 (0.3–10 mg/kg) and famotidine (0.1–3 mg/kg) suppressed the acid secretion stimulated by bethanechol dose-dependently. The ID₅₀ values were 6.0 and 2.6 mg/kg, i.d., respectively (Table 1).

In another experiment, the time course of the inhibitory effect of AG-1749 and famotidine on bethanechol-stimulated acid secretion was studied. Maximal inhibitory effects of AG-1749 (10 mg/kg, p.o.) and famotidine (10 and 30 mg/kg, p.o.) were observed when bethanechol was injected 30 min after drug administration. The antisecretory effect of famotidine was dose-related when observed 30 min after dosing. The inhibitory effect of AG-1749 at 10 mg/kg lasted longer than that of famotidine at 10 and 30 mg/kg. Reduction of acid secretion by 55% was observed 8 hr after the administration of AG-1749. However, the inhibition of acid secretion by famotidine at 10 and 30 mg/kg was barely observable 4 hr after dosing; and conversely, the acid secretion in the famotidine-treated group tended to be enhanced 8 hr after dosing (Fig. 3).

Fig. 2. Time course of the inhibitory effects of AG-1749 and famotidine on histamine-stimulated acid secretion in rats. The results are expressed as the % inhibition of the acid output in the vehicle group at each time point. Data are the mean values for 5–13 rats. *: $P < 0.05$, **: $P < 0.01$ vs. vehicle.

Fig. 3. Time course of the inhibitory effects of AG-1749 and famotidine on bethanechol-stimulated acid secretion in rats. The results are expressed as the % inhibition of the acid output in the vehicle group at each time point. Data are the mean values with S.E. for 8–10 rats. **: $P < 0.01$ vs. vehicle. AG-1749, 10 mg/kg, p.o. (▨); famotidine, 10 mg/kg, p.o. (▤); famotidine, 30 mg/kg, p.o. (□).

Pentagastrin-stimulated acid secretion: Total acid output stimulated by pentagastrin in the control group was $86.9 \pm 19.1 \mu\text{Eq H}^+ / 3 \text{ hr}$ ($n = 12$). AG-1749 (0.3–10 mg/kg) and famotidine (0.1–3 mg/kg) suppressed the acid secretion stimulated by pentagastrin dose-dependently. The ID_{50} values were 1.6 and 0.8 mg/kg, i.d., respectively (Table 1).

2-Deoxy-D-glucose-stimulated acid secretion: Total acid output stimulated by 2-deoxy-D-glucose in the control group was $274.3 \pm 30.5 \mu\text{Eq H}^+ / 3 \text{ hr}$ ($n = 13$). AG-1749 (1–30 mg/kg) inhibited the 2-deoxy-D-glucose-stimulated acid secretion dose-dependently with an ID_{50} value of 2.7 mg/kg, i.d. (Table 1, Fig. 4). On the other hand, famotidine at 30 and 100 mg/kg significantly inhibited the 2-deoxy-D-glucose-stimulated acid secretion by ~50% (Fig. 4). An ID_{50} value for famotidine was not obtained due to the lack of dose-dependency. In another experiment, it was demonstrated that, like AG-1749, omeprazole (1–30 mg/kg) inhibited the acid secretion dose-dependently with an ID_{50} value of 12.7 mg/kg, i.d.

Fig. 4. Effects of AG-1749 and famotidine on 2-deoxy-D-glucose-stimulated acid secretion in rats. Data are the mean values with S.E. for 11–13 rats. **: $P < 0.01$ vs. vehicle.

Basal and stress-induced acid secretion in pylorus-ligated rats

Basal secretion: Total acid output in control rats was $185.1 \pm 26.6 \mu\text{Eq H}^+ / 3 \text{ hr}$ ($n = 12$). AG-1749 (0.3–10 mg/kg) and famotidine (0.1–3 mg/kg) suppressed the basal acid secretion dose-dependently. The ID_{50} values were 3.6 and 0.3 mg/kg, i.d., respectively (Table 1).

