

Comparative Evaluation of the Role of Endogenous Gastrin in Basal Acid Secretion in Conscious Rats Provided with Chronic Fistula and Pylorus Ligation

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ABSTRACT—We determined the relative contributions of endogenous gastrin, histamine and cholinergic tone to basal acid secretion in chronic fistula rats. Results were compared with those for acid secretion in pylorus-ligated rats. In chronic fistula rats, YM022 {(R)-1-[2,3-dihydro-1-(2'-methylphenacyl)-2-oxo-5-phenyl-1*H*-1,4-benzodiazepin-3-yl]-3-(3-methylphenyl)urea} dose-dependently inhibited pentagastrin-stimulated acid secretion and abolished this secretion at 1 $\mu\text{mol/kg}$, s.c., but did not affect histamine- and carbachol-induced acid secretion even at 10 $\mu\text{mol/kg}$. In contrast, famotidine at 1 $\mu\text{mol/kg}$ completely inhibited not only the acid secretion induced by histamine but also those by pentagastrin and carbachol. Furthermore, atropine abolished carbachol- and pentagastrin-stimulated acid secretion and significantly suppressed histamine-stimulated acid secretion at 0.1 $\mu\text{mol/kg}$. YM022 dose-dependently inhibited basal acid secretion. The YM022 dosage required to inhibit basal acid secretion is consistent with that required to suppress pentagastrin-induced acid secretion. Famotidine (1 $\mu\text{mol/kg}$) and atropine (0.1 $\mu\text{mol/kg}$) also abolished basal acid secretion. In pylorus-ligated rats, YM022 inhibited acid secretion in a dose-dependent manner; the inhibition at 1 $\mu\text{mol/kg}$, i.v. was 65%. No additional effect was observed when rats were dosed at 30 $\mu\text{mol/kg}$. Famotidine partially inhibited acid secretion in these rats, whereas atropine abolished this secretion. These results indicate that the major part of basal acid secretion in rats is attributable to endogenous gastrin via histamine- and cholinergic tone-dependent pathways. Moreover, pylorus ligation reduces the relative contribution of gastrin to acid secretion due to the activation of cholinergic tone.

Keywords: YM022, Basal acid secretion, Gastrin receptor, Histamine H_2 -receptor, Cholinergic neuron

Gastrin is well-known as a stimulant of gastric acid secretion. Although the physiological significance of endogenous gastrin in the gastric phase of acid secretion has been well established (1, 2), the contribution of endogenous gastrin to basal acid secretion has remained unclear. Lotti and Chang reported that orally administered L-365,260, a gastrin receptor antagonist, does not inhibit basal acid secretion in pylorus-ligated rats (3). On the contrary, we and Pendley et al. reported that i.v. injection of this drug inhibited acid secretion in these rats (4, 5). However, L-365,260 also inhibits histamine- or cholinomimetic-stimulated acid secretion, whereas other gastrin antagonists do not affect this secretion (6–9). L-365,260, therefore, may have a non-specific antisecretory effect.

We recently reported that the potent and selective gastrin receptor antagonist YM022 inhibited basal acid

secretion in pylorus-ligated rats without affecting histamine- or bethanechol-induced acid secretion (10). However, we did not precisely evaluate the role of endogenous gastrin in basal acid secretion in this study. Moreover, since pylorus ligation has been shown to stimulate acid secretion via a cholinergic pathway (11), acid secretion in pylorus-ligated rats is not, strictly speaking, basal acid secretion.

