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Role of nitric oxide in regulation of gastric acid secretion in rats: effects of NO donors and NO synthase inhibitor

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1. The role of nitric oxide (NO) in the regulation of acid secretion was examined in the anaesthetized rat.

2. A rat stomach was mounted in an *ex vivo* chamber, instilled with 2 ml of saline every 15 min, and the recovered sample was titrated at pH 7.0 against 0.1 N NaOH by use of an automatic titrator for acid secretion. Gastric mucosal blood flow (GMBF) was measured simultaneously by laser Doppler flowmeter.

3. Intragastric application of NO donors such as FK409 (3 and 6 mg ml^{-1}) and sodium nitroprusside (SNP; 6 and 12 mg ml^{-1}) as well as i.p. administration of cimetidine (60 mg kg^{-1}), a histamine H_2-receptor antagonist, significantly inhibited the increase in acid secretion in response to pentagastrin (60 μg kg^{-1} h^{-1}, i.v.), in doses that increased gastric mucosal blood flow (GMBF).

4. Intragastric application of FK409 (6 mg ml^{-1}) increased both basal and stimulated acid secretion induced by YM-14673 (0.3 mg kg^{-1}, i.v.), an analogue of thyrotropin-releasing hormone (TRH), but had no effect on the acid secretory response induced by histamine (4 mg kg^{-1} h^{-1}, i.v.).

5. Pretreatment with N^G-nitro-L-arginine methyl ester (L-NAME; 10 mg kg^{-1}, i.v.) did not affect basal acid secretion, but significantly potentiated the increase in acid secretion induced by YM-14673 and slightly augmented the acid secretory response to pentagastrin.

6. Both pentagastrin and YM-14673 increased the release of nitrite plus nitrate (NO_x), stable NO metabolites, into the gastric lumen, and these changes were completely inhibited by prior administration of L-NAME (10 mg kg^{-1}, i.v.).

7. Pentagastrin caused an increase in luminal release of histamine and this response was significantly suppressed by intragastric application of FK409 (6 mg ml^{-1}).

8. These results suggest that either exogenous or endogenous NO has an inhibitory action on gastric acid secretion through suppression of histamine release from enterochromaffin-like (ECL) cells.

Keywords: Nitric oxide; acid secretion; gastric mucosal blood flow; FK409; sodium nitroprusside; pentagastrin: YM-14673

Introduction

The local release of endothelium-derived relaxing factor, identified as nitric oxide (NO), regulates the gastric mucosal microcirculation and maintains the mucosal integrity in collaboration with prostaglandins and sensory neurones (Whittle *et al*., 1990; Tepperman & Whittle, 1992). However, the influence of NO on the process that regulates gastric acid secretion remains unclear. In general, specific NO synthase inhibitors such as N^G-nitro-L-arginine methyl ester (L-NAME) are used to examine physiological actions of endogenous NO. In previous studies it was shown that the NO synthase inhibitor did not directly modulate basal or pentagastrin-stimulated acid secretion (Pique *et al*., 1992), but antagonized the antisecretory action of lipopolysaccaride and cytokines in rats (Martinez-Cuesta *et al*., 1992; Esplugues *et al*., 1993) and the inhibitory acid response observed in the stomach after damage (Takeuchi *et al*., 1994). In contrast, Bilski *et al*. (1994) found that the NO synthase inhibitor reduced the acid secretion stimulated by ordinary meat feeding or pentagastrin in dogs. On the other hand, Brown *et al*. (1993) and Barrachina *et al*. (1994) showed that the NO donors inhibited the acid secretion directly at the parietal cell level in both *in vitro* and *in vivo* experiments. Thus, the role of NO in the physiological regulation of acid secretion has not been fully understood.

The present study was therefore designed to investigate the effects of both NO donors and NO synthase inhibitor on basal and stimulated acid secretion by various secretagogues, in the attempt to clarify the role of endogenous NO in the regulation of gastric acid secretion.

Methods

Animals

Male Sprague Dawley rats (220–250 g, Charles River, Yokohama, Japan), kept in individual cages with mesh bottoms, were deprived of food but allowed free access to tap water for 18 h before the experiments. Studies were carried out with five rats per group.