Stress-induced acid secretion: Pylorus-ligated rats were subjected to restraint and water-immersion-stress for 150 min beginning 30 min after drug or vehicle was administered i.d. Total acid output in the control group was $560.8 \pm 58.1 \mu\text{Eq H}^+$ ($n = 12$), which was apparently higher than the acid output without stress. AG-1749 (1–30 mg/kg) inhibited the stress-induced acid secretion dose-dependently with an ID_{50} value of 1.0 mg/kg, i.d. (Table 1 and Fig. 5). On the other hand, famotidine (3–100 mg/kg) caused significant inhibition at each tested dose. However, the inhibitory effect of famotidine was moderate; the reduction in the acid secretion caused by the highest dose of famotidine was 56%. The ID_{50} value for famotidine was 76 mg/kg, i.d. (Table 1 and Fig. 5). In another experiment, like AG-

1749, omeprazole (1–30 mg/kg) caused a potent and dose-dependent inhibition of the acid secretion with an ID_{50} value of 5.0 mg/kg, i.d.

Gastric surface pH in conscious rats: Gastric surface pH in the control group receiving vehicle ranged from 2.2 to 2.7. AG-1749 (1–30 mg/kg), omeprazole (3–30 mg/kg) and famotidine (0.1–30 mg/kg) increased the surface pH dose-dependently. The doses of AG-1749, omeprazole and famotidine necessary to raise the surface pH to 4.0 were 3.7, 13.2 and 0.9 mg/kg, p.o., respectively.

In another experiment, the time course of the changes in surface pH following the administration of AG-1749, omeprazole and famotidine at a dose of 10 mg/kg, p.o., was examined. The surface pH in both the groups receiving AG-1749 and famotidine was elevated to more than 6 when observed 30 min after dosing; and thereafter, the pH gradually decreased. In the group receiving AG-1749, the surface pH was still significantly elevated 8 hr after dosing, while that in the group receiving famotidine had returned to the control level. In the omeprazole-treated group, a moderately elevated surface pH was observed through-

Fig. 5. Effects of AG-1749 and famotidine on the acid secretion induced by restraint and water-immersion stress in rats. Data are the mean values with S.E. for 12 rats. *: $P < 0.05$, **: $P < 0.01$ vs. vehicle.

Fig. 6. Effects of AG-1749, omeprazole and famotidine on the gastric surface pH in conscious rats. Data are the mean values with S.E. for 6 rats. *: $P < 0.05$. **: $P < 0.01$ vs. vehicle.

out the 8-hr experiment (Fig. 6).

Effect on the gastric acid secretion in Heidenhain pouch dogs

Histamine-stimulated acid secretion: Without stimulation, the amount of acid secreted was very small. Histamine ($30 \mu\text{g}/\text{kg}$, s.c.) caused an increase in gastric secretion, and the total acid output was $1348 \pm 309 \mu\text{Eq H}^+/\text{90 min}$ in the control group ($n = 6$). Oral administration of AG-1749 (0.1 – $1 \text{ mg}/\text{kg}$) and famotidine (0.01 – $0.3 \text{ mg}/\text{kg}$) suppressed the histamine-stimulated acid secretion dose-dependently. ID_{50} values, which were obtained from the inhibitory effects 30 min after dosing, were 0.27 and $0.01 \text{ mg}/\text{kg}$, p.o., respectively. From the study on the time course of the inhibitory effects of AG-1749 at $1 \text{ mg}/\text{kg}$ and famotidine at $0.1 \text{ mg}/\text{kg}$, it was demonstrated that inhibition by both agents reached the maximum 30 min after dosing and lasted over 24 hr, although the effect of AG-1749 tended to last a little longer than that of famotidine (Fig. 7).

