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Antisecretory effect of somatostatin on gastric acid via inhibition of histamine release in isolated mouse stomach

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Abstract

Somatostatin is known to inhibit gastric acid secretion via both inhibition of histamine release from gastric enterochromaffin-like cells and direct inhibition of parietal cell function. We tried to clarify which of these two mechanisms plays a more important role in the inhibition of gastric acid section by somatostatin using isolated mouse stomach preparations. The gastric acid secretion stimulated by histamine was not inhibited by pretreatment with somatostatin (1 μM), but somatostatin abolished acid secretion induced by 4-[[[(3-chlorophenyl)amino]-carbonyl]oxy]-*N*,*N*,*N*,-trimethyl-2-butynyl-1-aminium chloride (McN-A-343), a muscarinic M_1 receptor agonist. In addition, the histamine-H_2 receptor antagonist famotidine also completely inhibited the secretion stimulated by McN-A-343. Similarly, pretreatment with both somatostatin and famotidine completely abolished pentagastrin-induced acid secretion. Somatostatin partially inhibited the acid secretion stimulated by bethanechol. The late sustained acid secretion induced by bethanechol was reduced more strongly by somatostatin than the initial peak secretion. In addition, somatostatin had no effect on the transient increase in bethanechol-induced acid secretion in famotidine-pretreated preparations. Somatostatin had no effect on basal histamine secretion in isolated mouse stomach preparations, but markedly reduced histamine release induced by McN-A-343 and bethanechol. The present study showed that the acid secretory response via the endogenous histamine-mediated pathway was inhibited by somatostatin, but the response to a direct activation of gastric parietal cells was not. These results suggest that the inhibition of histamine release from enterochromaffin-like cells plays a more important role in the inhibition of gastric acid secretion by somatostatin than the direct inhibition of parietal cells. In addition, somatostatin inhibited the sustained acid secretion more strongly than the initial peak secretion after the cholinergic stimulation.

Keywords: Somatostatin; Gastric acid secretion; Histamine release; Gastric parietal cell; Enterochromaffin-like cell; Muscarinic acetylcholine receptor agonist; Stomach, isolated, mouse

1. Introduction

Somatostatin is a ubiquitous inhibitory peptide found in mammals (Chiba and Yamada, 1994). The peptide and its receptors are widely distributed in the stomach, pancreas, intestine, and central nervous system (Lewin, 1992; Bruno et al., 1993). In gut, somatostatin, which is released from D cells located in the fundus and the antral areas of the stomach (Penmann et al., 1983), inhibits gastric acid secretion (Bloom et al., 1974; Sachs et al., 1997; Chen et al., 1999). Several mechanisms of the antisecretory effect have been elucidated: one is a direct inhibitory effect on parietal cells (Park et al., 1987; Schubert et al., 1989; Schmidtler et al., 1992); the other is the inhibition of histamine release from gastric enterochromaffin-like cells (Chuang et al., 1993; Prinz et al., 1994; Sandor et al., 1996). Five distinct somatostatin receptors have been cloned, and many researchers have investigated which subtype mediates the diverse biological actions of this peptide (McKeen et al., 1994). Prinz et al. (1994) reported that enterochromaffin-like cells express predominantly type 2 somatostatin (sst_2) receptor mRNA. Immunohistchemical study disclosed the localization of somatostatin $sst_{2(a)}$ receptors in enterochromaffin-like cells and $sst_{2(b)}$ receptors in parietal cells (Schindler and Humphrey, 1999). It has also been revealed that the inhibition of

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gastric acid secretion is mediated by somatostatin sst_2 receptors located in enterochromaffin-like cells (Wyatt et al., 1996; Zaki et al., 1996; Aurang et al., 1997) and in parietal cells (Wyatt et al., 1996; Aurang et al., 1997). However, which of the two mechanisms plays the more important role in the inhibition of gastric acid secretion by somatostatin under physiological conditions is poorly understood. In the present study, we tried to elucidate peripheral inhibitory control mediated by somatostatin using an isolated mouse stomach preparation.

The isolated mouse stomach preparation was devised by Wan (1977) and Angus and Black (1979) and has been utilized to study peripheral regulation of gastric acid secretion. This experimental model permitted the present study on peripheral control mechanisms by somatostatin without the influence of complicated systemic factors such as blood flow, blood pressure, and control by the central nervous system (Horie et al., 1993, 1994). We have modified a method for quantitative measurement of gastric acid secretion using this preparation, and functionally differentiated gastric parietal cells and enterochromaffin-like cells using this preparation and several kinds of acid secretagogues (Hasebe et al., 1998, 2001; Fukamachi et al., 2001). The present results indicated that the inhibition of gastric acid secretion by somatostatin is mediated mainly by inhibition of histamine release from enterochromaffin-like cells.

2. Materials and methods

2.1. Animals

Four- or five-week-old male ddY mice (19–38 g, Japan SLC, Japan) were used after preliminary rearing on tap water and a pellet diet in cages. The experiments were carried out in strict accordance with "Guiding Principles for the Care and Use of Laboratory Animals" approved by the Japanese Pharmacological Society and the guidelines approved by the Ethical Committee on Animal Care and Animal Experiment of the Graduate School of Pharmaceutical Sciences, Chiba University.

