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Inhibitory effect of N^{ω}-nitro-L-arginine on gastric secretion induced by secretagogues and vagal stimulation in the isolated stomach

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Abstract

The involvement of endogenous nitric oxide (NO) in the control of gastric acid secretion induced by some secretagogues was studied in the mouse isolated whole stomach. The gastric acid secretion induced by McNeil A-343 {4-[[[(3-chlorophenyl)amino]carbonyl]oxy]-*N*,*N*,*N*,-trimethyl-2-butyn-1-aminium chloride}, a muscarinic M_1 receptor agonist, pentagastrin or electrical vagus nerve stimulation was markedly inhibited by pretreatment with the NO synthase inhibitor N^{ω}-nitro-L-arginine (L-NNA). This inhibitory effect of L-NNA was reversed by L-arginine, but not by D-arginine. Histamine-induced gastric acid secretion was not influenced by treatment with L-NNA. Famotidine completely inhibited the gastric acid secretion induced by McNeil A-343, pentagastrin or electrical vagus nerve stimulation, showing that these stimulations induced gastric acid secretion mainly through histamine release from histamine-containing cells in the gastric mucosa. Moreover, the pentagastrin- and bethanechol-induced histamine release from gastric mucosal cells was significantly inhibited by L-NNA. The NO donor, sodium nitroprusside, at a concentration not affecting histamine-induced gastric acid secretion, increased the acid secretory response, and this response was inhibited by famotidine. These results suggest that endogenous NO is involved in the gastric acid secretion via histamine release from histamine-containing cells.

Keywords: Nitric oxide (NO); Gastric acid secretion; Histamine; Stomach, mouse, isolated; Enterochromaffin-like cell

1. Introduction

The functional roles of nitric oxide (NO) in the physiology of the gastrointestinal tract have been widely studied, but they have not been fully elucidated yet. It is well-known that NO modulates gastric mucosal integrity in combination with prostaglandins through regulating microcirculation by its vasodilating effect (Whittle et al., 1990; Mariotto et al., 1995). It has been shown that exogenous NO inhibits neurally mediated gastric acid secretion in vivo in rats (Barrachina et al., 1994), as well as the acid secretory response to histamine in rat isolated parietal cells (Brown et al., 1993). It is reported that endogenous NO may contribute to the gastroprotection by pentagastrin against ethanol-induced gastric mucosal damage (Stroff et al., 1994), and endogenous NO is suggested to be, although not directly, involved in the pentagastrin-stimulated gastric acid secretion through mucosal vasodilation (Pique et al., 1992). Further, the activity of constitutive NO synthase in parietal cells is quite low (Brown et al., 1992).

Histamine H_2 receptor antagonists inhibit not only the histamine-induced gastric acid secretion but also the gastrin-induced or vagus-induced acid secretory response (Black and Shankley, 1987), suggesting that histamine plays an important role in the peripheral regulation of gastric acid secretion. The cells which synthesize and release histamine in rodent gastric mucosa are considered to be enterochromaffin-like cells (ECL cells) (Håkanson et al., 1986) which have histidine decarboxylase activity (Kubota et al., 1984), a histamine-producing enzyme. It is reported that some substances, for example, vasoactive intestinal polypeptide (VIP) and somatostatin, regulate histamine release from ECL cells (Sandor et al., 1996), and that they play a physiological role in the acid secretory mechanism (Håkanson et al., 1994b). However, the details are not yet known. We devised a method for quantitative measurement of gastric acid secretion of the isolated mouse

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whole stomach (Watanabe et al., 1993) with a modification of the method described by Wan (1977). By using this model, real time recording of the secretory response to vagus nerve stimulation was possible. This experimental model permitted research into the peripheral control of gastric acid secretion in the absence of complicating systemic factors such as blood pressure, blood flow and control by the central nervous system (Horie et al., 1993, 1994, 1996). In the present study, we investigated the involvement of endogenous NO in gastric acid secretion by the method of Wan (1977) modified by that of Watanabe et al. (1993).

2. Materials and methods

2.1. Procedures for setting up the preparation

Male mice, ddY strain (4 weeks old, 18–30 g) were fasted for 3–4 h with free access to water before the experiment. Gastric acid secretion was measured in the mouse isolated whole stomach as described previously (Watanabe et al., 1993). Briefly, under urethane (15 mg/10 g, i.p.) anesthesia, the stomach was exposed and incised about 2 mm on the forestomach. A dual polyethylene cannula was connected through the incision. After ligation of the pylorus and esophagus, the stomach was isolated and placed in a 20-ml organ bath containing a serosal side 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 gassed with 95% 0_2 and 5% CO_2), and kept at 37°C. The volume in the gastric lumen was about 2.5 ml; it was perfused at 1 ml/min with a mucosal 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, and gassed with 100% O_2) through the inlet tube of the dual cannula connected to a perfusion pump (Mini pump TMP-10H, Toyo Kagaku Sangyo, Japan), with the perfusate flowing out from the outlet tube. The intragastric pressure was kept at 20 cm H_2O. All drugs were applied via the serosal solution.