Bethanechol-stimulated acid secretion: Total acid output in the control group was $2050 \pm 467 \mu\text{Eq H}^+/\text{90 min}$ ($n = 6$). Oral doses of

AG-1749 (0.1 – $1 \text{ mg}/\text{kg}$) and famotidine (0.01 – $0.3 \text{ mg}/\text{kg}$) suppressed the bethanechol-stimulated acid secretion dose-dependently. The time course study demonstrated that the inhibitory effect of AG-1749 at $1 \text{ mg}/\text{kg}$ reached the maximum 4 hr after dosing and lasted for 24 hr. On the other hand, the inhibitory effect of famotidine at $0.1 \text{ mg}/\text{kg}$ reached the maximum 30 min after dosing and then rapidly decreased (Fig. 7). The ID_{50} values obtained from the results 30 min after dosing were 1.61 and $0.02 \text{ mg}/\text{kg}$, p.o., respectively, whereas the ID_{50} values obtained from the results 4 hr after dosing were 0.67 and $0.29 \text{ mg}/\text{kg}$, p.o., respectively.

Pentagastrin-stimulated acid secretion: Total acid output in the control group was $1366 \pm 298 \mu\text{Eq H}^+/\text{90 min}$ ($n = 6$). Oral administration of AG-1749 (0.1 – $1 \text{ mg}/\text{kg}$) and famotidine (0.01 – $0.1 \text{ mg}/\text{kg}$) suppressed the pentagastrin-stimulated acid secretion dose-dependently. ID_{50} values were 0.22 and $0.03 \text{ mg}/\text{kg}$, p.o. The inhibitory effects of both AG-1749 at $1 \text{ mg}/\text{kg}$ and famotidine at $0.1 \text{ mg}/\text{kg}$ reached a maximum 30 min after dosing. In the AG-1749-treated group, inhibition of acid secretion was still observed 24 hr after

Fig. 7. Effects of oral administration of AG-1749 and famotidine on histamine-, bethanechol- and pentagastrin-stimulated acid secretion in Heidenhain pouch dogs. Acid output is expressed as a percentage of the mean of the control acid output which was measured 3 times: 1 week and 1 day before and 1 week after each drug was administered. Data are the mean values for 3–6 dogs. Acid output hardly changed throughout the entire time course of the experiment in dogs receiving the vehicle. *: $P < 0.05$, **: $P < 0.01$ vs. the group receiving the vehicle. AG-1749, 1 mg/kg, p.o. (●); famotidine, 0.1 mg/kg, p.o. (○).

dosing, while in the famotidine-treated group, the acid secretion had returned to the control level 8 hr after dosing (Fig. 7).

Peptone meal-stimulated acid secretion: Administration of 100 ml of 20% peptone

meal caused a rather sustained increase in the acid secretion. Total acid output in the control group was $892 \pm 227 \mu\text{Eq H}^+$ ($n = 6$). AG-1749 (0.1–1 mg/kg) and famotidine (0.01–0.1 mg/kg) suppressed the peptone meal-stimulated acid secretion dose-dependently. The ID_{50} values were 0.36 and 0.03 mg/kg, p.o., respectively.

DISCUSSION

Substituted benzimidazoles such as AG-1749 and omeprazole suppress the gastric acid secretion by inhibiting the proton pump in gastric parietal cells (12, 17–19). We have previously reported that the proton pump inhibitors inhibit histamine-, carbachol- and db-cAMP-stimulated acid formation in isolated canine parietal cells (9); histamine-, bethanechol-, pentagastrin- and peptone meal-stimulated acid secretion in dogs (12) and basal and histamine-stimulated acid secretion in rats (12). In this study, it was demonstrated that AG-1749 suppresses the acid secretion induced by bethanechol, pentagastrin, 2-deoxy-D-glucose and water-immersion stress in rats. These results give further support to the idea that AG-1749 inhibits acid secretion regardless of the secretagogue.