The chronic fistula model is considered a suitable method for examination of basal acid secretion in rats. While a number of reports have described the use of gastrin receptor antagonists in chronic fistula rats, the role of gastrin in basal acid secretion has not been fully evaluated (9, 12, 13). In the present study, therefore, we examined the effect of gastrin, histamine H_2 - and muscarinic receptor antagonists on basal and stimulated acid secretion to evaluate the role endogenous gastrin in basal acid secre-

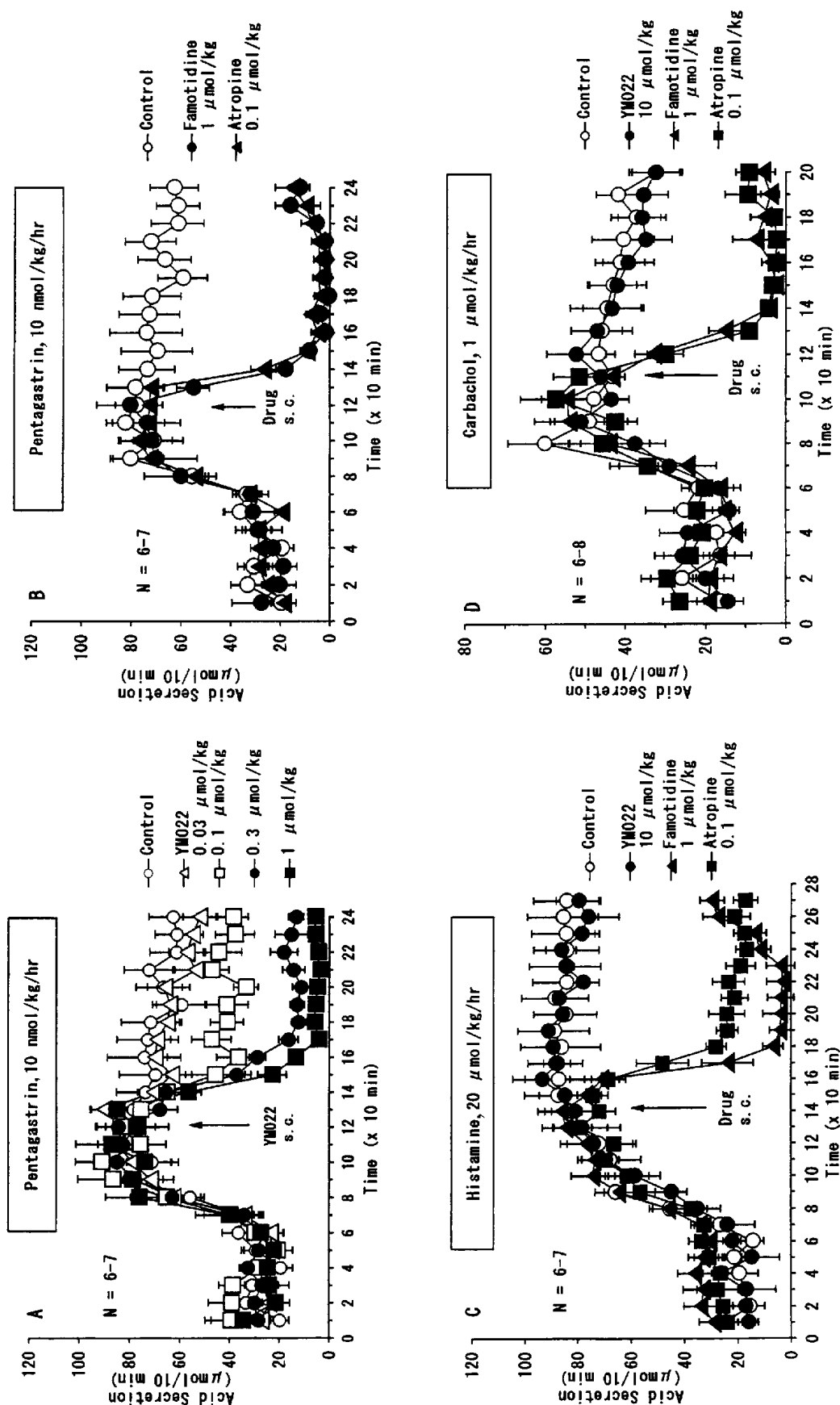


Fig. 1. Effect of YMO22, famotidine and atropine on maximal acid secretion response to pentagastrin (A and B), histamine (C) and carbachol (D) in conscious rats provided with chronic fistula. YMO22 (0.03–10 μmol/kg), famotidine (1 μmol/kg) and atropine (0.1 μmol/kg) were given s.c. 60 min after the start of pentagastrin (10 nmol/kg/hr) infusion, 90 min after the start of histamine (20 μmol/kg/hr) infusion and 50 min after the start of carbachol (1 μmol/kg/hr) infusion. Data are expressed as μmol/10 min, and are the mean ± S.E.M. from 6 to 8 rats.