2.2. Measurement of acid secretion induced by electrical vagus nerve stimulation

Electrical vagus nerve stimulation was performed via a pair of platinum electrodes (wire diameter: 0.25 mm, ring diameter: 1.8 mm, the distance between the electrodes: 1.5 mm) fixed at the lower part of the esophagus, as we described previously (Yamamoto et al., 1995). The acid output was continuously titrated with an automatic titrator (Toa Electronics, HM-5ES, HSM-10A, Tokyo). The digital pulse (2 μl/pulse) from the titrator was sent to a personal computer (FM-77, Fujitsu, Tokyo) equipped with a pulse counter (FM-77/8 Interface, Fujitsu, developed by our laboratory). After 30-min equilibration, the first vagal stimulation (5 Hz, 10 V, 0.3 ms) was applied for 5 min with an electric stimulator (SEN-7203, Nihon Koden, Tokyo). Acid secretion increased 10 min after the start of stimulation and returned to its basal level at 30 min. The second vagal stimulation was applied 30 min after the beginning of the first stimulation under the same conditions as the first stimulation. Thirty min after the second stimulation, the third vagal stimulation (2 Hz, 10 V, 0.3 ms) was applied for 5 min. When the second response was less than 1 μEq HCl/stimulation, the preparation was excluded from the experiment.

2.3. Determination of histamine release from gastric mucosal cells

Gastric mucosal cells were isolated from male Wistar rats weighing 280–360 g with a modification of the method described by Schepp et al. (1989) and Sakai et al. (1995). Briefly, rats were killed by exsanguination under ether anesthesia, and the stomach was removed promptly. Then the pylorus was ligated and the stomach was turned inside out. The everted stomach was filled with 5 ml solution A containing 0.1% pronase E and was incubated twice in solution A at 37°C for 45 min with gentle shaking under continuous gassing (95% O_2–5% CO_2). The isolated cells were discarded, and then the everted stomach was incubated in solution B containing 0.2% pronase E at 37°C for 50 min. Separated cells were collected and resuspended in solution D, and then the stomach was stirred three times in ice-cold solution B for 10 min under continuous gassing (95% O_2–5% CO_2). The separated cells were also collected in solution D. About 4×10^9 cells were obtained and their viability was determined to be more than 95% in the trypan blue exclusion test.

A Percoll density-gradient was formed by centrifuging (at 25 000 $\times g$ for 50 min) a mixture of Percoll, solution C and 1.5 M NaCl (45:52:3 in v/v), and isolated cells were laid on the Percoll gradient and centrifuged at 400 $\times g$ for 10 min. The cells obtained were used for the experiments as the ECL cell-enriched fraction of gastric mucosal cells. Their viability was determined to be more than 95% by the same method as described above.

One milliliter of cell suspension (consisting of about 5×10^8 cells) was suspended in microtubes in solution D for 60 min at 37°C in a shaking incubator under basal conditions or after addition of test agents at a volume of 10 μl. The incubation was stopped after 60 min by centrifugation (at 4°C, 400 $\times g$ for 5 min). The supernatant, 400 μl, was added with the same volume of 0.1 M HCl for determination of histamine release.

Histamine was measured by high-performance liquid chromatography (Saito et al., 1982). The histamine levels were calculated using a standard curve with 3 concentrations of histamine standards and expressed as μg/ml.

We used four kinds of nutrient solutions, and their compositions were as follows: Solutions A and C con-

tained (in mM): NaCl 70, KCl 5, NaH_2PO_4 0.5, Na_2HPO_4 1, $NaHCO_3$ 20, glucose 11, ethylenediaminetetraacetic acid (EDTA) 2 and HEPES–NaOH (pH 7.4) 50; 20 mg/ml (solution A) or 1 mg/ml (solution C) bovine albumin F-V was supplemented. Solutions B and D were made by substituting 1 mM $CaCl_2$ plus 1.5 mM $MgCl_2$ for 2 mM EDTA in solutions A and C, respectively.

2.4. Drugs

McNeil A-343, pentagastrin, N^{ω}-nitro-L-arginine (L-NNA), famotidine, L-arginine hydrochloride (L-Arg), D-arginine hydrochloride (D-Arg), carbamyl-β-methylcholine chloride (bethanechol chloride), Percoll and pronase E were purchased from Sigma Chemicals (St. Louis, MO, USA). Histamine dihydrochloride, atropine sulfate and bovine albumin F-V were obtained from Nakalai Tesque (Kyoto, Japan). Sodium pentacyanonitrosylferrate (III) dihydrate (sodium nitroprusside) was purchased from Wako Pure Chemical (Osaka, Japan). YM022 {(*R*)-1-[2,3-dihydro-1-(2′-methylphenacyl)-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl]-3-(3-methylphenyl)urea} was a kind gift from Yamanouchi Pharmaceutical (Tokyo, Japan). Most of the test solutions were prepared just before the beginning of each experiment. McNeil A-343, L-Arg, D-Arg, bethanechol chloride, atropine sulfate, histamine dihydrochloride and sodium nitroprusside were dissolved in saline. Famotidine was prepared in saline after being dissolved with a small amount of 0.1 M HCl. L-NNA was prepared in saline after being dissolved with a small volume of 1 M HCl. Pentagastrin and YM022 were dissolved in dimethyl sulfoxide.

2.5. Statistics

All data are shown as the means ± S.E.M. for 3–5 mice. Statistical analysis was performed with Student's two-tailed *t*-test for unpaired observations or one-way analysis of variance (ANOVA) followed by the Bonferroni multiple comparison test. A P value < 0.05 was considered statistically significant.