In our previous study, ranitidine selectively inhibited histamine-stimulated acid formation in isolated canine parietal cells with an IC_{50} value of $5.9 \mu\text{M}$ and suppressed histamine-stimulated acid secretion in dogs and rats with ID_{50} values of 0.1 and 22 mg/kg, respectively (12). In this study, famotidine inhibited histamine-stimulated acid formation selectively with an IC_{50} value of $0.6 \mu\text{M}$ and histamine-stimulated acid secretion in dogs and rats with ID_{50} values of 0.01 and 0.4 mg/kg, respectively. These results indicate that famotidine is 10 and 50 times as potent as ranitidine in dogs and rats, respectively.

It is generally accepted that selective blockade of the histamine H_2 -receptor results in the inhibition of acid secretion induced by other stimuli because endogenous histamine enhances the acid secretion. In this study famoti-

dine inhibited bethanechol- and pentagastrin-stimulated acid secretion as well as histamine-stimulated acid secretion. However, famotidine at 100 mg/kg only partially inhibited the acid secretion induced by 2-deoxy-D-glucose and water-immersion stress in rats. It is reported that 2-deoxy-D-glucose enhances acid secretion via stimulation of the vagal nerve by acting on the central nervous system (20–22) and that the acid secretion is suppressed centrally by GABA antagonists or opioids (23, 24) and peripherally by prostaglandins or histamine H₂-receptor antagonists (25). Restraint and water-immersion stress also stimulates acid secretion via the central nervous system (26). Katz et al. (25) reported that histamine H₂-receptor antagonists inhibit 2-deoxy-D-glucose-stimulated acid secretion as well as the acid secretion induced by other stimuli in gastric fistula dogs. Our results using rats are not consistent with their results. As we have found that, like famotidine, ranitidine at 100 mg/kg caused only partial inhibition of the water-immersion stress- and 2-deoxy-D-glucose-induced acid secretion (H. Nagaya and H. Satoh, unpublished data), it is suggested that histamine H₂-receptor antagonists have only a slight inhibitory effect on vagally stimulated acid secretion. By contrast, the finding that AG-1749 and omeprazole inhibited vagally stimulated acid secretion as well as peripherally stimulated acid secretion might indicate that proton pump inhibitors are more effective than histamine H₂-receptor antagonists in inhibiting the gastric acid hypersecretion induced by stress. In principle, stimulation of the vagus enhances acid secretion via the cholinergic pathway. Because H₂-receptor antagonists potently inhibit acid secretion induced by cholinergic agents, it is difficult to explain why they do not potently inhibit vagally stimulated acid secretion. Soll (27) reported that db-cAMP as well as histamine potentiates carbachol-induced acid formation in isolated parietal cells. It might be possible that vagal stimulation enhances the acid secretion induced by cholinergic agents through an unknown pathway which is independent of en-

dogenous histamine.

In rats, the inhibitory effects of AG-1749 on bethanechol- and histamine-stimulated acid secretion were long lasting, although the ID₅₀ value of AG-1749 for histamine-stimulated acid secretion was several times lower than that for bethanechol-stimulated acid secretion. On the other hand, the inhibitory effect of famotidine on bethanechol-stimulated acid secretion decreased much more rapidly than that on histamine-stimulated acid secretion. As the ID₅₀ value of famotidine for histamine-stimulated acid secretion was several times lower than that for bethanechol-stimulated acid secretion, the inhibitory effect on bethanechol-stimulated acid secretion would seem to decrease more rapidly as the blood level of famotidine decreases. However, the duration of the inhibitory effect of famotidine on bethanechol-stimulated acid secretion was not prolonged by increasing the dose of famotidine. Therefore, the time-dependent inhibition of acid secretion, at least bethanechol-stimulated acid secretion, by famotidine could not be explained simply by the blood concentration of famotidine.