Measurement of acid secretion and gastric mucosal blood flow (GMBF)

Animals were anaesthetized with urethane (1.25 g kg^{-1}, i.p.), and the trachea was cannulated to ensure a patent airway. Simultaneous measurement of acid secretion and GMBF was performed in the chambered stomach as described previously (Kato *et al*., 1993). In brief, the abdomen was incised, the stomach exposed and mounted in an *ex vivo* chamber. At the

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beginning of each experiment, the stomach was rinsed several times with physiological saline. After the gastric exudate became clear, 2 ml of the saline was instilled in the chamber, and 15 min later the gastric contents were recovered from the chamber. This procedure was repeated every 15 min. Acid output was determined from the analyses of the collected gastric contents. Each sample was titrated by 0.1 N NaOH to pH 7.0 with an automatic titrator (Autoburette, model Comtite; Hiranuma, Tokyo) and expressed as microequivalents per 15 min or microequivalents per hour. GMBF was measured during a test period by laser Doppler flowmeter (ALF-21, Advance, Tokyo, Japan) and placing a probe lightly on the surface of the corpus mucosa by use of a balancer (Medical Agent, Kyoto, Japan). Blood pressure was also measured via the femoral artery by a pressure transducer and amplifier system (TP-200TL, AP-100F, RTA-1100A Nihon Koden). The body temperature was kept at $36 \pm 1^{\circ}C$ using a heating lamp.

Measurement of nitrite plus nitrate contents in the gastric lumen

The stomach was mounted in an *ex vivo* chamber under urethane anaesthesia, and 2 ml of saline was instilled in the chamber. Thirty minutes later, the gastric contents were recovered from the chamber and this procedure was repeated every 30 min. Nitrite plus nitrate (NO_x) concentrations in gastric contents were measured by Griess method (Green *et al*., 1982) after reduction of nitrate to nitrite with 0.05 units ml^{-1} of nitrate reductase (from Aspergillus, Sigma) in the presence of 5 mM NADPH for 1 h at 37°C. Nitrites were incubated with Griess reagent (0.1% naphthylene diamine dihydrochloride and 1% sulphanilamide in 2.5% H_3PO_4) for 10 min at room temperature, and the absorbance at 550 nm was measured. For the standard curve, sodium nitrate was used under the same measurement. In a preliminary experiment, we confirmed that nitrate reductase quantitatively converted nitrate and that nitrate up to the concentration of 1000 nmol ml^{-1} was reduced over 98% to nitrate in the presence of the reductase at 0.05 units ml^{-1}.

Measurement of histamine contents in the gastric lumen

The stomach was mounted in an *ex vivo* chamber under urethane anaesthesia, and 2 ml of saline was instilled in the chamber. Thirty minutes later the gastric contents were recovered from the chamber and this procedure was repeated every 30 min. The amount of histamine in gastric contents was determined by enzyme immunoassay (Histamine EIA kit, Immunotech, Marseille, France).

Experimental protocols

After both acid output and GMBF stabilized for at least 30 min they were considered as basal values. Thereafter, the animals were subjected to a continuous i.v. infusion of pentagastrin (60 $\mu g\ kg^{-1}\ h^{-1}$) or histamine (4 mg $kg^{-1}\ h^{-1}$) via a tail vein for over 135 min. Sixty minutes after the onset of infusion, 2 ml of NO donors such as FK409 (3 and 6 mg ml^{-1}) and sodium nitroprusside (SNP; 6 and 12 mg ml^{-1}) or the vehicle of NO donors were instilled in the chamber in place of saline. Fifteen minutes later, the NO donor was removed and replaced with fresh saline again. In some cases, acid secretion was stimulated by YM-14673 given i.v. in a dose of 0.3 mg kg^{-1} as a bolus injection, and FK409 was topically applied to the chamber 30 min after YM-14673. The doses of secretagogues were selected to stimulate submaximally acid secretion (Takeuchi *et al*., 1991; Kato *et al*., 1993). In some cases, the effects of N^G-nitro-L-arginine methyl ester (L-NAME; 10 mg kg^{-1}), the NO synthase inhibitor, in the presence or absence of L-arginine (500 mg kg^{-1}) and cimetidine (60 mg kg^{-1}), a histamine H_2-receptor antagonist, on acid secretion were examined. In separate experiments, the effects of L-NAME (10 mg kg^{-1}), omeprazole (30 mg kg^{-1}) or vagotomy on luminal NO_x released under basal and stimulated conditions (induced by YM-14673 and pentagastrin) were examined. L-NAME was administered i.v. 15 min before the onset of pentagastrin infusion or the administration of YM-14673, while omeprazole and cimetidine was given i.p. 30 min before these treatments. L-Arginine was administered i.p. 15 min before L-NAME treatment. Vagotomy was performed bilaterally at the cervical portion 60 min before the experiments. Furthermore, the effect of FK409 (6 mg ml^{-1}) on the luminal histamine release in response to pentagastrin (60 $\mu g\ kg^{-1}\ h^{-1}$) was examined. FK409 was applied intragastrically for 15 min 1 h after the onset of pentagastrin infusion.