3. Results

3.1. Gastric acid secretion induced by electrical vagus stimulation

Basal acid secretion before stimulation was stable for 2–3 h, and the basal secretory rate was approximately 50 nEq HCl/min. Gastric acid secretion was reproducibly induced by electrical vagus nerve stimulation (5 Hz (high-frequency), 10 V, 0.3 ms, for 5 min). Augmentation of the acid secretory response started 2 min after the stimulation, reaching a peak at 9 min (200–350 nEq HCl/min). The acid output gradually returned to the control level 20 min after the end of the stimulation. The response to the second stimulation (5 Hz, 10 V, 0.3 ms, for 5 min) was greater than that the first (300–400 nEq HCl/min). The third weak stimulation (2 Hz (low-frequency), 10 V, 0.3 ms, for 5 min) also induced gastric acid secretion (150–200 nEq HCl/min) (Fig. 1 shows a typical recording), which was

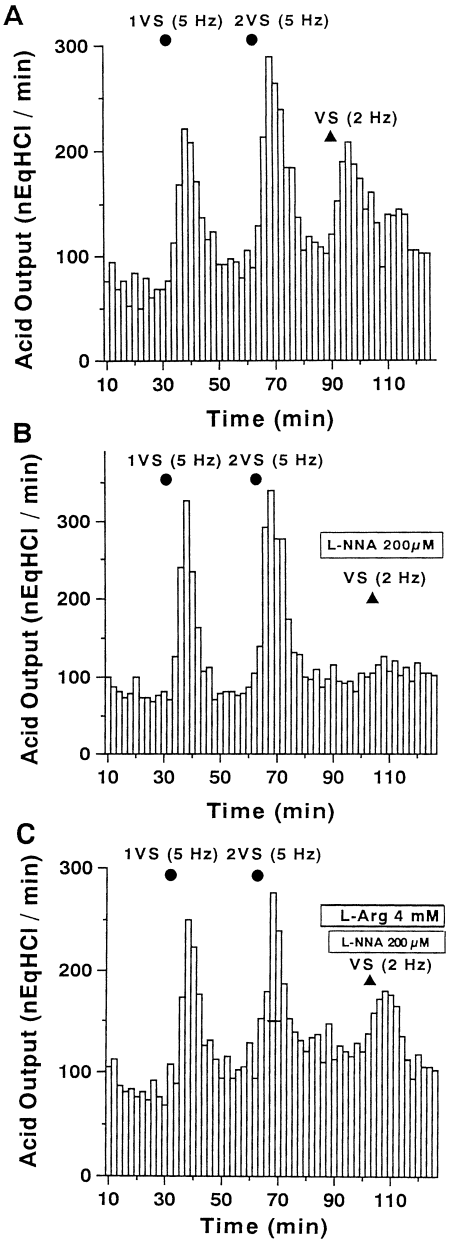

Fig. 1. Typical patterns of gastric acid response to vagus nerve stimulation (VS) in the isolated mouse whole stomach. Each column represents the acid output for 2 min. The 1st (1 VS) and 2nd vagal stimulations (2 VS) were 5 Hz, 10 V, 0.3 ms, for 5 min (●) and the 3rd vagal stimulation (VS) was 2-Hz frequency with the same voltage and duration (▲). (A) Typical pattern of acid response to vagus nerve stimulation. (B) The effect of L-NNA on gastric acid secretion induced by vagus nerve stimulation. (C) The reversal effect of L-Arg on the inhibition by L-NNA of gastric acid secretion induced by vagus nerve stimulation.

found to be sensitive to famotidine, a histamine H_2 receptor antagonist. As shown in Fig. 1, the acid output with the third vagal stimulation was about 50% of that with the second stimulation. The administration of famotidine (10 μM) 10 min before stimulation inhibited the acid response by up to about 95%.

In the vagal stimulation experiments, we regarded the second response as an internal standard, and expressed the electrically stimulated gastric acid secretion as the ratio of the third response to the second response. When the preparation receives the same frequency of electrical vagal stimulation (5 Hz, 10 V, 0.3 ms, for 5 min) three times, the third response has almost the same amplitude as the second response in each preparation, although these responses varied somewhat according to the inherent sensitivities of the preparations. The treatment with L-NNA (200 μM), a NO synthase inhibitor, attenuated the secretory response to the electrical vagal stimulation to a similar degree as did famotidine. The inhibitory effect of L-NNA was dose dependently reversed by L-arginine, a substrate of NO synthase, but not by D-arginine (Fig. 2). Moreover, we found in the present experiment that L-NNA did not affect the response to high-frequency vagal stimulation (control $102 \pm 7\%$; L-NNA treatment $89 \pm 9\%$; $P = 0.29$ vs. control group, $n = 4$). On the other hand, famotidine did not greatly affect the acid secretory response to high-frequency (5 Hz) vagal stimulation (about 30% inhibition, data not shown). The response to vagal stimulation was completely abolished by tetrodotoxin (0.3 μM), atropine (1 μM) or hexamethonium (100 μM) (data not shown). These results are consistent with our previous reports (Watanabe et al., 1993; Yamamoto et al., 1995).

