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Stimulatory effects of nitric oxide donors on gastric acid secretion in isolated mouse stomach

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Abstract

We previously reported on the stimulatory role of endogenous nitric oxide (NO) in gastric acid secretion. In the present study, we investigated the effects of NO donors on acid secretion in isolated mouse stomach. Nitroprusside (100 μM–1 mM) inhibited the gastric acid secretion induced by histamine (500 μM) in a concentration-dependent manner. In addition, nitroprusside abolished the acid secretion induced by bethanechol (100 μM) and by electrical stimulation (10 Hz) of the vagus nerve. On the other hand, nitroprusside, 75 μM, which did not affect the acid secretion induced by histamine, itself elicited an increase in acid secretion. The acid secretion induced by 75 μM nitroprusside was inhibited by 10 μM famotidine, a histamine H_2 receptor antagonist. These results suggest that NO donors at high doses act on gastric parietal cells, resulting in inhibition of the stimulated acid secretion, and, at lower doses, facilitate histamine release from histamine-containing cells, leading to the increased acid secretion.

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

1. Introduction

Nitric oxide (NO) is known to regulate many physiological responses. In the gastrointestinal tract, NO donors inhibit neurally mediated gastric acid secretion in in vivo experiments with rats (Barrachina et al., 1994) and reduce histamine-induced acid production in isolated rat parietal cells (Brown et al., 1993) and in isolated rabbit gastric glands (Kim and Kim, 1996). The inhibitory effect of endogenous NO on acid secretion induced by gastric distension has also been demonstrated in rats (Kitamura et al., 1999). Thus, NO is generally thought to play an inhibitory role during gastric acid secretion.

We previously reported that *N*-nitro-L-arginine (L-NNA) reduces bethanechol- and pentagastrin-induced histamine release in isolated rat gastric mucosal cell preparations, and that L-NNA inhibits gastric acid secretion induced via histamine release in isolated mouse whole stomach preparations (Hasebe et al., 1998). In addition, dibutyryl-cyclic GMP induces histamine release from histamine-containing cells (Horie et al., 2000). Our results suggest that the NO-cyclic GMP system is involved in the gastric acid secretion mediated by histamine. However, it has not yet been studied whether NO donors induce an increase in gastric acid secretion via histamine release from enterochromaffin-like cells (ECL cells). In the present study, we studied the effects of NO donors on gastric acid secretion in isolated mouse stomach.

2. Materials and methods

2.1. Procedures for setting up the preparation of isolated mouse whole stomach

Male mice, ddY strain (4–5 weeks old, 18–32 g), were fasted for 3–4 h with free access to water before experiments. Gastric acid secretion was measured in the isolated mouse whole stomach preparation as described previously (Watanabe et al., 1993; Horie et al., 1994). 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 contain-

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ing 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 gassed with 95% O_2 and 5% CO_2) and kept at 37°C. The volume in 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)