2.2. Chemicals

Histamine dihydrochloride, famotidine, 4-[[[(3-chlorophenyl)amino]carbonyl]oxy]-*N*,*N*,*N*,-trimethyl-2-butynyl-1-aminium chloride (McN-A-343), carbamyl-β-choline chloride (bethanechol), *N*-*t*-BOC-β-Ala-Trp-Met-Asp-Phe-amide (pentagastrin) and *S*-[4-(*N*,*N*-dimethylamino)-butyl]isothiourea dihydrochloride (SKF-91488) were purchased from Sigma (St. Louis, MO, USA). Somatostatin-14 was purchased from Novabiochem (Läufelfingen, Switzerland). Atropine sulfate was purchased from Nacalai Tesque (Kyoto, Japan). Famotidine was dissolved in saline containing a small amount of 0.1 N HCl. Other agents were dissolved in distilled water.

2.3. Preparation of isolated mouse stomach and measurement of acid secretion

Male mice were fasted for 3–4 h with free access to water before experiments. Gastric acid secretion was measured in isolated mouse stomachs as described previously (Wan, 1977; Angus and Black, 1979) with a slight modification (Watanabe et al., 1993; Horie et al., 2000). Briefly, the stomach was exposed and a 2-mm incision was made in the forestomach under urethane anesthesia (1.5 g/kg, i.p.). A dual polyethylene cannula was inserted into the incision. After ligation of the pylorus and the esophagus, the stomach was isolated, and placed in a 20-ml organ bath containing a serosal nutrient solution (128 mM NaCl, 4.8 mM KCl, 1.2 mM $MgSO_4$, 1.3 mM $CaCl_2$, 30 mM glucose, 10 mM HEPES, adjusted to pH 7.0 with NaOH and aerated with 95% O_2 and 5% CO_2) and kept at 37 °C. The organ bath is made of a plastic tube. The initial intragastric pressure was kept at 20 cm H_2O before the perfusion of the mucosal nutrient solution. The volume of the gastric lumen was about 2.5 ml. The gastric lumen was perfused at 1 ml/min with a mucosal nutrient solution (137 mM NaCl, 4.8 mM KCl, 1.2 mM $MgSO_4$, 1.3 mM $CaCl_2$, 30 mM glucose, adjusted to pH 5.0 with HCl) through the inlet tube of the dual cannula connected to the perfusion pump (Mini Pump TMP-10H, Toyo Kagaku Sangyo, Japan). The perfusate exited through the outlet tube and was recovered in 10-min fractions using a fraction collector. The acid output in each 10-min perfusate fraction was measured by titrating it with an automatic titrator (AUT-201, Toa Electronics, Tokyo, Japan). The basal acid output in these experiments was unstable just after the set-up of the preparations, and it took at least 30 min to stabilize basal acid secretion. The experiments were started at least 30 min after the set-up of the preparations. The value of basal acid output is 0.35 ± 0.05 μEq H^+/10 min after the stabilization. The amount of acid output shown in all figures is expressed as a net value against a value in a 10-min fraction before the addition of the secretagogue in each preparation. Namely, the amount of acid output in a 10-min fraction before the addition of the secretagogue is subtracted from that in each fraction. The acid output values at the 5th, 15th, 25th min, etc., in the figures for time course indicate the amount of acid output collected from 0 to 10 min, from 10 to 20 min, from 20 to 30 min, etc., respectively, after the stimulation. All drugs were added to the serosal solution. Famotidine, atropine and somatostatin were added 10, 10 and 40 min, respectively, before the application of secretagogue. The preparation was exposed to the drugs all through the experiment after the drugs were added into the organ bath.

2.4. Measurement of histamine release from whole isolated mouse stomach

The whole isolated mouse stomachs were prepared as described above. The experiments for determination of hista-

mine were performed independently of those for measurement of gastric acid secretion. The 300 μl serosal nutrient solution was recovered 40 and 110 min after the addition of somatostatin. 4-(*N*,*N*-Dimethylamino)butylthioisurea dihydrochloride (SKF-91488, 100 μM), an inhibitor of the histamine *N*-methyltransferase, was applied 15 min before the administration of somatostatin in order to inhibit histamine metabolism. All drugs were added to the serosal solution. The preparation was exposed to the drugs all through the experiment after the drugs were added into the organ bath. It took about 50 min to stabilize basal values of histamine release after the set-up of the preparations. So, the experiments were started 50 min after the set-up of the preparations. The samples were stored at −20 °C until the determination of histamine. The amount of histamine released in the serosal solution was measured using an enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI, USA). The control experiments were performed using the same lot of animals simultaneously in order to exclude the difference of the reactivity of each preparation.

2.5. Statistical analyses

Data are presented as mean ± S.E.M. Statistical analyses were performed by two-tailed Student's *t*-test for comparison of two groups, and by a one-way analysis of variance followed by a Dunnett's multiple comparison test for comparison of more than two groups. Values of $P < 0.05$ were regarded as significant.