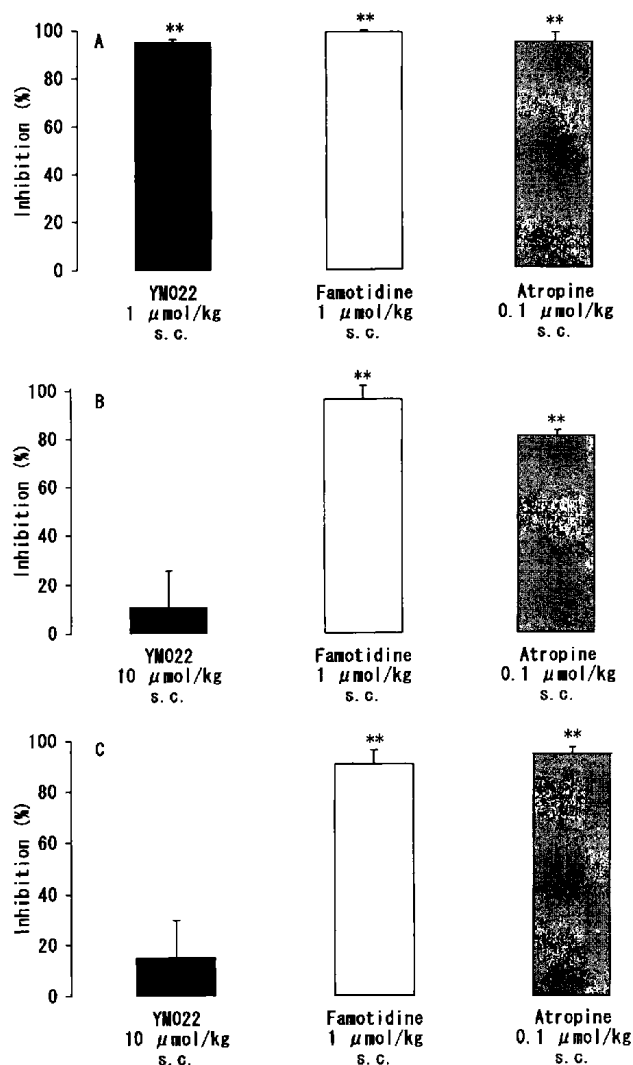


Fig. 2. Maximal inhibitory effect of YM022, famotidine and atropine on maximal acid secretion response to pentagastrin (A), histamine (B) and carbachol (C) in conscious chronic fistula rats. YM022 (1 and 10 μ mol/kg), famotidine (1 μ mol/kg), atropine (0.1 μ mol/kg) and vehicle were given s.c. 60 min after the start of pentagastrin (10 nmol/kg/hr) infusion, 90 min after the start of histamine (20 μ mol/kg/hr) infusion and 50 min after the start of carbachol (1 μ mol/kg/hr) infusion. Maximal inhibition (%) was determined by comparing acid output in corresponding control and treated groups. Data represent the mean \pm S.E.M. from 6 or 7 rats. ** $P < 0.01$, compared with the control groups (Student's *t*-test).

tion in conscious rats provided with chronic fistula. Furthermore, with regard to the effect of antagonists on basal acid secretion, the results in chronic fistula rats were compared with those in pylorus-ligated rats.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Charles River Japan, Inc., Yokohama) weighing 180–620 g were housed in individual

cages with a mesh bottom. They were allowed free access to tap water but were deprived of food for 18 hr before the experiments.