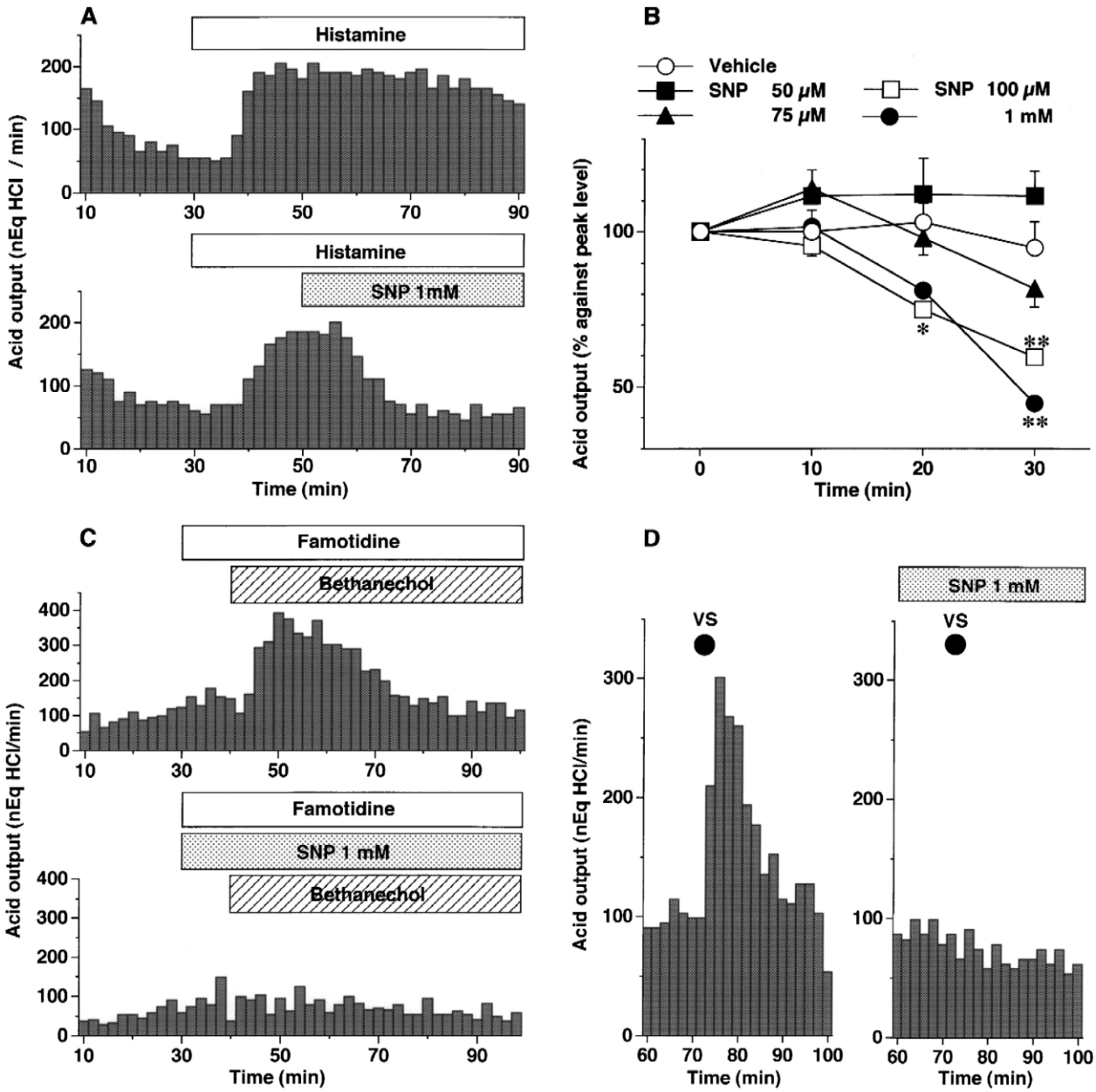

Fig. 1. Effect of sodium nitroprusside (SNP) on gastric acid secretion induced by (A, B) histamine, (C) bethanechol and (D) electrical vagal stimulation in isolated mouse stomach. (A) Typical patterns showing effect of sodium nitroprusside (1 mM) on histamine (500 μM)-induced acid secretion. Abscissa is the time after setting up of the preparation. Each column represents the acid output for 2 min. (B) Concentration-dependent effect of sodium nitroprusside on histamine-induced acid secretion. Sodium nitroprusside (50 μM–1 mM) was applied 20 min after the addition of histamine (500 μM). Abscissa is the time after the addition of sodium nitroprusside. Each value is expressed as a percentage of the maximum acid output 20 min after the addition of histamine. Data represent the means ± S.E.M. for four mice. Statistical analysis was performed by one-way analysis of variance (Kruskal–Wallis test) followed by nonparametric Dunnett's multiple comparison test. $^{*}P < 0.05$ and $^{**}P < 0.01$, significantly different from the control (vehicle-treated) group. (C) Typical patterns showing the effect of sodium nitroprusside on bethanechol-induced acid secretion in the presence of famotidine. Sodium nitroprusside (1 mM) and famotidine (10 μM) were applied 10 min before the addition of bethanechol (100 μM). Abscissa is the time after the setting up of the preparation. This trace shown is from one of three experiments. (D) Typical patterns showing effect of sodium nitroprusside on electrical vagus stimulation-induced acid secretion. Sodium nitroprusside (1 mM) was added 10 min before the electrical vagus stimulation. Vagus stimulation (VS, shown as ● in figure, 10 Hz, 10 V, 0.3 ms) was applied to the preparation for 5 min. Abscissa is the time after the setting up of the preparation. This trace shown is from one of three experiments.