3. Results

3.1. Effect of somatostatin on histamine-induced acid secretion

The application of histamine (500 μM) increased gastric acid secretion that reached a peak at 30 min in isolated mouse stomach preparations. The gastric acid secretion stimulated by histamine was not inhibited by addition of 1 μM somatostatin into the serosal solution (Fig. 1A). Even 10 μM of somatostatin had no effect, or rather, a stimulatory effect on histamine-induced acid secretion (Fig. 1B). But the data dispersion is large, so the stimulatory effect is obscure.

The application of somatostatin (10 μM) into the mucosal solution also failed to inhibit the histamine-induced acid secretion (data not shown). The application of somatostatin to the serosal solution after histamine addition did not also inhibit acid secretion (data not shown). Thus, it is supposed that somatostatin does not affect histamine-induced acid secretion in isolated mouse stomach.

The histamine-stimulated gastric acid secretion was completely inhibited by serosal application of the histamine-H_2 receptor antagonist famotidine 10 μM (control, 13.27 ± 1.21 μEq H^+/70 min, $n = 6$; famotidine treatment, 2.41 ± 0.52 μEq H^+/70 min, $n = 5$, $P < 0.001$ vs. control group) as reported previously (Watanabe et al., 1993; Yamamoto et al., 1995).

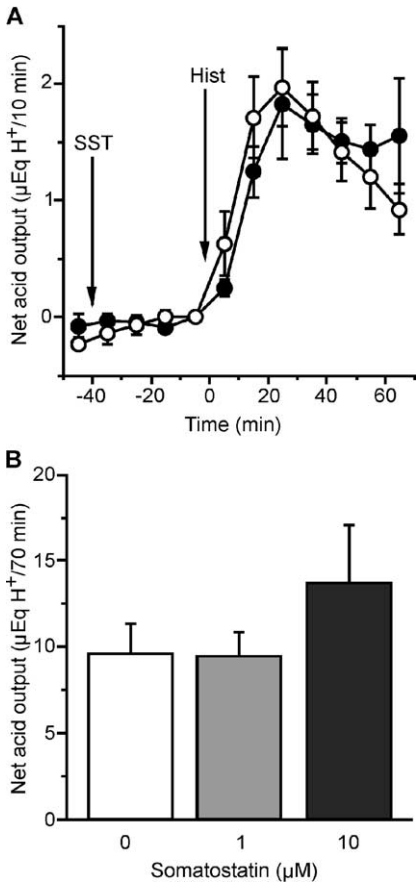

Fig. 1. Effect of somatostatin on histamine-stimulated gastric acid secretion in isolated mouse whole stomach. (A) Time course of acid output measured every 10 min. Somatostatin (SST, 1 μM) was applied 40 min before the addition of histamine (Hist, 500 μM). Control (○); SST-treated group (●). The amount of acid output is expressed as a net value against a value in a 10-min fraction before the addition of Hist in each preparation. (B) Total acid output. SST (1–10 μM) was applied 40 min before the addition of Hist (500 μM). Total acid output collected for 70 min after the stimulation was presented. Each value represents the mean ± S.E.M. of five to nine mice.

3.2. Effect of somatostatin on McN-A-343-induced acid secretion

The muscarinic M_1 receptor agonist McN-A-343 (300 μM) produced an increase in gastric acid secretion. The McN-A-343-stimulated gastric acid secretion was inhibited by 1 μM atropine (control, 11.07 ± 2.89 μEq H^+/70 min, $n = 5$; atropine treatment, 1.07 ± 0.78 μEq H^+/70 min, $n = 5$, $P < 0.05$ vs. control group). Pretreatment with 1 μM somatostatin, which did not inhibit histamine-induced acid secretion, abolished McN-A-343-induced acid secretion when added 40 min before the stimulation (Fig. 2A). The inhibitory effect of somatostatin was dependent on the concen-