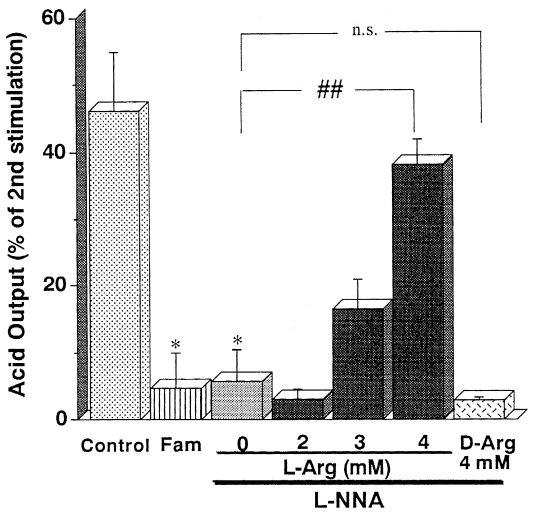

Fig. 2. Effects of famotidine (Fam) and the pretreatment with combinations of L-NNA, L-arginine (L-Arg) and D-arginine (D-Arg) on gastric acid secretion induced by vagus nerve stimulation (2 Hz, 10 V, 0.3 ms, for 5 min). Famotidine (10 μM) or L-NNA (200 μM) was given 10 min before stimulation, whereas L- (2–4 mM) or D-arginine (4 mM) was given 5 min before L-NNA. Each value represents the mean $\pm$ S.E.M. for 3–5 mice. Statistical significance at $* P < 0.05$ from the control group and $^{\#\#}P < 0.01$ from the L-NNA group in the absence of L-arginine. Acid output at the peak response (9–10 min after the stimulation) was as follows: control = 206 ± 16; L-NNA group = 123 ± 4; L-arginine group = 195 ± 13 nEq HCl/min.

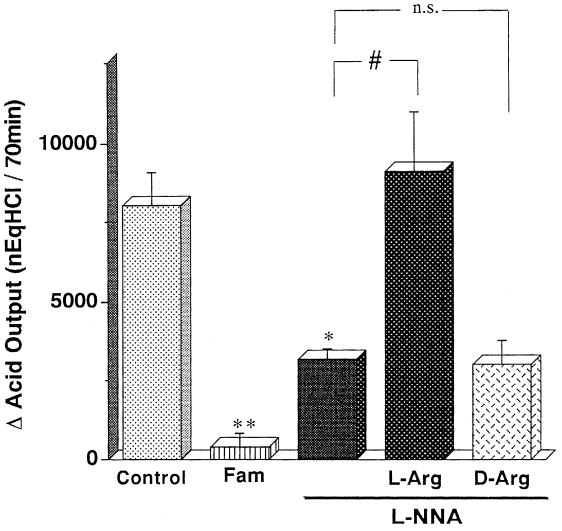

Fig. 3. Effects of famotidine (Fam) and the pretreatment with combinations of L-NNA, L-arginine (L-Arg) and D-arginine (D-Arg) on gastric acid secretion stimulated by McNeil A-343. Famotidine (10 μM) or L-NNA (200 μM) was given 10 min before McNeil A-343 (50 μM), whereas L- (4 mM) or D-arginine (4 mM) was given 5 min before L-NNA. Each value represents the mean $\pm$ S.E.M. for 3–5 mice. Statistical significance at $* P < 0.05$, $** P < 0.01$ from the control group, and $^{\#}P < 0.05$ from the group treated with L-NNA alone. n.s.: not significant.

3.2. McNeil A-343-stimulated gastric acid secretion

Gastric acid secretion began to increase about 10 min after the administration of McNeil A-343, a muscarinic M_1 receptor agonist, and attained a maximum level of 2300 nEq HCl/10 min at 50 min. This maximum response was maintained for at least 30 min. Pretreatment with famotidine (10 μM) blocked the McNeil A-343-stimulated gastric acid secretion (by about 95%, Fig. 3). The pretreatment with L-NNA (200 μM) also markedly reduced the gastric acid secretion induced by McNeil A-343 (about 60%). The inhibitory effect of L-NNA was reversed to the control level by L-arginine (4 mM), the substrate of NO synthase, but not by D-arginine (Fig. 3).

3.3. Pentagastrin-stimulated gastric acid secretion

Gastric acid secretion was immediately augmented by the administration of pentagastrin (3 μM), reaching a submaximal response 5 min after the treatment. This increase was maintained for more than 70 min. Pretreatment with famotidine (10 μM) reduced the pentagastrin-stimulated gastric acid secretion by over 95% (Fig. 4). Pretreatment with L-NNA (200 μM) inhibited the secretion induced by pentagastrin by about 85%, thus less than did famotidine but more than that induced by McNeil A-343