Drugs

We used urethane (Tokyo Kasei, Tokyo, Japan), pentagastrin, SNP, L-NAME, L-arginine, cimetidine (Sigma Chemicals, St. Louis, Missouri), histamine 2HCl (Nacali Tesque, Kyoto, Japan), FK409 (kindly supplied by Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan), YM-14673 (kindly supplied by Yamanouchi Pharmaceutical Co. Ltd., Tokyo, Japan) and omeprazole (Astra, Osaka, Japan). The chemical structures of FK409 and YM14673 are shown in Figure 1. FK409 was suspended in saline, while omeprazole and cimetidine in 0.5% carboxymethylcellulose solution (Wako, Osaka, Japan). Other drugs were dissolved in saline. Each drug was prepared immediately before use. Agents were administered; intraperitoneally in a volume of 5 ml kg^{-1} body weight, intragastrically 2 ml/animal, and by i.v. infusion from tail vein 5 ml $kg^{-1}\ h^{-1}$, or by i.v. bolus injection 1 ml kg^{-1} body weight.

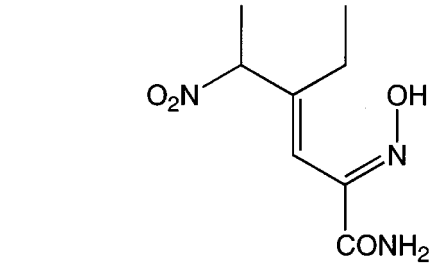
Statistics

Data are presented as the means ± s.e. from five rats per group. Statistical analyses were performed by a two-tailed Student's *t* test and Dunnett's multiple comparison test. Values of $P < 0.05$ were regarded as significant.

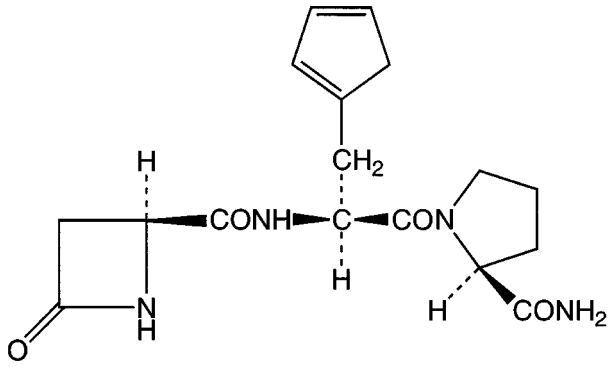
Results

Effect of FK409 and SNP on acid secretory and GMBF responses induced by pentagastrin

Pentagastrin (60 $\mu g\ kg^{-1}\ h^{-1}$) caused a progressive increase of acid secretion, reaching a plateau (15–17 μEq 15 min^{-1}) within 30 min. Intragastric application of FK409 (3 and 6 mg ml^{-1}) reduced the pentagastrin-stimulated acid secretion in a dose-dependent manner, the inhibition being 27.6% and 53.2%, respectively, 30 min after FK409 application (Figure 2). The GMBF was gradually increased in response to pentagastrin, and the increase was markedly enhanced by FK409, reaching values several fold bigger than control; the responses observed at 15 min after the application were 208.8 ± 17.6% and 340.6 ± 20.6% of basal values at 3 mg ml^{-1} and 6 mg ml^{-1}, respectively, which are significantly greater than control (176.4 ± 6.0%). Similarly, sodium nitroprusside



FK409



YM-14673

Figure 1 Chemical structures of FK409, a NO donor, and YM14673, an analogue of TRH.