In Heidenhain pouch dogs, the maximal inhibitory effects of AG-1749 on histamine-, pentagastrin- and bethanechol-stimulated acid secretion were observed at 0.5, 0.5 and 4 hr, respectively, after AG-1749 administration. The inhibitory effects lasted for more than 4–8 hr. On the other hand, the maximal inhibitory effects of famotidine against the above stimuli were all observed 0.5 hr after famotidine administration. The inhibitory effect on histamine-stimulated acid secretion tended to last longer than that on pentagastrin- or bethanechol-stimulated acid secretion. In addition, acid secretion in response to bethanechol or pentagastrin was slightly enhanced 24 hr after the administration of famotidine.

From these results, it is suggested that the proton pump inhibitor AG-1749 persistently inhibits the acid secretion induced by either central or peripheral stimuli, whereas the anti-secretory actions of the histamine H₂-receptor antagonist famotidine depend on the nature of

the stimuli.

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REFERENCES

- 1 Soll, A.H.: The actions of secretagogues on oxygen uptake by isolated mammalian parietal cells. *J. Clin. Invest.* **61**, 370–380 (1978)
- 2 Chew, C.S., Hersey, S.J., Sachs, G. and Berglindh, T.: Histamine responsiveness of isolated gastric glands. *Am. J. Physiol.* **238**, G312–G320 (1980)
- 3 Muallem, S. and Sachs, G.: Ca^{2+} metabolism during cholinergic stimulation of acid secretion. *Am. J. Physiol.* **248**, G216–G228 (1985)
- 4 Sachs, G., Muallem, S. and Hersey, S.J.: Passive and active transport in the parietal cell. *Comp. Biochem. Physiol. [A]* **90**, 722–731 (1988)
- 5 Helander, H.F. and Hirschowitz, B.I.: Quantitative ultrastructural studies on gastric parietal cells. *Gastroenterology* **63**, 951–961 (1972)
- 6 Wolosin, J.M. and Forte, J.G.: Changes in the membrane environment of the $(\text{K}^+ + \text{H}^+)\text{-ATPase}$ following stimulation of the gastric oxyntic cell. *J. Biol. Chem.* **256**, 3149–3152 (1981)
- 7 Im, W.B., Blakeman, D.P., Fieldhouse, J.M. and Rabon, E.C.: Effect of carbachol or histamine stimulation on rat gastric membranes enriched in $(\text{H}^+ + \text{K}^+)\text{-ATPase}$. *Biochim. Biophys. Acta* **772**, 167–175 (1984)
- 8 Nagaya, H., Satoh, H., Kubo, K. and Maki, Y.: Possible mechanism for the inhibition of gastric $(\text{H}^+ + \text{K}^+)\text{-adenosine triphosphatase}$ by the proton pump inhibitor AG-1749. *J. Pharmacol. Exp. Ther.* **248**, 799–805 (1989)
- 9 Nagaya, H., Satoh, H. and Maki, Y.: Possible mechanism for the inhibition of acid formation by the proton pump inhibitor AG-1749 in isolated canine parietal cells. *J. Pharmacol. Exp. Ther.* **252**, 1289–1295 (1990)
- 10 Fellenius, E., Berglindh, T., Sachs, G., Olbe, L., Elander, B., Sjöstrand, S.E. and Wallmark, B.: Substituted benzimidazoles inhibit gastric acid secretion by blocking $(\text{H}^+ + \text{K}^+)\text{ATPase}$. *Nature* **290**, 159–161 (1981)
- 11 Wallmark, B., Jaresten, B.M., Larsson, H., Ryberg, B., Brändström, A. and Fellenius, E.: Differentiation among inhibitory actions of omeprazole, cimetidine, and SCN^- on gastric acid secretion. *Am. J. Physiol.* **245**, G64–G71 (1983)