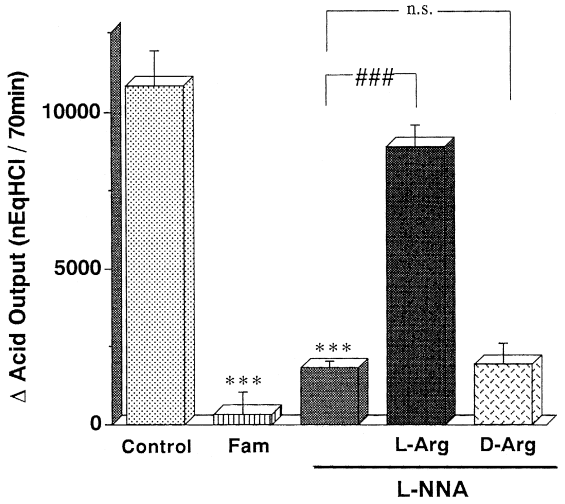

Fig. 4. Effects of famotidine (Fam) and the pretreatment with combinations of L-NNA, L-arginine (L-Arg) and D-arginine (D-Arg) on gastric acid secretion stimulated by pentagastrin. Famotidine (10 μM) or L-NNA (200 μM) was given 10 min before pentagastrin (3 μM), whereas L- (4 mM) or D-arginine (4 mM) was given 5 min before L-NNA. Each value represents the mean ± S.E.M. for 3–5 mice. Statistical significance at $***P < 0.001$ from the control group, and $^{###}P < 0.001$ from the group treated with L-NNA alone. n.s.: not significant.

(Figs. 3 and 4). The inhibitory effect of L-NNA was restored by L-arginine, but not by D-arginine.

3.4. Histamine-stimulated gastric acid secretion

Gastric acid secretion gradually increased after the application of histamine (500 μM), reaching a peak at 30 min. This response level was maintained for at least 2 h (Fig. 5). The treatment with L-NNA (200 μM) did not affect the secretion evoked by histamine, and this response

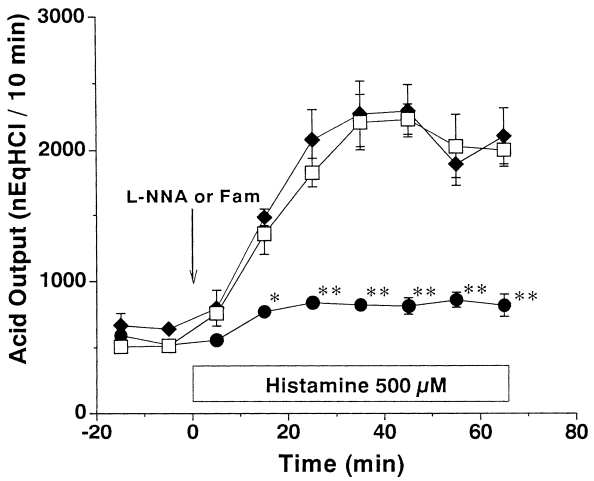

Fig. 5. Effect of L-NNA on gastric acid secretion stimulated by histamine. L-NNA (200 μM) or famotidine (Fam, 10 μM) was given 10 min before histamine (500 μM). Each value represents the mean ± S.E.M. for four mice. Statistical significance at $*P < 0.05$, $**P < 0.01$ from the control group. Control (□); L-NNA (■); famotidine (●).

was also not influenced by a higher dose of L-NNA (400 μM, data not shown), but it was almost completely inhibited by famotidine (10 μM) (Fig. 5). The peak acid outputs in the control group and the treated groups were as follows: control, 2424 ± 139 nEq HCl/10 min; L-NNA, 2425 ± 146 nEq HCl/10 min, $P > 0.8$ vs. control; famotidine, 859 ± 56 nEq HCl/10 min, $P < 0.001$ vs. control; $n = 4$ each (Bonferroni test).

3.5. Histamine release from gastric mucosal cells

Treatment with pentagastrin (20 nM) markedly increased the histamine release from gastric mucosal cells compared with that in the vehicle group, and this response was significantly inhibited by L-NNA (2 μM) and also by YM022 (300 nM), a gastrin/CCK_B receptor antagonist (72% and 75% inhibition, respectively), (Table 1). Bethanechol (2 μM) also induced histamine release, and this response was antagonized by a higher dose of L-NNA (0.2 mM) and atropine (100 nM) (77% and 87% inhibition, respectively) (Table 1).

3.6. Effect of sodium nitroprusside on acid secretory response

The NO donor, sodium nitroprusside, was applied to the stomach preparation during a stable basal acid secretion 30 min after the 2nd vagal stimulation. Sodium nitroprusside at 75 μM, a concentration that did not affect the histamine-induced gastric acid secretion, significantly increased the acid secretory response. This response was significantly inhibited by famotidine (10 μM). The net total acid output for 30 min was as follows: control, 0.1 ± 1 nEq HCl/30 min; SNP, 30 ± 4 nEq HCl/30 min, $P < 0.002$ vs. control; SNP plus famotidine, 0.3 ± 4 nEq HCl/30 min, $P < 0.001$ vs. SNP; $n = 5$ each.

Table 1
Effects of L-NNA on histamine release induced from gastric mucosal cells by pentagastrin or bethanechol

Treatment	n	Histamine release ($\mu g/10^8$ cells)	Inhibition (%)
Pentagastrin treatment			
Vehicle	4	0.10 ± 0.05	
Control (20 nM)	4	3.61 ± 0.56	
+ L-NNA 2 μM	4	1.02 ± 0.25[a]	72
+ YM022 0.3 μM	4	0.89 ± 0.18[a]	75
Bethanechol treatment			
Vehicle	4	0.36 ± 0.22	
Control (2 μM)	4	1.82 ± 0.20	
+ L-NNA 0.1 mM	4	0.90 ± 0.20	51
+ L-NNA 0.2 mM	4	0.41 ± 0.10[b]	77
+ Atropine 0.1 μM	4	0.24 ± 0.24[b]	87

Each value represents the mean ± S.E.M. for four rats. L-NNA, YM022 or atropine was added 5 min before pentagastrin or bethanechol.
[a] $P < 0.05$, [b] $P < 0.05$, compared with the corresponding group.