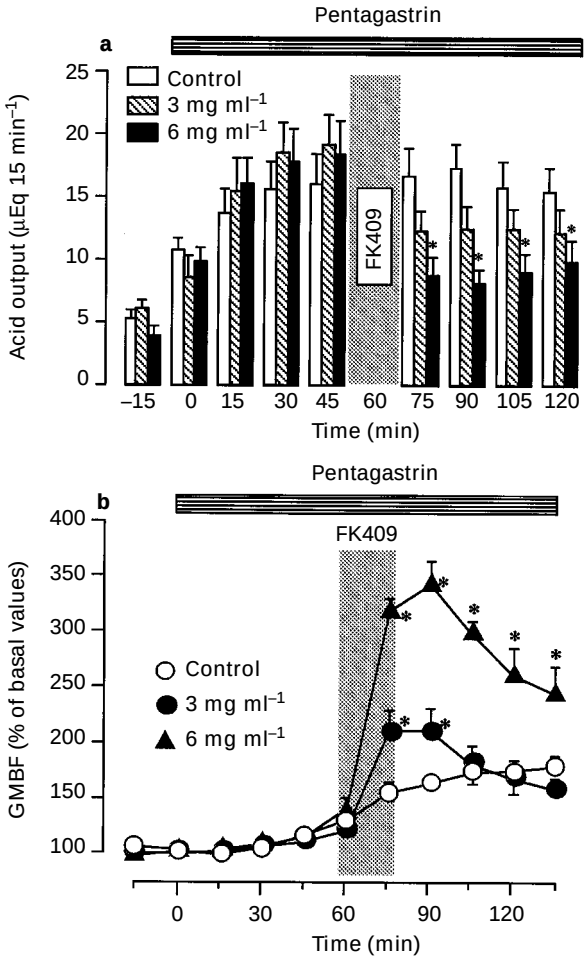


Figure 2 Effect of FK409 (3 and 6 mg ml^{-1}) on the increase of acid secretion (a) and GMBF (b) induced by pentagastrin (60 μg kg^{-1} h^{-1}) in the anaesthetized rat. Pentagastrin was continuously infused i.v. from a tail vein, while FK409 (2 ml) was applied intragastrically for 15 min, 1 h after the onset of pentagastrin infusion. The values of acid secretion are expressed as μEq 15 min^{-1}, while those of GMBF are expressed as % of basal values. Data are presented as the means $\pm$ s.e. (vertical lines) of values determined every 15 min from 5 rats. *Statistically significant difference from control, at $P<0.05$.

(SNP; 6 and 12 mg ml^{-1}) dose-dependently reduced the acid secretory response but enhanced the GMBF response induced by pentagastrin. At 12 mg ml^{-1} the acid secretion was inhibited to 63.9%, and the GMBF was further increased to 248.3 $\pm$ 29.3% of basal values 15 min post-application of SNP, which is significantly greater than control (169.6 $\pm$ 13.2%) (Figure 3).

Effect of FK409 on acid secretory and GMBF responses induced by histamine and YM-14673

Acid secretion increased progressively following i.v. infusion of histamine (4 mg kg^{-1} h^{-1}) or i.v. bolus injection of YM-14673 (0.3 mg kg^{-1}), an analogue of TRH. The GMBF remained relatively unchanged during histamine infusion, but markedly increased in response to YM-14673, reaching about 250% of basal values (Figures 4 and 5). The acid secretory response induced by histamine was not significantly affected by intragastric application of FK409 even at the dose of 6 mg ml^{-1}, the inhibition being 8.8% 30 min post-application, although this treatment markedly increased GMBF during histamine infusion. On the other hand, FK409 at 6 mg ml^{-1} significantly decreased the acid secretion in response to YM-14673, with a further increase in GMBF; the inhibition of acid secretion was 42.3%. Intragastric application of FK409 at the dose of 6 mg ml^{-1} also significantly reduced basal acid secretion with a marked increase of GMBF; at 30 min post-application the inhibition of acid secretion was 48.4% and the increase of GMBF was 214.9 $\pm$ 29.3% of basal values.