Determination of acid secretion in conscious chronic gastric fistula rats

Four weeks before the experiment, rats were surgically provided, under ether anesthesia, with a chronic stainless steel cannula inserted into the glandular part of the stomach wall.

The stomach was rinsed with warmed saline (37°C) through the gastric fistula until clear washing solution was obtained. The animals were then placed in modified Bollman cages, and gastric juice was drained under gravity into a reservoir. Acid secretion was measured at pH 7.0 with 0.1 M NaOH using an automatic titrator (AUT-201; Toa Electronics, Tokyo). For basal acid secretion, YM022, atropine, famotidine or vehicle was administered subcutaneously (s.c.) to the rat 120 min after basal secretion had stabilized. Data were calculated every 30 min and are expressed as μ mol/30 min. Maximal inhibition (%) was determined by comparing acid output in the control and treated groups.

For stimulant-induced acid secretion, pentagastrin (10 nmol/kg/hr), histamine (20 μ mol/kg/hr) or carbachol (1 μ mol/kg/hr) was infused s.c. 60 min after the basal secretion had stabilized. YM022, atropine, famotidine or vehicle were administered s.c. 60 min after the start of pentagastrin infusion, 50 min after the start of carbachol infusion and 90 min after the start of histamine infusion. Data were calculated every 10 min and are expressed as μ mol/10 min. Maximal inhibition (%) was determined by comparing acid output in corresponding control and treated groups.

Determination of gastric acid secretion in pylorus-ligated rats

Under ether anesthesia, the abdomen was incised and the pylorus ligated. Four hours later, the animals were killed with ether, and the gastric contents were collected and analyzed for volume and acidity. Acidity was determined by automatic titration of the gastric juice against 0.05 N NaOH to pH 7.0 (Comtite-7; Hiranuma, Tokyo). YM022, atropine, famotidine or their corresponding vehicle was intravenously dosed immediately after ligation. Inhibition (%) was determined by comparing acid output in corresponding control and treated groups.

Drugs

YM022 {(R)-1-[2,3-dihydro-1-(2'-methylphenacyl)-2-oxo-5-phenyl-1*H*-1,4-benzodiazepin-3-yl]-3-(3-methylphenyl)urea} and famotidine were prepared by Yamanouchi Pharmaceutical Co. Atropine sulfate mono-