4. Discussion

In the isolated gastric whole stomach, electrical vagal stimulation reproducibly induced gastric acid secretion, and some other secretagogues, such as McNeil A-343, pentagastrin and histamine also stimulated the acid secretory response. The electrical stimulation used in this study was considered to selectively activate the parasympathetic preganglionic fibers because of the complete block by tetrodotoxin, atropine and hexamethonium. Our preparation, the isolated mouse whole stomach, is suitable for determination of the neuronal mechanisms of gastric acid secretion, and has advantages because it is independent of systemic control (blood flow, central nervous system, etc.). This procedure enables a more precise evaluation of the acid secretory mechanism than do in vivo experiments. L-NNA, an NO synthase inhibitor, inhibited the vagally induced acid secretory response to the same extent as did famotidine. This inhibition was dose dependently reversed by L-arginine, the substrate of NO synthase. The results indicate that endogenous NO is related to the mechanism of vagally stimulated gastric acid secretion, and suggest that endogenous NO is involved in the acid secretory response induced by histamine released from histamine-containing cells. The results also suggest a stimulatory role of NO in the acid secretory process, quite in contrast to reports that NO inhibits parietal cell function in vitro (Brown et al., 1993; Kim and Kim, 1996). Thus, it appears that endogenous NO might play some role in a process involved in histamine release from histamine-containing cells.

There are two major types of histamine-containing cells in the gastric mucosa: (i) mucosal mast cells (Soll et al., 1988) and (ii) enterochromaffin-like cells (ECL cells) (Håkanson et al., 1986). It has been reported that, in rodents such as rats and mice, gastric mucosal histamine-containing cells are mainly ECL cells. It has been reported that the depletion of ECL cell histamine decreases the gastrin-evoked acid response (Andersson et al., 1996; Chen et al., 1996). On the other hand, isolated mucosal mast cells do not respond to gastrin or carbachol (Rangachari, 1992). Based on these observations, ECL cells are considered to be the site of origin of endogenous histamine involved in peripheral regulation of gastric acid secretion (Hersey and Sachs, 1995).

It is known that there are muscarinic, histamine and gastrin receptors exist on parietal cells (Soll, 1978). A subtype of muscarinic receptors on parietal cells was identified as M_3 (Hirschowitz et al., 1995; Hersey and Sachs, 1995). McNeil A-343 is known to be a muscarinic M_1 receptor agonist, but in fact, this compound is a non-selective muscarinic partial agonist on M_1, M_2 and M_3 receptors(Eglan et al., 1987). McNeil A-343 was used as a muscarinic receptor agonist in the present study for the following reasons: (1) McNeil A-343 induces acid secretion, an effect which is abolished by famotidine but not by tetrodotoxin (Yamamoto et al., 1996). These results are consistent with other reports (Black and Shankley, 1985, 1987). (2) Acetylcholine, bethanechol and carbachol induce acid secretion, an effect which is partially inhibited by famotidine (5–20% inhibition) (Yamamoto et al., 1996). Because these results suggested that McNeil A-343 induced gastric acid secretion through histamine release from histamine-containing cells, we considered it the most suitable secretagogue for this study. The most important observation from the present study was that the acid secretory response to all the secretagogues tested, except histamine, was inhibited by L-NNA, and this inhibition was reversed by L-arginine. These findings suggest that endogenous NO mediates the histamine release from histamine-containing cells.

Many investigators have reported on the relationship between ECL cells and gastrin-related substances. Gastrin has a trophic effect on ECL cells (Tielemans et al., 1990; Håkanson et al., 1994a) and allows the release of histamine from ECL cells (Prinz et al., 1993; Chen et al., 1994). It was shown that pentagastrin induces an acid secretory response through gastrin/CCK_B receptors (Prinz et al., 1994), and these receptors exist on both ECL cells and parietal cells (Hirschowitz et al., 1995). In the present study, famotidine abolished the pentagastrin-stimulated gastric acid secretion. Together, the results suggest that acetylcholine released by low-frequency vagal stimulation, McNeil A-343 and pentagastrin acts on ECL cells to release histamine, and that endogenous NO is involved in the mechanisms inducing histamine release from ECL cells. On the other hand, it is considered that acetylcholine released by high-frequency stimulation, such as at 5 or 10 Hz, acts not only on ECL cells but also on parietal cells. Accordingly, this response could not be inhibited by famotidine or L-NNA.