Effect of L-NAME on basal and stimulated acid secretion

Pretreatment with L-NAME (10 mg kg^{-1}, i.v.) enhanced the acid secretory response to YM-14673 significantly from 162.1 $\pm$ 7.4 μEq h^{-1} to 251.1 $\pm$ 27.3 μEq h^{-1}. This change was almost completely antagonized by co-administration of L-arginine (500 mg kg^{-1}, i.p.) (Figure 6). The acid secretion in response to pentagastrin was slightly increased in the presence of L-NAME, from 61.6 $\pm$ 8.6 μEq h^{-1} to 86.2 $\pm$ 9.9 μEq h^{-1}, but this effect was not significant. Similarly, basal acid secretion was not affected by pretreatment with L-NAME, and the acid output was 25.0 $\pm$ 4.5 μEq h^{-1} or 28.3 $\pm$ 5.9 μEq h^{-1} in the absence and presence of L-NAME, respectively. L-NAME itself increased basal GMBF temporarily for the initial 10 min after administration, but significantly reduced the increased GMBF response to pentagastrin and YM-14673; the decrease in the GMBF response was 18.3% (from 162.4 $\pm$ 12.1% to 132.6 $\pm$ 9.2% of basal values) and 35.9% (from 249.2 $\pm$ 33.6% to 159.8 $\pm$ 13.0% of basal values), respectively.

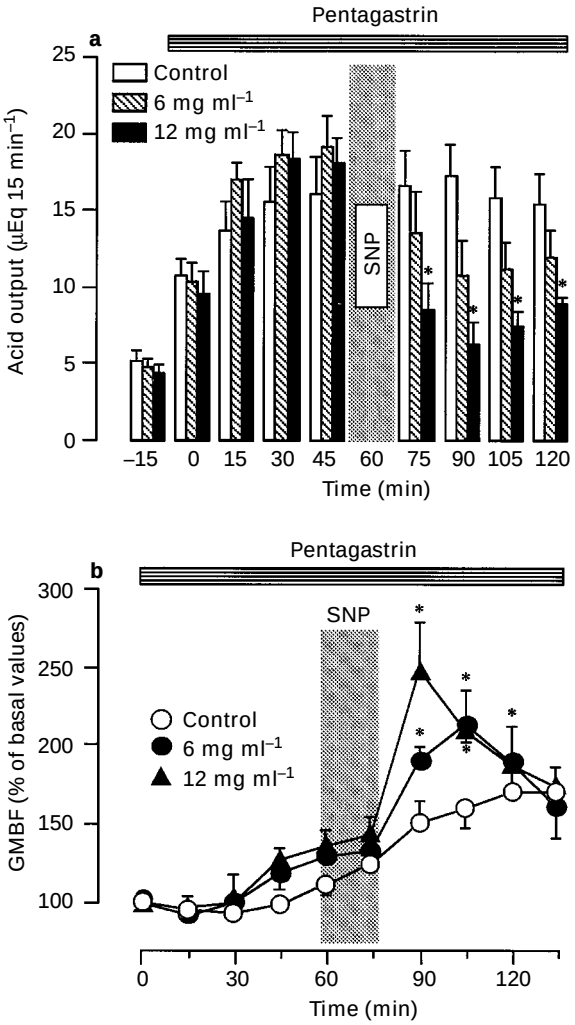


Figure 3 Effect of SNP (6 and 12 mg ml^{-1}) on the increase of acid secretion (a) and GMBF (b) induced by pentagastrin (60 μg kg^{-1} h^{-1}) in the anaesthetized rat. Pentagastrin was continuously infused i.v. from a tail vein, while SNP (2 ml) was applied intragastrically for 15 min, 1 h after the onset of pentagastrin infusion. The values of acid secretion are expressed as μEq 15 min^{-1}, while those of GMBF are expressed as % of basal values. Data are presented as the means $\pm$ s.e. (vertical lines) of values determined every 15 min from 5 rats. *Statistically significant difference from control, at $P<0.05$.