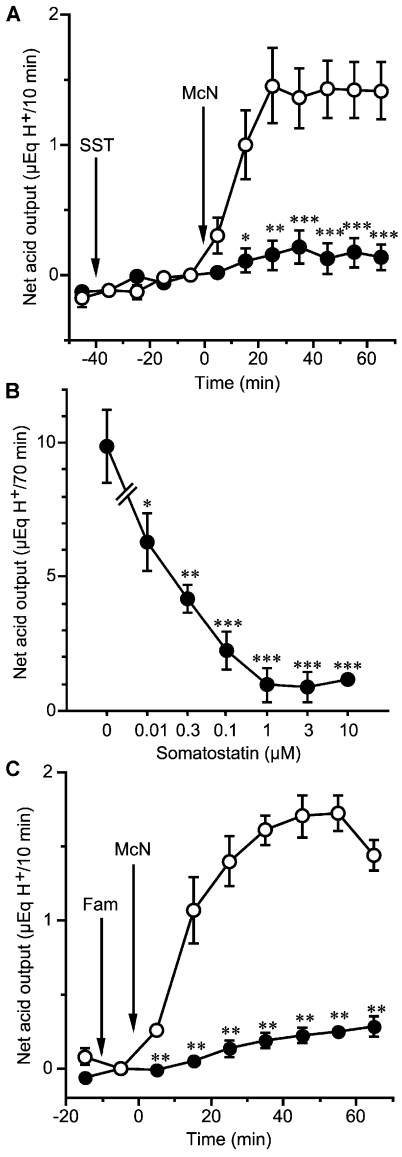

Fig. 2. Effects of somatostatin (A, B) and famotidine (C) on McN-A-343-stimulated gastric acid secretion in isolated mouse whole stomach. (A) Time course of acid output measured every 10 min ($n=8-9$). Control (○); somatostatin (SST, 1 μM)-treated group (●). (B) Concentration dependency of effect of SST ($n=6-9$). SST (0.01–10 μM) was applied 40 min before the addition of McN-A-343 (McN, 300 μM). (C) Time course of the acid output measured every 10 min ($n=5$). Famotidine (Fam, 10 μM) was applied 10 min before the addition of McN (300 μM). Control (○); Fam-treated group (●). The amount of acid output is expressed as a net value against a value in a 10-min fraction before the addition of McN in each preparation. Each value represents the mean ± S.E.M. $*P<0.05$, $**P<0.01$, $***P<0.001$, significantly different from the corresponding control group.

tration used (10 nM–1 μM) (Fig. 2B). On the other hand, somatostatin did not inhibit McN-A-343-induced acid secretion when added 10 min before the stimulation (data not shown).

The pretreatment with famotidine (10 μM) also completely inhibited acid secretion stimulated by McN-A-343 (Fig. 2C) as reported previously (Hasebe et al., 1998).

3.3. Effect of somatostatin on pentagastrin-induced acid secretion

Pentagastrin (3 μM) produced an increase in gastric acid secretion in isolated mouse stomach preparation. Pretreatment with 10 μM somatostatin abolished pentagastrin-induced acid secretion (Fig. 3A). Famotidine (10 μM) also completely inhibited pentagastrin-induced acid secretion (Fig. 3B) as reported previously (Hasebe et al., 1998).

3.4. Effect of somatostatin on bethanechol-induced acid secretion

We investigated the effect of somatostatin on acid secretion induced by the cholinergic agent bethanechol at

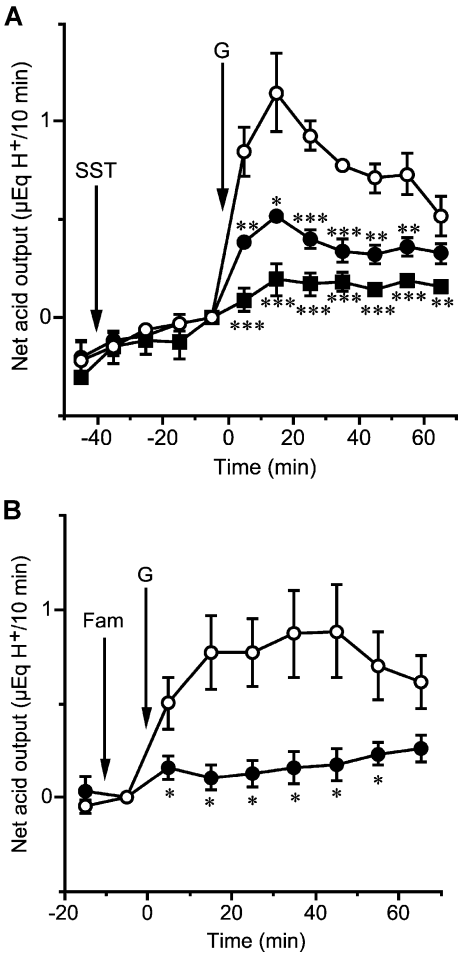

Fig. 3. Effects of somatostatin (A) and famotidine (B) on pentagastrin-stimulated gastric acid secretion in isolated mouse whole stomach. Time course of acid output measured every 10 min. (A) Somatostatin (SST, 1–10 μM) was applied 40 min before the addition of pentagastrin (G, 3 μM). Control (○); SST-treated group (1 μM: ●; 10 μM, ■). $n=4$. (B) Famotidine (Fam, 10 μM) was applied 10 min before the addition of G (3 μM). Control (○); Fam-treated group (●). $n=5$. The amount of acid output is expressed as a net value against a value in a 10-min fraction before the addition of G in each preparation. Each value represents the mean ± S.E.M. $*P<0.05$, $**P<0.01$, $***P<0.001$, significantly different from the corresponding control group.

two doses: 10 and 100 μM. Bethanechol at 10 μM produced an increase in gastric acid secretion (Fig. 4A). Addition of bethanechol at 100 μM also produced a relatively long-lasting increase in gastric acid secretion (Fig. 5A). Bethanechol at 100 μM elicits a maximum effect, judging from the concentration–response curve reported previously (Yamamoto et al., 1995). The acid secretion stimulated by bethanechol at both 10 and 100 μM was completely inhibited by 1 μM atropine (data not shown).