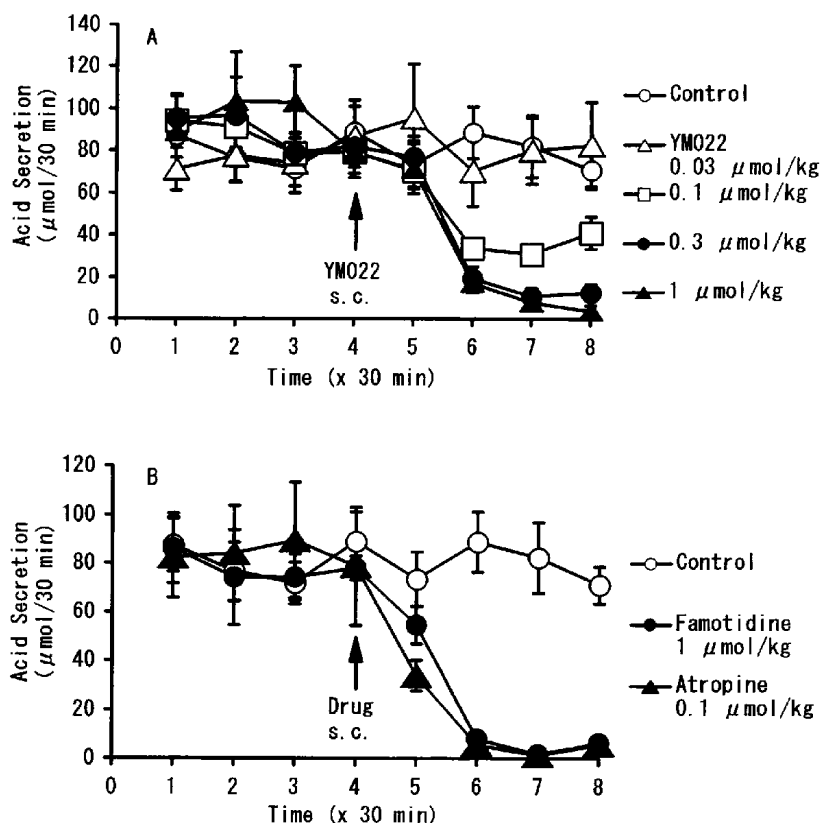


Fig. 3. Effect of YM022 (A), famotidine and atropine (B) on basal acid secretion in conscious chronic fistula rats. YM022 (0.03–1 $\mu\text{mol/kg}$), famotidine (1 $\mu\text{mol/kg}$), atropine (0.1 $\mu\text{mol/kg}$) and vehicle were given s.c. 120 min after basal acid secretion had stabilized. Data are expressed as $\mu\text{mol/30 min}$ and are the mean \pm S.E.M. from 7 rats.

hydrate was purchased from Wako Pure Chemical Industries Ltd. (Osaka). YM022, famotidine and atropine were suspended in Tween 80 and saline for s.c. injection. For i.v. injection, YM022 was dissolved in *N,N*-dimethylformamide. Famotidine was dissolved in a small volume of 0.1 M HCl. The pH of this solution was adjusted to 6 with 0.1 M NaOH, and then the solution was diluted with physiological saline. Atropine was dissolved in saline. Pentagastrin, histamine dihydrochloride, bethanechol hydrochloride and carbachol chloride were from Sigma Chemical Co. (St. Louis, MO, USA) and were prepared with physiological saline for appropriate concentrations at 5 ml/kg/hr.

Statistical evaluation

Results are expressed as the mean \pm S.E.M. from 6 to 10 rats per group. Statistical comparisons of vehicle and test compound treatment groups in secretagogue-induced and basal acid secretion were made by Student's *t*-test. Statistical comparisons in pylorus-ligated rats were made by Dunnett's multiple range test. Probabilities of less than 5% ($P < 0.05$) were considered significant.

RESULTS

Effect of YM022, famotidine and atropine on secretagogue-induced acid secretion in conscious chronic fistula rats

Maximal secretory rates in response to pentagastrin (10 nmol/kg/hr), histamine (20 $\mu\text{mol/kg/hr}$) and carbachol (1 $\mu\text{mol/kg/hr}$) were 51.7 ± 3.1 , 55.9 ± 5.9 and 27.1 ± 2.2 $\mu\text{mol/10 min}$, respectively. YM022 (0.03–1 $\mu\text{mol/kg}$, s.c.) dose-dependently inhibited pentagastrin-induced acid secretion (Fig. 1A) and abolished this secretion at a dose of 1 $\mu\text{mol/kg}$ (Fig. 2A). YM022 did not affect histamine- and carbachol-stimulated acid secretion even at a dose of 10 $\mu\text{mol/kg}$ (Figs. 1 and 2). In contrast, famotidine at a dose of 1 $\mu\text{mol/kg}$ abolished not only histamine-induced acid secretion but also pentagastrin- and carbachol-induced acid secretion (Figs. 1 and 2). Atropine at a dose of 0.1 $\mu\text{mol/kg}$ completely suppressed carbachol- and pentagastrin-induced secretion. Atropine also markedly but not completely inhibited histamine-induced acid secretion (Figs. 1C and 2B).