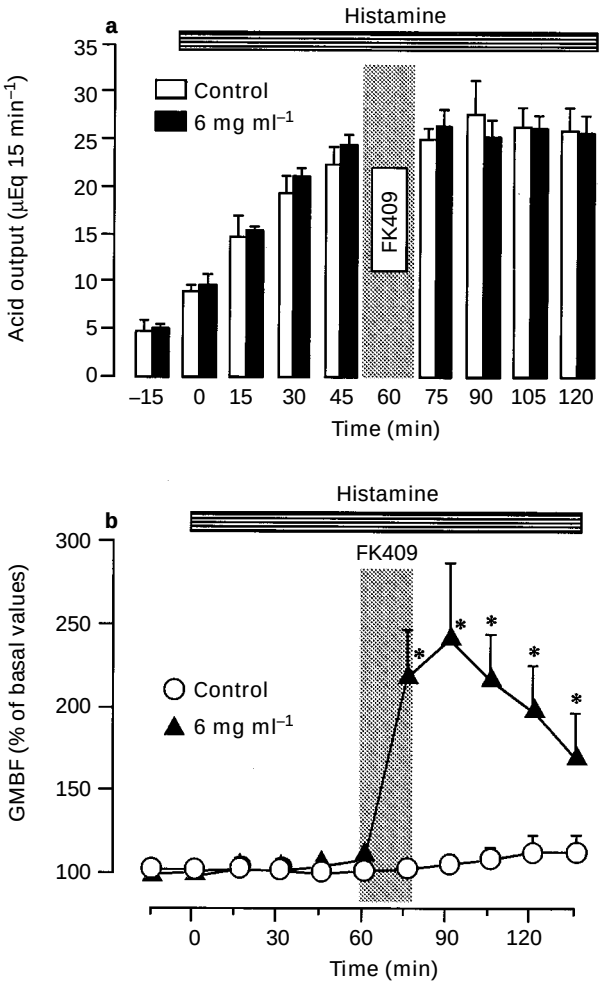


Figure 4 Effect of FK409 (6 mg ml^{-1}) on the increase of acid secretion (a) and GMBF (b) induced by histamine (4 mg kg^{-1} h^{-1}) in the anaesthetized rat. Histamine was continuously infused i.v. from a tail vein, while FK409 (2 ml) was applied intragastrically for 15 min, 1 h after the onset of histamine infusion. The values of acid secretion are expressed as μEq 15 min^{-1}, while those of GMBF are expressed as % of basal values. Data are presented as the means $\pm$ s.e. (vertical lines) of values determined every 15 min from 5 rats. *Statistically significant difference from control, at $P<0.05$.

Effect of FK409 and SNP on systemic blood pressure

Under urethane anaesthesia, systemic blood pressure was maintained at between 82–85 mmHg during the test period (Table 1). Intragastric application of FK409 at the doses of 3 and 6 mg ml^{-1} produced a rapid and dose-dependent decrease in systemic blood pressure, persisting for at least 30 min, with a maximal reduction within 15 min after application, the reduction being 17.6% and 26.7%, respectively. In contrast, SNP (6 and 12 mg ml^{-1}) caused only a slight reduction of blood pressure even at the dose which inhibited the acid secretion, the reduction being 10.8% at 12 mg ml^{-1} 15 min after the application.

Effect of L-NAME on NO_x release in the gastric lumen

In normal animals, spontaneous NO_x release into the gastric lumen was 28.8 ± 2.5 nmol h^{-1}, and this process was not affected by either omeprazole (30 mg kg^{-1}) or vagotomy, but significantly reduced by L-NAME (10 mg kg^{-1}) (Table 2). The luminal release of NO_x was increased by i.v. infusion of pentagastrin, reaching the value of 42.3 ± 1.9 nmol h^{-1} which is significantly greater than control. Likewise, i.v. injection of YM-14673 also increased NO_x release to 97.4 ± 5.8 nmol h^{-1}. The increased NO_x release in response to pentagastrin was significantly inhibited by prior administration of L-NAME, the inhibition being 106.7%. Omeprazole (30 mg kg^{-1}) slightly reduced the NO_x release in response to pentagastrin, but this effect was not significant. On the other hand, the increased NO_x release caused by YM-14673 was significantly inhibited by vagotomy or prior administration of L-NAME but not omeprazole, the inhibition being 89.8%, 95.5% and 23.3%, respectively.