It is reported that NO has an inhibitory effect on gastric acid secretion that is exerted through both central (Barrachina et al., 1995; Yokotani et al., 1997) and peripheral (Brown et al., 1993; Kim and Kim, 1996) pathways. In the present study, L-NNA inhibited the acid secretory response to pentagastrin and McNeil A-343, and L-arginine reversed this effect to the control level. These findings suggest that the NO-producing system is concerned with acid secretion stimulated via histamine-releasing cells. The acid response to exogenous histamine was not affected by L-NNA. Thus, it was shown that L-NNA decreases the histamine release induced from ECL cells by electrical stimulation, McNeil A-343 and pentagastrin. Our experiments showed that pentagastrin and bethanechol increased histamine release from gastric mucosal cells. These responses were significantly inhibited by L-NNA and the corresponding receptor antagonists (YM022 and atropine). Accordingly, it could be confirmed that, indeed, these acid secretagogues induce histamine release through a mechanism related to NO synthesis. Moreover, the NO donor, sodium nitroprusside, at a concentration which did not affect histamine-induced

acid secretion, significantly increased the basal acid response, an effect which was then inhibited by famotidine. Furthermore, this also suggested that NO induces gastric acid secretion through histamine release. Our findings seem to contradict previous reports which suggested an inhibitory effects of exogenous NO on gastric acid secretion. The site of action of NO in these reports was considered to be on parietal cells. This discrepancy may be explainable by differences in the quantity of NO.

In conclusion, the present results showed that acid secretagogue-stimulated endogenous NO production may be involved in the gastric acid secretion induced via histamine release from histamine-containing cells, possibly ECL cells. NO may function as a paracrine or autocrine mediator in the mechanisms of histamine release from ECL cells.

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References

Andersson, K., Cabero, J.L., Mattsson, H., Håkanson, R., 1996. Gastric acid secretion after depletion of enterochromaffin-like cell histamine. Scand. J. Gastroenterol. 31, 24–30.

Barrachina, D., Calatayud, S., Esplugues, J., Whittle, B.J.R., Moncada, S., Esplugues, J.V., 1994. Nitric oxide donors preferentially inhibit neurally mediated rat gastric acid secretion. Eur. J. Pharmacol. 262, 181–183.

Barrachina, M.D., Whittle, B.J.R., Moncada, S., Esplugues, J.V., 1995. Endotoxin inhibition of distension-stimulated gastric acid secretion in rat: mediation by NO in the central nervous system. Br. J. Pharmacol. 114, 8–12.

Black, J.W., Shankley, N.P., 1985. Pharmacological analysis of the muscarinic receptors involved when McNeil A-343 stimulates acid secretion in the mouse isolated stomach. Br. J. Pharmacol. 86, 609–617.

Black, J.W., Shankley, N.P., 1987. How does gastrin act to stimulate oxyntic cell secretion?. Trends Pharmacol. Sci. 8, 486–490.

Brown, J.F., Tepperman, B.L., Hanson, P.J., Whittle, B.J.R., Moncada, S., 1992. Differential distribution of nitric oxide synthase between cell fractions isolated from the rat gastric mucosa. Biochem. Biophys. Res. Commun. 184, 680–685.

Brown, J.F., Hanson, P.J., Whittle, B.J.R., 1993. The nitric oxide donor, *S*-nitroso-*N*-acetyl-penicillamine, inhibits secretory activity in rat isolated parietal cells. Biochem. Biophys. Res. Commun. 195, 1354–1359.

Chen, D., Monstein, H., Nylander, A., Zhao, C., Sundler, F., Håkanson, R., 1994. Acute response of rat stomach enterochromaffin-like cells to gastrin: secretory activation and adaptation. Gastroenterology 107, 18–27.

Chen, D., Marvik, R., Rønning, K., Andersson, K., Waldum, H.L., Håkanson, R., 1996. Gastrin-evoked secretion of pancreastatin and histamine from ECL cells and of acid from parietal cells in isolated, vascularly perfused rat stomach. Effects of isobutylmethylxanthine and α-fluoromethylhistamine. Regul. Pept. 65, 133–138.

Eglan, R.M., Kenny, B.A., Michel, A.D., Whiting, R.L., 1987. Muscarinic activity of McNeil A-343 and its value in muscarinic receptor classification. Br. J. Pharmacol. 90, 693–700.

Håkanson, R., Böttcher, G., Ekblad, E., Panula, P., Simonsson, M., Dohlsten, M., Hallberg, T., Sundler, F., 1986. Histamine in endocrine cells in the stomach. Histochemistry 86, 5–17.

Håkanson, R., Chen, D., Sundler, F., 1994a. The ECL cells. In: Johnson, L.R. (Ed.), Physiology of the Gastrointestinal Tract, 3rd edn. Raven Press, New York, pp. 1171–1184.

Håkanson, R., Chen, D., Andersson, K., Monstein, H.-J., Zhao, C.M., Ryberg, B., Sundler, F., Mattsson, H., 1994b. The biology and physiology of the ECL cell. Yale J. Biol. Med. 67, 123–134.

Hersey, S.J., Sachs, G., 1995. Gastric acid secretion. Pharmacol. Rev. 75, 155–189.

Hirschowitz, B.I., Keeling, D., Lewin, M., Okabe, S., Parsons, M., Sewing, K., Wallmark, B., Sachs, G., 1995. Pharmacological aspects of acid secretion. Dig. Dis. Sci. 40, 3–23.

Horie, S., Yano, S., Watanabe, K., 1993. Inhibition of gastric acid secretion in vivo and in vitro by an inhibitor of $Cl^- - HCO_3^-$ exchanger, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid. J. Pharmacol. Exp. Ther. 265, 1313–1318.

Horie, S., Yano, S., Watanabe, K., 1994. Differential effects of Na^+, K^+-ATP-ase inhibition by ouabain on acid secretory response to histamine and bethanechol in the mouse isolated stomach. Br. J. Pharmacol. 112, 87–92.