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. The perfusate flowing out of the outlet tube was collected as fractions with a fraction collector (Eyela fraction collector DC-1000, Tokyo Rikakikai, Japan). Acid output was measured by titrating with 2 mN NaOH to pH 5.0 as nEq HCl with an automatic titrator (AUT 210, Toa Electronics, Tokyo, Japan). The intragastric pressure was kept at 20 cm H_2O. Drugs were added into the serosal solution.

2.2. Measurement of acid secretion induced by electric vagus stimulation

The gastric acid secretion induced by sodium nitroprusside was measured after electrical stimulation of the vagus twice in order to stabilize the acid secretory response in each individual preparation. 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 esophagus, according to our previously reported method (Yamamoto et al., 1995). The acid output was continuously titrated with an automatic titrator (Toa Electronics, HM-5ES, HSM-10A). The digital pulse (2 μl/pulse) from the titrator was sent to a personal computer (FM-77, Fujitsu, Tokyo, Japan) equipped with a pulse counter (FM-77/8 Interface, Fujitsu, developed by our laboratory). After a 30-min equilibration, the first vagal stimulation (10 Hz, 10 V, 0.3 ms, for 5 min) was applied with an electric stimulator (SEN-7203, Nihon Kohden, Tokyo, Japan). 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.

2.3. Drugs

Famotidine was purchased from Sigma (St. Louis, MO, USA). Histamine hydrochloride was obtained from Nacalai Tesque (Kyoto, Japan). Sodium pentacyanonitrosylferrate (|||) dihydrate (sodium nitroprusside) was purchased from Wako (Osaka, Japan). Histamine hydrochloride and sodium nitroprusside were dissolved in saline. Famotidine was prepared in saline after being dissolved with a small amount of 0.1 N HCl.

2.4. Statistics

All data are given as the means ± S.E.M. Statistical analyses of raw data for two groups and for more than three groups were performed by two-tailed Student's *t*-test and by one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison test, respectively. Statistical analysis of percent data from more than three groups was performed by one-way analysis of variance (Kruskal–Wallis test) followed by the nonparametric Dunnett's multiple comparison test. A P value < 0.05 was considered statistically significant.

3. Results

Histamine (500 μM) elicited an increase in acid secretion up to approximately 200 nEq HCl/min. This maxi-