As shown in Fig. 4A, pretreatment with somatostatin (1 μM) inhibited the acid secretion stimulated by 10 μM bethanechol. The sustained acid secretion induced by 10 μM bethanechol was reduced more strongly by somatostatin

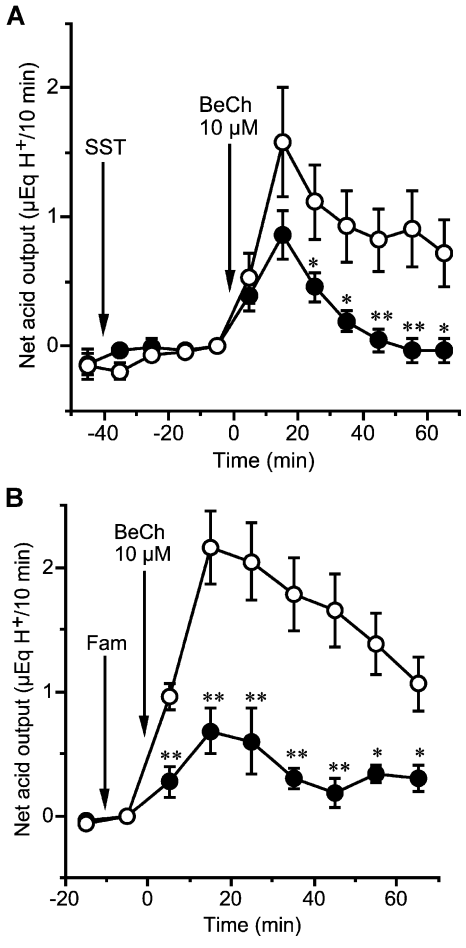

Fig. 4. Effects of somatostatin (A) and famotidine (B) on bethanechol-stimulated gastric acid secretion in isolated mouse whole stomach. Time course of acid output measured every 10 min. (A) Somatostatin (SST, 1 μM) was applied 40 min before the addition of bethanechol (BeCh, 10 μM). Control (○); SST-treated group (●). $n=5$. (B) Famotidine (Fam, 10 μM) was applied 10 min before the addition of BeCh (10 μM). Control (○); Fam-treated group (●). $n=4–5$. The amount of acid output is expressed as a net value against a value in a 10-min fraction before the addition of BeCh in each preparation. Each value represents the mean ± S.E.M. $*P<0.05$, $**P<0.01$, significantly different from the corresponding control group.

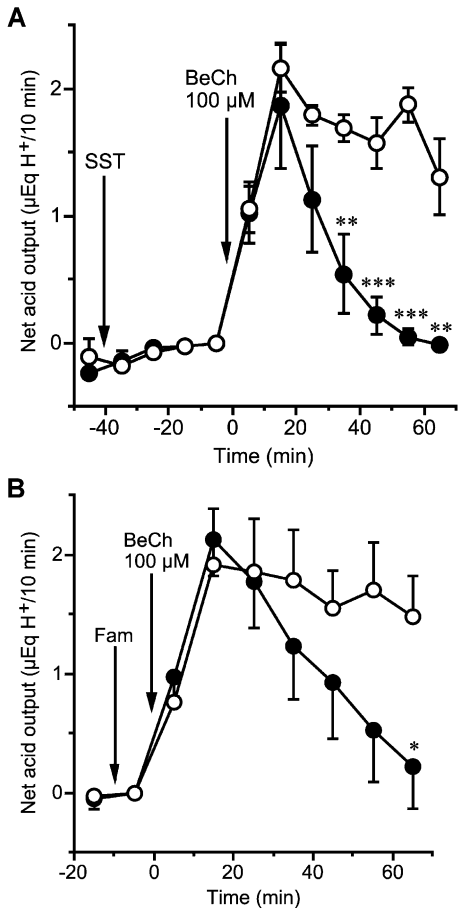

Fig. 5. Effects of somatostatin (A) and famotidine (B) on bethanechol-stimulated gastric acid secretion in isolated mouse whole stomach. Time course of acid output measured every 10 min. (A) Somatostatin (SST, 1 μM) was applied 40 min before the addition of bethanechol (BeCh, 100 μM). Control (○); SST-treated group (●). $n=5$. (B) Famotidine (Fam, 10 μM) was applied 10 min before the addition of BeCh (100 μM). Control (○); Fam-treated group (●). $n=5–6$. The amount of acid output is expressed as a net value against a value in a 10-min fraction before the addition of BeCh in each preparation. Each value represents the mean ± S.E.M. $*P<0.05$, $**P<0.01$, $***P<0.001$, significantly different from the corresponding control group.

than the initial peak secretion. Famotidine completely inhibited the late component of the stimulated acid secretion, while it moderately inhibited the initial component (Fig. 4B). Then the inhibitory effect of somatostatin was evaluated by the comparison of acid output during the periods of 0 to 20 min (the initial half) and 50 to 70 min (the latter half) after the addition of bethanechol. As shown in Table 1, somatostatin completely inhibited the acid secretion during the latter half in a dose-dependent manner, while it moderately inhibited it during the initial half.