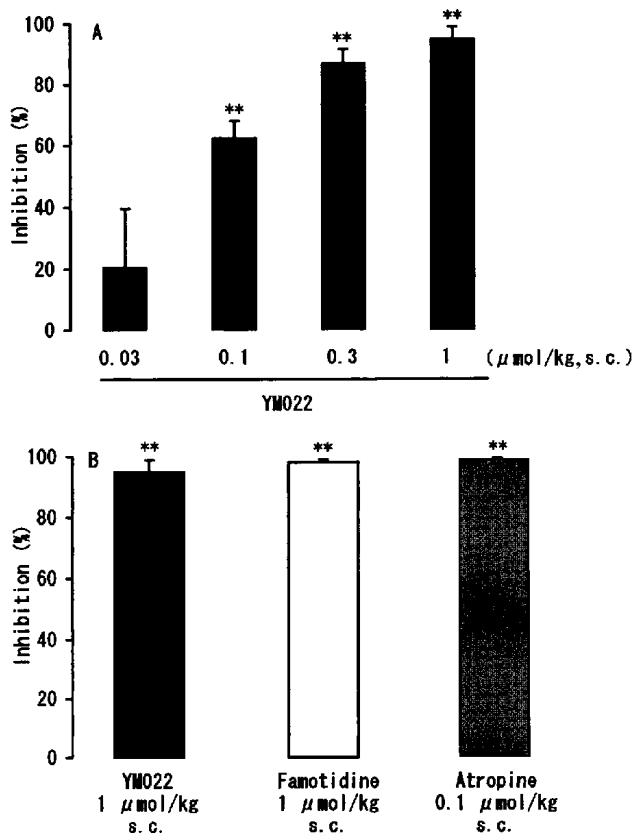


Fig. 4. Maximal inhibitory effect of YM022 (A), famotidine and atropine (B) on basal acid secretion in conscious chronic fistula rats. YM022 (0.03–1 $\mu\text{mol/kg}$), famotidine (1 $\mu\text{mol/kg}$), atropine (0.1 $\mu\text{mol/kg}$) and vehicle were given s.c. 120 min after basal acid secretion had stabilized. Maximal inhibition (%) was determined by comparing acid output in control and treated groups. Data represent the mean \pm S.E.M. from 7 rats. ** $P < 0.01$, compared with the control group (Student's *t*-test).

Effect of YM022, famotidine and atropine on basal acid secretion in conscious chronic fistula rats

Basal acid output in conscious chronic fistula rats was $83.3 \pm 2.4 \mu\text{mol/30 min}$ (Fig. 3). YM022 (0.03–1 $\mu\text{mol/kg}$, s.c.) dose-dependently inhibited this basal acid secretion (Figs. 3A and 4A) and abolished it at a dose of 1 $\mu\text{mol/kg}$ (Figs. 3A and 4). Atropine and famotidine at doses that antagonize the corresponding receptor also abolished basal acid secretion (Figs. 3B and 4B).

Effect of YM022, famotidine and atropine on acid secretion in pylorus-ligated rats

The acid output in pylorus-ligated rats was $812.6 \pm 40.6 \mu\text{mol/4 hr}$. YM022 (0.1–1 $\mu\text{mol/kg}$, i.v.) decreased gastric acid secretion in a dose-dependent manner, with an inhibition of 64.8% at 1 $\mu\text{mol/kg}$, i.v. (Fig. 5). Higher doses of this drug (3–30 $\mu\text{mol/kg}$) did not produce an additional inhibitory effect. Atropine (0.01–10 $\mu\text{mol/kg}$,

i.v.) dose-dependently inhibited acid secretion with complete inhibition at 10 $\mu\text{mol/kg}$ in pylorus-ligated rats (Fig. 5). Famotidine (0.3–30 $\mu\text{mol/kg}$, i.v.) also inhibited this secretion in a dose-dependent manner, with a maximal degree of inhibition of 85.1% at 30 $\mu\text{mol/kg}$, i.v. No additional effect was observed when rats were dosed at 100 $\mu\text{mol/kg}$ (Fig. 5).