Effect of FK409 on histamine release into the gastric lumen

Under normal conditions, the amount of histamine released into the gastric lumen was 76.6 ± 6.9 pmol h^{-1}. The luminal release of histamine was markedly increased by i.v. infusion of pentagastrin, reaching the value of 317.8 ± 60.3 pmol h^{-1} (Figure 7). Intragastric application of FK409 at 6 mg ml^{-1}

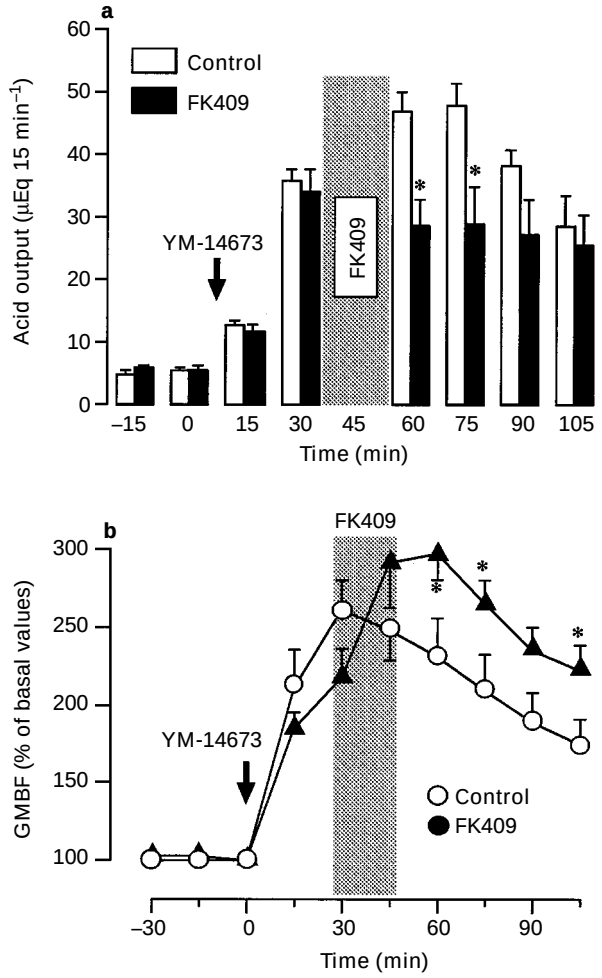


Figure 5 Effect of FK409 (6 mg ml^{-1}) on the increase of acid secretion (a) and GMBF (b) induced by YM-14673 (0.3 mg kg^{-1}) in the anaesthetized rat. YM-14673 was administered i.v. as a bolus injection, while FK409 (2 ml) was applied intragastrically for 15 min, 1 h after YM-14673. The values of acid secretion are expressed as μEq 15 min^{-1}, while those of GMBF are expressed as % of basal values. Data are presented as the means ± s.e. (vertical lines) of values determined every 15 min from 5 rats. *Statistically significant difference from control, at $P<0.05$.

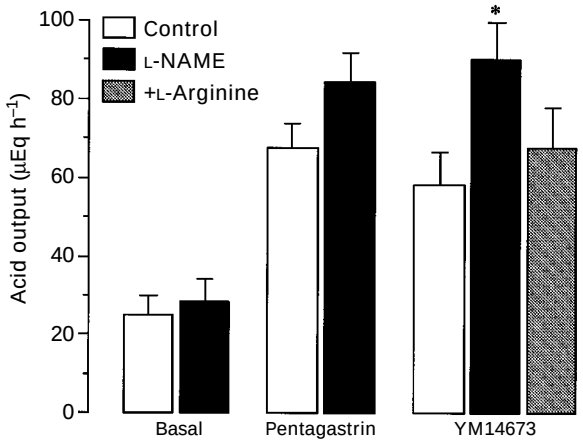


Figure 6 Effects of L-NAME on basal and stimulated acid secretion by pentagastrin and YM-14673 in the anaesthetized rat. Pentagastrin (60 μg kg^{-1} h^{-1}) was continuously infused i.v. from a tail vein, while YM-14673 (0.3 mg kg^{-1}) was administered i.v. as a bolus injection. L-NAME (10 mg kg^{-1}) was administered i.v. 15 min before the onset of pentagastrin infusion or YM-14673 injection. L-Arginine (500 mg kg^{-1}) was given i.p. 15 min before L-NAME. Values indicate total acid output for 1 h after pentagastrin infusion or YM-14673 injection and are expressed as μEq h^{-1}. Data are presented as the means ± s.e. from 5 rats. *Statistically significant difference from the control, at $P<0.05$.