Next, we investigated effect of somatostatin on acid secretion induced by bethanechol at 100 μM. Somatostatin (1 μM) had no effect on the bethanechol-induced gastric acid secretion for about 20 min after the stimulation, but a significant inhibition was observed after that time (Fig. 5A).

Table 1
Effect of somatostatin on bethanechol (10 μM)-induced acid secretion in isolated mouse stomach

	Concentration	Net acid output after stimulation	
		0–20 min (μEq H^+/20 min)	50–70 min (μEq H^+/20 min)
Experiment I			
Control		2.11 ± 0.57 (5)	1.63 ± 0.55 (5)
Somatostatin	10 nM	1.48 ± 0.36 (5)	1.03 ± 0.23 (5)
	1 μM	1.26 ± 0.30 (5)	−0.06 ± 0.18[a] (5)
Experiment II			
Control		3.13 ± 0.39 (5)	2.45 ± 0.46 (5)
Famotidine	10 μM	0.97 ± 0.18[b] (4)	0.65 ± 0.17[a] (4)

The inhibitory effect of somatostatin was evaluated using acid output during the period of 0 to 20 min (the initial half) and 50 to 70 min (the latter half) after the addition of bethanechol (10 μM). Somatostatin and famotidine were applied to the organ bath 40 and 10 min, respectively, before the addition of bethanechol. Each value represents the mean ± S.E.M. Number of animals (*n*) is shown in parenthesis.

[a] $P < 0.05$, significantly different from the corresponding control group.

[b] $P < 0.01$, significantly different from the corresponding control group.

A similar result was obtained in the case of pretreatment with famotidine in 100 μM bethanechol-stimulated preparations (Fig. 5B). Then the inhibitory effect of somatostatin was evaluated by the comparison of acid output during the period of 0 to 20 min (the initial half) and 50 to 70 min (the latter half) after the addition of bethanechol. Table 2 shows that somatostatin inhibited acid secretion during the latter half in a dose-dependent manner, but did not during the initial half. The latter half acid secretion induced by 10 and 100 μM bethanechol was inhibited to the basal level by somatostatin or famotidine.

Table 2
Effect of somatostatin on bethanechol (100 μM)-induced acid secretion in isolated mouse stomach

	Concentration	Net acid output after stimulation	
		0–20 min (μEq H^+/20 min)	50–70 min (μEq H^+/20 min)
Experiment I			
Control		3.20 ± 0.33 (5)	3.18 ± 0.39 (5)
Somatostatin	10 nM	3.07 ± 0.36 (5)	2.70 ± 0.46 (5)
	1 μM	2.89 ± 0.73 (5)	0.03 ± 0.09[a] (5)
Experiment II			
Control		2.67 ± 0.65 (6)	3.18 ± 0.73 (6)
Famotidine	10 μM	3.10 ± 0.45 (5)	0.74 ± 0.77[b] (5)

The inhibitory effect of somatostatin was evaluated using acid output during the period of 0 to 20 min (the initial half) and 50 to 70 min (the latter half) after the addition of bethanechol (100 μM). Somatostatin and famotidine were applied to the organ bath 40 min and 10 min, respectively, before the addition of bethanechol. Each value represents the mean ± S.E.M. Number of animals (*n*) is shown in parenthesis.

[a] $P < 0.001$, significantly different from the corresponding control group.

[b] $P < 0.05$, significantly different from the corresponding control group.

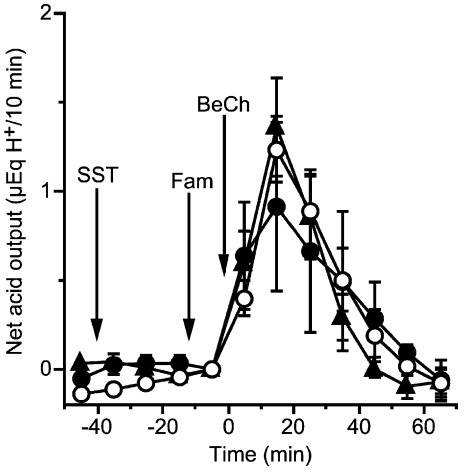

Fig. 6. Effect of somatostatin on gastric acid secretion stimulated by bethanechol in the presence of famotidine in isolated mouse whole stomach. Time course of the acid output measured every 10 min. Somatostatin (SST, 1–10 μM) was applied 40 min before the addition of bethanechol (BeCh, 100 μM). Famotidine (Fam, 10 μM) was applied 10 min before the addition of BeCh. Control (○); SST (1 μM)-treated group (●); SST (10 μM)-treated group (▲). The amount of acid output is expressed as a net value against a value in a 10-min fraction before the addition of BeCh in each preparation. Each value represents the mean ± S.E.M. of five to seven mice.