DISCUSSION

The role of endogenous gastrin in basal acid secretion in rats has been poorly understood. The present study shows that the gastrin/cholecystokinin (CCK)-B receptor antagonist YM022 dose-dependently and completely inhibits basal acid secretion in conscious rats provided with chronic fistula. YM022 also inhibited pentagastrin-induced acid secretion, but did not affect histamine- and carbachol-stimulated acid secretion in these rats. Moreover, the YM022 dosage required to inhibit basal secretion agreed well with that required to suppress pentagastrin-induced secretion in these conscious rats. It is therefore likely that the inhibitory effect of YM022 on basal acid secretion is due to antagonization of gastrin receptors. We previously reported that YM022 is a potent gastrin/CCK-B receptor antagonist with slight affinity for pancreatic CCK-A receptors (7); However, because the CCK-A receptor antagonist L-364,718 has no effect on basal acid secretion (14), the inhibitory effect of YM022 does not result from any CCK-A receptor antagonism. The further specificity of YM022 for gastrin/CCK-B receptors has also been shown by its lack of activity ($\text{IC}_{50} > 10 \mu\text{M}$) in various radioligand binding assays (10). These results suggest that YM022 inhibits basal acid secretion through gastrin receptor antagonism. Furthermore, we confirmed that acid secretion stimulated by a maximal dose of pentagastrin was completely blocked by histamine H_2 - and muscarinic receptor blockade in conscious rats (15, 16). These results indicate that endogenous gastrin accounts for the major part of the basal acid secretion and is implicated in this secretion via histamine- and cholinergic nerve-dependent pathways.

Although whether gastrin acts solely by releasing histamine from histamine-containing cells or by an additional direct action on parietal cells is still a matter of debate (17), the participation of endogenous histamine in the regulation of gastrin-mediated acid secretion is well known (18). Andersson et al. (19) reported that 4-day treatment with α -fluoromethyl-histidine, an inhibitor of histidine decarboxylase, abolished pentagastrin-stimulated acid secretion in rats, an effect that was accompanied by a decrease in histamine levels of gastric mucosa. Gastrin may therefore stimulate gastric acid secretion mainly via histamine release from enterochromaffin-like

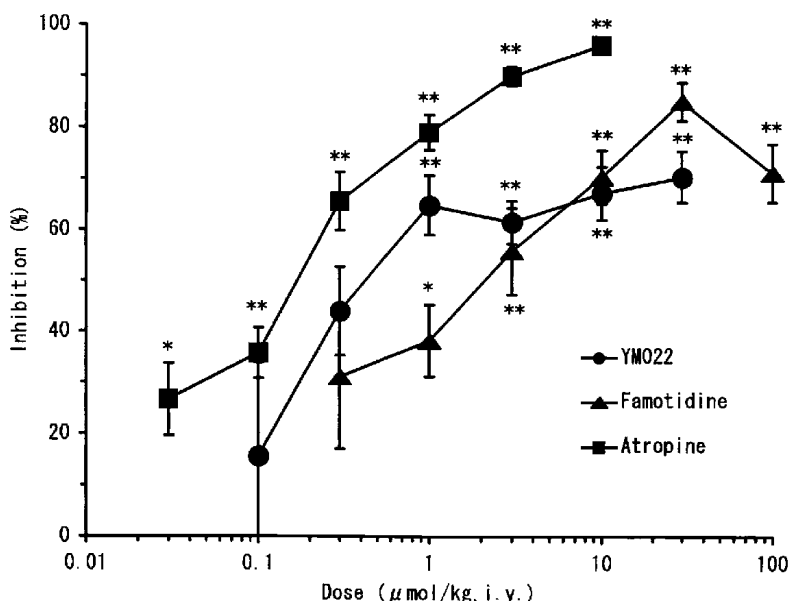


Fig. 5. Effect of YM022, famotidine and atropine on gastric acid secretion in pylorus-ligated rats. YM022 (0.1–30 $\mu\text{mol/kg}$), famotidine (0.3–100 $\mu\text{mol/kg}$), atropine (0.03–10 $\mu\text{mol/kg}$) and vehicle were given i.v. immediately after pylorus ligation. Data represent the mean \pm S.E.M. from 9 or 10 rats. * $P < 0.05$, ** $P < 0.01$, compared with the corresponding control groups (Dunnett's multiple range test).