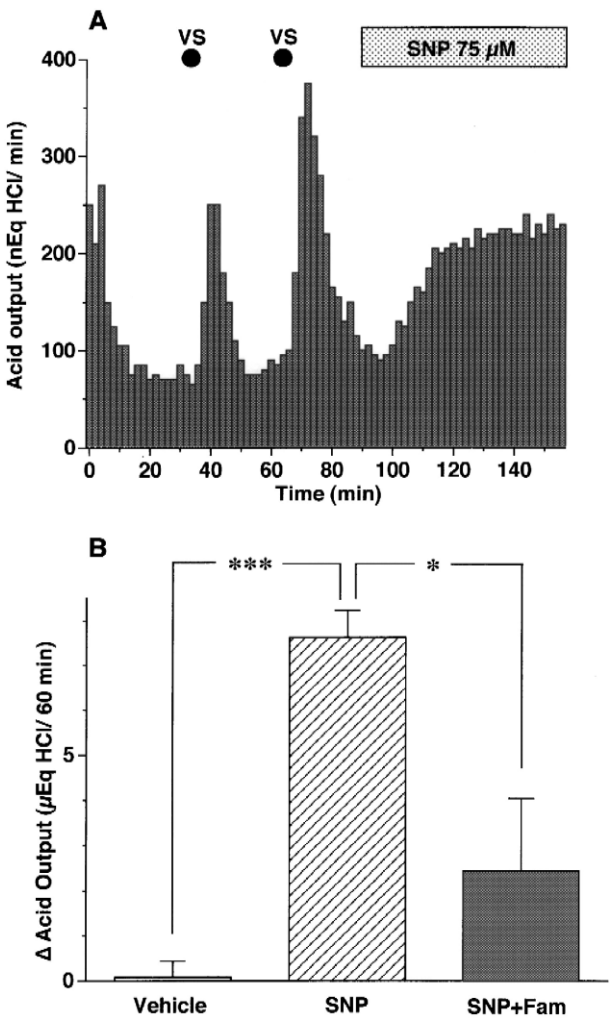

Fig. 2. Stimulation by sodium nitroprusside (SNP) of gastric acid secretion and the inhibition by famotidine in isolated mouse whole stomach. (A) Typical patterns showing effect of sodium nitroprusside (75 μM) on basal acid secretion. Sodium nitroprusside (75 μM) was applied to the preparation 25 min after the second vagal stimulation. Abscissa is the time after setting up of the preparation. Each column represents the acid output for 2 min. The first and the second vagal stimulation (VS, shown as ● in figure, 10 Hz, 10 V, 0.3 ms) were applied to the preparation for 5 min. (B) Quantitative data. Famotidine (Fam, 10 μM) was applied 10 min before addition of sodium nitroprusside (75 μM). The change in acid output after the addition of sodium nitroprusside is expressed as the Δ acid output for 60 min. Each value represents the means ± S.E.M. for four to five mice. Statistical analysis was performed with Student's *t*-test. $^{*}P < 0.05$, $^{***}P < 0.001$, significantly different from the group treated with sodium nitroprusside alone.

mum response was maintained for at least 1 h. Sodium nitroprusside (1 mM) reduced the acid secretion stimulated by histamine (Fig. 1A). Sodium nitroprusside (100 μM–1 mM) showed an inhibitory effect on the histamine-induced acid secretory response in a concentration-dependent manner (Fig. 1B). In addition, the pretreatment with sodium nitroprusside abolished the acid secretion induced by bethanechol (100 μM) in the presence of famotidine (10 μM) and by electrical vagus nerve stimulation (10 Hz). These inhibitory effects are shown in Fig. 1C and D. These results are consistent with previous reports for other preparations (Barrachina et al., 1994; Brown et al., 1993; Kim and Kim, 1996).

Next, we investigated the effect of sodium nitroprusside on basal acid secretion. Basal acid secretion was stable for 1–2 h, and the basal secretory rate was approximately 50 nEq HCl/min as previously reported by Horie et al. (1993, 1996). Interestingly, sodium nitroprusside at 75 μM, a concentration which did not affect the histamine-induced gastric acid secretion (Fig. 1B), significantly increased the acid secretory response (Fig. 2A). Sodium nitroprusside at 50 and 100 μM did not affect the basal acid secretion. The following effect of sodium nitroprusside on basal acid secretion was expressed as a net increase in acid output over the basal level before the addition of sodium nitroprusside: control, 0.08 ± 0.03 μEq/60 min, $n = 4$; sodium nitroprusside 50 μM, 0.34 ± 0.22 μEq/60 min, $n = 4$, $P > 0.05$ vs. control group; 75 μM, 7.63 ± 0.60 μEq/60 min, $n = 4$, $P < 0.001$ vs. control group; 100 μM, 0.25 ± 0.21 μEq/60 min, $n = 4$, $P > 0.05$ vs. control group. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by the Bonferroni multiple comparison test. The increase in acid secretion induced by sodium nitroprusside, 75 μM, was significantly inhibited by 10 μM famotidine, a histamine H_2 receptor antagonist (Fig. 2B).

4. Discussion

In the gut, the cells that synthesize and release histamine are mucosal mast cells (Soll et al., 1988) and ECL cells (Håkanson et al., 1986). ECL cells are considered to be at the origin of endogenous histamine which is related to peripheral regulation of gastric acid secretion in rodents (Hersey and Sachs, 1995).

We previously reported that L-NNA inhibits the acid secretion induced by a cholinergic agent, gastrin, and electrical vagus nerve stimulation in isolated mouse stomach. Moreover, the increased histamine release in response to pentagastrin and bethanechol is reduced by L-NNA in isolated rat gastric mucosal cells (Hasebe et al., 1998). We speculate that endogenous NO promotes the acid secretory response through histamine release. Furthermore, we have shown that dibutyryl-cyclic GMP, a cyclic GMP analogue, induces an increase in acid secretion in isolated mouse stomach, and histamine release from gastric mucosal cells (Horie et al., 2000). These findings suggest that the NO-cyclic GMP system is involved in promotion of gastric acid secretion under physiological conditions. However, the effects of NO donors on gastric acid secretion have not yet been investigated. We now studied the acid secretory effect of NO donors in isolated mouse stomach. When sodium nitroprusside at a lower dose (about 75 μM), which did not affect histamine-stimulated gastric acid secretion, was applied to the preparation, an augmented acid secretion was observed. The acid secretion was almost completely inhibited by pretreatment with famotidine. We have also observed that, in isolated mouse stomach, dibutyryl-cyclic GMP at a low dose induced the acid secretion which was abolished by famotidine (Horie et al., 2000). It is thus likely that the NO-cyclic GMP system in gastric mucosal cells plays a role in promoting acid secretion through the released histamine.