3.5. Effect of somatostatin on bethanechol-induced acid secretion in the presence of famotidine

We investigated the effect of somatostatin on acid secretion induced by bethanechol (100 μM) in the presence of famotidine (10 μM). In the presence of famotidine, bethanechol induced transient acid secretion that reached a peak at 20 min and that was not sustained. Somatostatin (1–10 μM) did not affect the transient increase in gastric acid secretion (Fig. 6).

Table 3
Effect of somatostatin on histamine release stimulated by McN-A-343 or bethanechol in mouse isolated whole stomach

Treatment	Histamine release (pmol/stomach)	
	None	Somatostatin 1 μM
Control	82.3 ± 5.3	78.3 ± 21.0
McN-A-343	452.0 ± 42.8[a]	163.0 ± 29.7[b]
Bethanechol	1929.0 ± 375.6[c]	570.8 ± 260.3[d]

Somatostatin was applied to the organ bath 40 min before the addition of McN-A-343 (300 μM) or bethanechol (100 μM). Each value represents the mean ± S.E.M. of four mice.

[a] $P < 0.01$, significantly different from the corresponding control group.

[b] $P < 0.01$, significantly different from the corresponding somatostatin-untreated groups (none).

[c] $P < 0.05$, significantly different from the corresponding control group.

[d] $P < 0.05$, significantly different from the corresponding somatostatin-untreated groups (none).

3.6. Effect of somatostatin on histamine release stimulated by McN-A-343 or bethanechol

McN-A-343 (300 μM) and bethanechol (100 μM) elicited increases in histamine release for 70 min after the stimulation (Table 3). Bethanechol released a larger amount of histamine than McN-A-343 did. Somatostatin (1 μM) had no effect on basal histamine secretion, but it significantly inhibited histamine release induced by McN-A-343 and bethanechol (Table 3).

4. Discussion

4.1. Inhibition by somatostatin of McN-A-343- and pentagastrin-induced acid secretion

A subtype of muscarinic receptors on gastric parietal cells was identified as muscarinic M_3 receptors, but it has not yet been determined on enterochromaffin-like cells (Hirschowitz et al., 1995; Hersey and Sachs, 1995). McN-A-343 is as a muscarinic M_1 receptor agonist, but, in fact, this compound is a non-selective partial agonist of muscarinic M_1, M_2 and M_3 receptors (Eglen et al., 1987). Despite its relatively low efficacy as a muscarinic receptor agonist, McN-A-343 was used in the present study because its ability to induce acid secretion is abolished by histamine H_2 receptor agonists, but not by tetrodotoxin, a blocker of neurotransmission (Black and Shankley, 1985, 1987; Kromer et al., 1989). In the present study, we confirmed that famotidine completely inhibited McN-A-343-induced acid secretion. Taken together, McN-A-343 is considered to induce gastric acid secretion not via direct stimulation of gastric parietal cells but via histamine release from gastric enterochromaffin-like cells (the famotidine-sensitive pathway). Kromer et al. (1990) have claimed that the muscarinic receptors which is involved in acid secretion induced by McN-A-343 in isolated mouse stomach have muscarinic M_1 receptor nature. On the other hand, pentagastrin, a cholecystokinin CCK_2 receptor agonist, induced acid secretion, an effect that was abolished by famotidine. This shows that pentagastrin also induces gastric acid secretion via histamine release from enterochromaffin-like cells. So we considered McN-A-343 and pentagastrin the most suitable secretagogues to investigate whether somatostatin affects the histamine-mediated pathway of acid secretion.

In the present study, somatostatin inhibited gastric acid secretion stimulated by McN-A-343 in a concentration-dependent manner. The stimulated acid secretion was reduced to the basal level by somatostatin at more than 0.1 μM. In addition, we investigated an inhibitory effect on histamine release in isolated mouse stomach preparations. As expected, somatostatin reduced histamine release elicited by McN-A-343. These results are consistent with the previous reports that somatostatin inhibits histamine release from the enterochromaffin-like cells in experiments with isolated human, dog, and rat gastric antrum (Zaki et al., 1996) and cultured canine gastric mucosal cells (Chuang et al., 1993).

In the present study, somatostatin inhibited gastric acid secretion stimulated by pentagastrin. Prinz et al. (1994) reported that somatostatin acts on somatostatin sst_2 receptors, which are predominantly expressed in cultured enterochromaffin-like cells, leading to the inhibition of gastrin-induced histamine release by reducing the increased intracellular Ca^{2+}. Taken together, it is suggested that somatostatin acts on gastric enterochromaffin-like cells and then inhibits histamine release. This mechanism is probably responsible for the inhibitory effect of somatostatin on the stimulated acid secretion.