Horie, S., Maruyama, T., Yano, S., Watanabe, K., 1996. Neurogenic stimulation of gastric acid secretion by the Na^+, K^+-ATP-ase inhibitor ouabain in mouse isolated stomach. Gen. Pharmacol. 27, 905–909.

Kim, H., Kim, K.H., 1996. Effects of a nitric oxide donor and nitric oxide synthase inhibitors on acid secretion of isolated rabbit gastric glands. Pharmacology 53, 331–339.

Kubota, H., Taguchi, Y., Tohyama, M., Matsuura, N., Shiosaka, S., Ishihara, T., Watanabe, T., Shiotani, Y., Wada, H., 1984. Electron microscopic identification of histidine decarboxylase-containing endocrine cells of the rat gastric mucosa. Gastroenterology 87, 496–502.

Mariotto, S., Menegazzi, M., Carcereri de Prati, A., Cuzzolin, L., Adami, A., Suzuki, H., Benoni, G., 1995. Protective effect of NO on gastric lesions and inhibition of expression of gastric inducible NOS by flurbiprofen and its nitro-derivative, nitroflurbiprofen. Br. J. Pharmacol. 116, 1713–1714.

Pique, J.M., Esplugues, J.V., Whittle, B.J.R., 1992. Endogenous nitric oxide as a mediator of gastric mucosal vasodilation during acid secretion. Gastroenterology 102, 168–174.

Prinz, C., Kajimura, M., Scott, D.R., Mercier, F., Helander, H.F., Sachs, G., 1993. Histamine secretion from rat enterochromaffin-like cells. Gastroenterology 105, 449–461.

Prinz, C., Scott, D.R., Hurwitz, D., Sundler, H.F., Sachs, G., 1994. Gastrin effects on isolated rat enterochromaffin-like cells in primary culture. Am. J. Physiol. 267, G663–G675.

Rangachari, P.K., 1992. Histamine: mercurial messenger in the gut. Am. J. Physiol. 262, G1–13.

Saito, K., Horie, M., Nose, N., 1982. High-performance liquid chromatography of histamine and 1-methylhistamine with on-column fluorescence derivation. J. Chromatography 595, 163–168.

Sakai, H., Tabuchi, Y., Kakinoki, B., Seike, H., Kumagai, S., Matsumoto, C., Takeguchi, N., 1995. Ca^{2+}-activated outward-rectifier K^+ channels and histamine release by rat gastric enterochromaffin-like sells. Eur. J. Pharmacol. 291, 153–158.

Sandor, A., Kidd, M., Lawton, G.P., Miu, K., Tang, L.H., Modlin, I.M., 1996. Neurohormonal modulation of rat enterochromaffin-like cell histamine secretion. Gastroenterology 110, 1084–1092.

Schepp, W., Kath, D., Tatge, C., Zimmerhackl, B., Scusdziarra, V.,

Classen, M., 1989. Leukotriene C_4 and D_4 potentiate acid production by isolated rat parietal cells. Gastroenterology 97, 1420–1429.

Soll, A.H., 1978. The action of secretagogues on oxygen uptake by isolated mammalian parietal cells. J. Clin. Invest. 61, 370–380.

Soll, A.H., Toomey, M., Culp, D., Shanahan, F., Beaven, M.A., 1988. Modulation of histamine release from canine fundic mucosal mast cells. Am. J. Physiol. 254, G40–G48.

Stroff, T., Lambrecht, N., Peskar, B.M., 1994. Nitric oxide as mediator of the gastroprotection by cholecystokinin-8 and pentagastrin. Eur. J. Pharmacol. 260, R1–R2.

Tielemans, Y., Axelson, J., Sundler, F., Willems, G., Håkanson, R., 1990. Serum gastrin concentration affects the self-replication rate of the enterochromaffin-like cells in the rat stomach. Gut 31, 274–278.

Wan, B.Y.C., 1977. Metiamide and stimulated acid secretion from the isolated nondistended and distended mouse stomach. J. Physiol. 266, 327–346.

Watanabe, K., Yano, S., Yamamoto, M., Kanaoka, S., 1993. Comparative effects of cimetidine and famotidine on the vagally stimulated acid secretion in the isolated mouse whole stomach. Jpn. J. Pharmacol. 61, 229–236.

Whittle, B.J.R., Lopez-Belmonte, J., Moncada, S., 1990. Regulation of gastric mucosal integrity by endogenous nitric oxide: interactions with prostanoids and sensory neuropeptides in the rat. Br. J. Pharmacol. 99, 607–611.

Yamamoto, M., Yano, S., Watanabe, K., 1995. Comparison of effects of famotidine on vagally and field-electrically stimulated acid secretion in the isolated mouse whole stomach. Jpn. J. Pharmacol. 69, 259–268.

Yamamoto, M., Yano, S., Watanabe, K., 1996. Functional determination of muscarinic receptor subtypes involved in vagally stimulated acid secretion in the isolated mouse whole stomach. Jpn. J. Pharmacol. 71, 206P.

Yokotani, K., Murakami, Y., Okuma, Y., Osumi, Y., 1997. Centrally applied nitric oxide donors inhibit vagally evoked rat gastric acid secretion: involvement of sympathetic outflow. Jpn. J. Pharmacol. 74, 337–340.