(ECL) cells. On the other hand, although atropine has been shown to render the parietal cell unresponsive to circulating stimuli (16), its inhibitory mechanism on gastrin-mediated acid secretion remains unclear. Since atropine's effect is mimicked by bilateral vagotomy (20), vagal inhibition is thought to be implicated in these mechanisms. Vagal cholinergic neurons stimulate gastric acid secretion not only via activation of muscarinic receptors on parietal cells and histamine release from ECL cells (21) but also suppression of somatostatin secretion from somatostatin-containing cells (22, 23). Therefore, it is likely that the inhibition of cholinergic neurons by atropine augments somatostatin release. The present study confirmed that atropine also inhibits histamine-induced acid secretion (16, 24). This result is consistent with the fact that somatostatin inhibits acid secretion induced by not only pentagastrin but also by histamine (25). Somatostatin also reduces gastrin-stimulated histamine release from ECL cells (26, 27). Possible mechanisms for the inhibitory effect of atropine on gastrin-induced acid secretion may therefore be inhibition of the secretory activity of parietal cells and attenuation of histamine release from ECL cells via somatostatin release.

Previous reports showed that intravenous injection of L-365,260, a gastrin receptor antagonist, inhibited acid secretion in pylorus-ligated rats. However, i.v. administration of this drug inhibits histamine- and cholinomimetic-induced acid secretion in anesthetized and pylorus-ligated rats, whereas other gastrin receptor antagonists

do not affect these secretions (6–9). These results suggest that L-365,260 may have a non-specific antisecretory effect. The dose range of L-365,260 required to suppress acid secretion in pylorus-ligated rats overlaps with that required to inhibit histamine-induced secretion in these rats (5). Therefore, the role of endogenous gastrin in acid secretion in pylorus-ligated rats has remained unclear. The present study also shows that intravenously administered YM022 dose-dependently inhibits acid secretion in pylorus-ligated rats. In contrast to the complete inhibition by YM022 of basal acid secretion in chronic fistula rats, YM022 exhibited partial inhibition in pylorus-ligated rats. Gastrin receptor blockade by YM022 therefore demonstrates that endogenous gastrin, at least in part, plays a role in acid secretion in pylorus-ligated rats. Pylorus ligation has been shown to stimulate acid secretion via the vago-vagal reflex and cholinergic muscarinic mechanism (11, 28, 29). YM022 did not suppress carbachol-induced acid secretion in rats. It appears that pylorus ligation decreases the relative contribution of endogenous gastrin to acid secretion due to activation of cholinergic mechanisms. In the present study, atropine abolished acid secretion in pylorus-ligated rats, whereas high doses of famotidine did not completely inhibit this secretion. This latter result may also indicate the activation of cholinergic nerves by pylorus ligation. The present study therefore indicates that the relative contribution of endogenous gastrin to acid secretion is less in pylorus-ligated rats than in chronic fistula rats.

Famotidine abolished cholinomimetic-induced acid secretion in conscious rats. This result seems contradictory to the fact that famotidine partially inhibited this secretion in pylorus-ligated rats, and it disagrees with previous studies in which a histamine H_2 -receptor antagonist exhibited partial inhibition against cholinomimetic-stimulated acid secretion in conscious acute fistula rats and anesthetized rats (15, 30, 31). Cholinomimetics stimulate not only acid secretion but also salivary secretion. Because saliva is swallowed into the stomach and neutralizes gastric acid, the antisecretory activity of drugs may therefore be occasionally overestimated in chronic fistula rats. Furthermore, histamine H_2 -receptor blockade inhibits acid secretion but not salivary secretion. Thus, the inhibitory effect of famotidine on cholinomimetic-stimulated acid secretion in conscious rats may have been also overestimated in this study.

In summary, the present study demonstrates that basal acid secretion is mainly regulated by endogenous gastrin through histamine- and cholinergic neurone-dependent pathways in conscious rats provided with chronic fistula. Pylorus ligation reduces the relative contribution of endogenous gastrin to acid secretion in rats.

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