4.2. Inhibition by somatostatin of bethanechol-induced acid secretion

We investigated the effect of famotidine on acid secretion induced by bethanechol at two doses: 10 and 100 μM. Pretreatment with famotidine reduced the latter sustained acid secretion stimulated by 10 μM bethanechol to the basal level, whereas its inhibition of the initial peak secretion is moderate. In addition, famotidine also inhibited the late response to 100 μM bethanechol to the basal level, but it did not affect the initial peak response at all. These results suggest that bethanechol-induced acid secretion is mediated both by direct activation of parietal cells (the famotidine-insensitive pathway) and by histamine release from enterochromaffin-like cells (the famotidine-sensitive pathway) in our preparations. The initial peak acid output caused by bethanechol is a consequence of direct activation of parietal cells, and the late acid output is a consequence of histamine release from enterochromaffin-like cells. We previously observed slight inhibition of bethanechol-induced acid secretion by famotidine, and suggested that the cholinergic stimulation of acid secretion is not largely mediated by histamine release (Watanabe et al., 1993). In that previous report, famotidine did not reduce the initial acid output obtained for 30 min after the stimulation. Thus, our previous findings are consistent with the present results that famotidine did not affect the initial peak acid secretion induced by 100 μM bethanechol.

We investigated effect of somatostatin on acid secretion induced by bethanechol. Pretreatment with somatostatin reduced the late response to bethanechol to the basal level, whereas the inhibition on the initial peak response is slight. This effect of somatostatin on bethanechol-induced acid secretion is quite similar to the effect of famotidine described above. We further investigated effects of somatostatin on bethanechol-induced histamine release in isolated mouse stomach. Bethanechol induced the release of a larger amount of histamine than McN-A-343 did. Somatostatin markedly inhibited histamine release stimulated by bethanechol. Taken together, these results suggest that the effect of somatostatin is largely due to the blockade of histamine release rather than the direct inhibition of parietal cells. Namely, somatostatin

does not inhibit parietal cells, but does inhibit enterochromaffin-like cells.

4.3. Lack of inhibitory effect of somatostatin on acid secretion stimulated by a direct stimulation of gastric parietal cells

Somatostatin, even at 10 μM, a 10-fold higher concentration than that which elicited the maximum inhibitory effect on McN-A-343-induced acid secretion, had no effect on acid secretion stimulated by histamine. In addition, somatostatin did not inhibit a transient increase in acid secretion induced by bethanechol in the presence of famotidine. These results show that somatostatin does not affect the acid secretory mechanisms via histamine H_2 receptors or muscarinic M_3 receptors in parietal cells. Taken together, somatostatin had no effect on the acid secretion stimulated by a direct activation of parietal cells. Athmann et al. (2000) reported that somatostatin does not inhibit increases of intracellular Ca^{2+} levels in parietal cells stimulated by histamine, gastrin, or carbachol, but it abolishes enterochromaffin-like cell Ca^{2+} responses to gastrin in rabbit gastric gland preparations. Our conclusion is in good agreement with this finding of Athmann et al. (2000).

We previously reported that the acid secretion elicited by the electrical stimulation of vagal nerves is not affected by famotidine in isolated mouse stomach (Yamamoto et al., 1995). It may be interpreted that endogenous somatostatin released by the electrical stimulation does not affect the acid secretion via the famotidine-insensitive pathway, namely via direct activation of parietal cells. This is consistent with the present conclusion. Further study must be done to elucidate role of endogenous somatostatin under physiological conditions.

Somatostatin inhibited aminopyrine accumulation stimulated by histamine in a rat parietal cell preparation (Schmidtler et al., 1992) and aminopyrine accumulation stimulated by carbachol in a canine parietal cell preparation (Park et al., 1987). These findings seem to contradict the present results. Most of the findings that somatostatin directly inhibits the acid secretory mechanisms in parietal cells are obtained from investigations using isolated parietal cell preparations. In the present study, we used a whole stomach preparation, and clarified its inhibitory mechanisms of peripheral acid secretion at the whole tissue level. It has been so far reported that the antisecretory effect of somatostatin results from an inhibition of histamine release from gastric enterochromaffin-like cells (Chuang et al., 1993; Prinz et al., 1994; Sandor et al., 1996; Wyatt et al., 1996; Aurang et al., 1997). These findings are consistent with our conclusion.

On the other hand, somatostatin inhibited McN-A-343-induced acid secretion when added 40 min before the application of the secretagogue, but not when added 10 min before in the present study. These results may mean that it is difficult for somatostatin to gain access to its receptors under our experimental conditions. Possibly, they may mean that it takes time to induce the inhibitory effect of somatostatin. The different conclusions described above are attributed to the differences of the experimental models, the protocol and methods for measurement of acid secretion.

4.4. Inhibition by somatostatin of acid secretion in isolated stomach

The most important observation from the present study using isolated mouse stomach is that the acid secretory response via the endogenous histamine-mediated pathway was inhibited by somatostatin, but the response to a direct activation of gastric parietal cells was not. These results suggest that the inhibition of histamine release from enterochromaffin-like cells plays a more important role in the inhibition of gastric acid secretion by somatostatin than the direct inhibitory effect on parietal cells.

It is noteworthy that cholinergic stimulation elicits sustained acid secretion through the activation of parietal cells by histamine released from gastric enterochromaffin-like cells, and somatostatin inhibits the sustained acid secretion more strongly than the initial peak secretion after the cholinergic stimulation.

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