Bilski et al. (1994) also reported a contribution of endogenous NO to stimulated acid secretion in the in vivo experiments with dogs. Kunikata et al. (1999) showed that the NO donor, NOR-3, at high concentrations induces, in isolated bullfrog stomach preparation, acid secretion via endogenous histamine release. It was reported that endogenous NO is involved in an increase of postprandial acid secretion in humans (Konturek et al., 1999). These reports and the present results suggest the hypothesis that NO stimulates gastric acid secretion under physiological conditions. In addition, stimulated secretory responses through the NO-cyclic GMP mechanism have been reported for other secretory cell systems (Nguyen et al., 1991; Florucci et al., 1995; Okayama et al., 1995).

Kato et al. (1998) reported that intragastrically applied NO donors inhibit the pentagastrin-induced acid secretion and luminal release of histamine in anesthetized rats. The authors suggest that the NO donor inhibition of acid secretion results from a decrease in histamine release due to the inhibition of ECL cell function. This finding seems to contradict our results for the stimulatory effect of NO donors on acid secretion. Kato et al. (1998) studied the effects of intragastric application of NO donors on acid secretion in in vivo experiments. They used sodium nitroprusside at higher doses, about 20–40 mM, although the concentration around ECL cells could not be estimated. In the present study, we studied the effects of serosal-side application of NO donors on acid secretion in an in vitro experiment. We observed acid secretion induced by NO donors only at a lower dose, 75 μM. The discrepancy may be explained by (1) preparation differences, (2) drug administration differences, and (3) dose differences.

It has been reported that *S*-nitroso-*N*-acetylpenicillamine, a NO donor, at high doses, inhibits histamine-induced acid production in isolated rat parietal cells, suggesting a direct inhibitory action on parietal cells (Brown et al., 1993). Barrachina et al. (1994) showed that *S*-nitrosoglutathione, another NO donor, reduces the acid secretion

stimulated by 2-deoxy-D-glucose and by gastric distension. Inhibition by NO donors of stimulated acid secretion has also been reported for isolated rabbit gastric glands (Kim and Kim, 1996). The present study also showed that sodium nitroprusside at higher doses (100 μM–1 mM) inhibited the histamine-induced acid secretion, which is consistent with previous reports. It was found in the experiment using bethanechol in the presence of famotidine that sodium nitroprusside, 1 mM, also inhibited the acid secretion induced by the stimulation of muscarinic receptors on parietal cells. The vagally stimulated acid secretion was inhibited by sodium nitroprusside at 1 mM. Moreover, we reported that dibutyryl-cyclic GMP at a higher dose (1 mM) also reduced the histamine-induced acid secretory response (Horie et al., 2000). Accordingly, the NO-cyclic GMP pathway is thought to play a role in the inhibition of acid secretion through a direct effect on gastric parietal cells. In contrast, our findings clearly show that the NO-cyclic GMP pathway is involved in promotion of gastric acid secretion through ECL cells. We speculated that the amount of NO determines whether NO stimulates or inhibits gastric acid secretion. A small amount of NO has a stimulatory effect on ECL cells, resulting in increased acid secretion, while a large amount of NO has an inhibitory effect on parietal cells, leading to decreased acid secretion.

Sodium nitroprusside, 100 μM, did not induce an increase in basal acid secretion. On the other hand, sodium nitroprusside, only at 75 μM, elicited an increase in basal acid secretion. Because sodium nitroprusside, 100 μM, shows an inhibitory effect on parietal cell function, we considered that it abolishes the acid secretion induced by histamine released from ECL cells. The apparent bell-shaped dose–response curve for sodium nitroprusside in the experiment with isolated mouse stomach is ascribed to promotion of acid secretion by histamine released by NO donors at a low concentration and inhibition of gastric parietal cells by NO donors at a high concentration.

In conclusion, it was found that NO donors increase gastric acid secretion via histamine released from ECL cells. In addition, NO donors also reduced gastric acid secretion, most likely via inhibition of parietal cell function. NO functions as a mediator responsible for both stimulation and inhibition of gastric acid secretion.

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