- 12 Satoh, H., Inatomi, N., Nagaya, H., Inada, I., Nohara, A., Nakamura, N. and Maki, Y.: Anti-secretory and antiulcer activities of a novel proton pump inhibitor AG-1749 in dogs and rats. *J. Pharmacol. Exp. Ther.* **248**, 806–815 (1989)
- 13 Fryklund, J., Wallmark, B., Larsson, H. and Helander, H.F.: Effect of omeprazole on gastric secretion in $\text{H}^+/\text{K}^+\text{-ATPase}$ and in pepsinogen-rich cell fractions from rabbit gastric mucosa. *Biochem. Pharmacol.* **33**, 273–280 (1984)
- 14 Berglindh, T., Helander, H.F. and Obrink, K.J.: Effects of secretagogues on oxygen consumption, aminopyrine accumulation and morphology in isolated gastric glands. *Acta Physiol. Scand.* **97**, 401–414 (1976)
- 15 Soll, A.H.: Secretagogue stimulation of $[^{14}\text{C}]$ aminopyrine accumulation by isolated canine parietal cells. *Am. J. Physiol.* **238**, G366–G375 (1980)
- 16 Finney, D.J.: Statistical Method in Biological Assay, p. 27–29, Charles Griffin and Company, London (1964)
- 17 Larsson, H., Carlsson, E., Junggren, U., Olbe, L., Sjöstrands, S.E., Skånberg, I. and Sundell, G.: Inhibition of gastric acid secretion by omeprazole in the dog and rat. *Gastroenterology* **85**, 900–907 (1983)
- 18 Konturek, S.J., Cieszkowski, M., Kwiecien, N., Konturek, J., Tasler, J. and Bilski, J.: Effects of omeprazole, a substituted benzimidazole, on gastrointestinal secretions, serum gastrin, and gastric mucosal blood flow in dogs. *Gastroenterology* **86**, 71–77 (1984)
- 19 Yamamoto, O., Okada, Y. and Okabe, S.: Effects of a proton pump inhibitor, omeprazole, on gastric secretion and gastric and duodenal ulcers or erosions in rats. *Dig. Dis. Sci.* **29**, 394–401 (1984)
- 20 Eisenberg, M.M., Emas, G.S. and Grossman, M.I.: Comparison of the effect of 2-deoxy-D-glucose and insulin on gastric acid secretion in dogs. *Surgery* **60**, 111–117 (1966)
- 21 Hirschowitz, B.I. and Sachs G.: Vagal gastric secretory stimulation by 2-deoxy-D-glucose. *Am. J. Physiol.* **209**, 452–460 (1965)
- 22 Shiraishi, T. and Simpson, A.D.: Lateral hypothalamus neuron response to electroosmotic 2-deoxy-D-glucose. *Brain Res. Bull.* **5**, 245–250 (1980)
- 23 Lin, W.C., Yano, S. and Watanabe, K.: Inhibitory effect of intravenous GABA antagonists on gastric acid secretion stimulated by secretagogues in rats. *Japan. J. Pharmacol.* **49**, 267–274 (1989)
- 24 Ho, M.M., Dai, S. and Ogle, C.W.: Morphine reduces vagal-stimulated gastric acid secretion through a central action. *Eur. J. Pharmacol.* **139**,

- 251–257 (1987)
- 25 Katz, L.B., Tobia, A.J. and Shriver, D.A.: Effects of ORF 17583, other histamine H_2 -receptor antagonists and omeprazole on gastric acid secretory states in rats and dogs. *J. Pharmacol. Exp. Ther.* **242**, 437–442 (1987)
- 26 Arai, I., Hirose, H., Muramatsu, M. and Aihara, H.: Effects of restraint and water-immersion stress and insulin on gastric acid secretion in rats. *Physiol. Behav.* **40**, 357–361 (1987)
- 27 Soll, A.H.: Potentiating interactions of gastric stimulants on [^{14}C]aminopyrine accumulation by isolated canine parietal cells. *Gastroenterology* **83**, 216–223 (1982)