Table 1 Effects of FK409 and SNP on arterial blood pressure in urethane anaesthetized rats

Drugs	*Dose* (mg ml^{-1})	*Before* (mmHg)	*15 min after* (mmHg)	*30 min after* (mmHg)
FK409	3	82.0 ± 1.8	67.6 ± 2.7*	72.0 ± 3.4*
	6	82.4 ± 2.1	60.4 ± 2.4*	69.6 ± 2.6*
SNP	6	83.6 ± 3.0	83.6 ± 2.7	82.4 ± 3.1
	12	84.8 ± 3.3	75.6 ± 2.3*	73.6 ± 2.7*

FK409 or SNP was applied intragastrically for 15 min. All data represent values determined at 15 and 30 min after treatment with FK409 or SNP and are presented as the means ± s.e. from five rats per group. *Statistically significant difference from before values, at $P<0.05$.

significantly reduced the luminal release of histamine in response to pentagastrin, the inhibition being 42.6%.

Effect of cimetidine on acid secretory responses induced by pentagastrin and YM-14673

To confirm the involvement of endogenous histamine in the mechanism of acid secretion induced by pentagastrin and YM-14673, the effects of cimetidine a histamine H_2-receptor antagonist on the acid secretory response to these agents were examined. Cimetidine (60 mg kg^{-1}, i.p.) potently inhibited histamine-induced acid secretion, the degree of inhibition being 83.3%. Likewise, this agent at the same dose caused a significant decrease in the acid secretory response induced by either pentagastrin or YM-14673, the inhibition being 74.1% or 53.8%, respectively (Figure 8).

Discussion

The present study showed that intragastric application of NO donors such as FK409 and SNP significantly decreased both basal and stimulated acid secretion by pentagastrin and YM-14673, an analogue of TRH, but not by histamine. Furthermore, we observed that FK409 significantly reduced the luminal histamine release into the gastric lumen in response to pentagastrin. On the other hand, the inhibition of NO biosynthesis by L-NAME potentiated the increase of acid secretion induced by YM-14673 and pentagastrin but had no effect on basal acid secretion. These findings suggest that NO, either generated endogenously or administered exogenously, is capable of reducing gastric acid secretion through inhibition of histamine release from enterochromaffin-like (ECL) cells.

There have been several studies investigating the effect of NO synthase inhibitors on acid secretion, although the results remained controversial. Pique *et al.* (1992) found that the NO synthase inhibitor L-NMMA did not affect either basal or pentagastrin-stimulated acid secretion in rats. Martinez-Cuesta *et al.* (1992) and Esplugues *et al.* (1993) showed that the NO synthase inhibitor L-NAME antagonized the inhibitory action of lipopolysaccharide or cytokines on acid secretion induced by gastric distension or pentagastrin in rats. We have also shown that the inhibitory acid response in the stomach after

Table 2 Effects of pentagastrin and YM-14673 on NO_x release into the gastric lumen in urethane anaesthetized rats

Treatments	*Luminal NO_x release* (nmol h^{-1})
Saline	
Control	28.8±2.5
+L-NAME	19.6±2.4[b]
+omeprazole	29.3±5.0
+vagotomy	24.6±4.3
Pentagastrin	
Control	42.3±1.9[a]
+L-NAME	27.9±2.0[a,b]
+omeprazole	35.1±5.2
YM-14673	
Control	97.4±5.8[a]
+L-NAME	35.8±2.7[a,b]
+omeprazole	81.4±9.9[a]
+vagotomy	31.9±0.9[b]

Pentagastrin (60 μg kg^{-1} h^{-1}) was continuously infused i.v. during a test period, while YM-14673 (0.3 mg kg^{-1}) was administered i.v. as a bolus injection. L-NAME (10 mg kg^{-1}) was administered i.v. as a bolus injection 15 min before the onset of pentagastrin infusion or the injection of YM-14673, respectively, while omeprazole (30 mg kg^{-1}) was given i.p. 30 min before these treatments. Values represent total amount of NO_x obtained for 1 h after pentagastrin infusion or YM-14673 injection and are presented as the means±s.e. from five rats. Statistically significant difference, [a]from saline group; [b]from control group, at $P<0.05$.

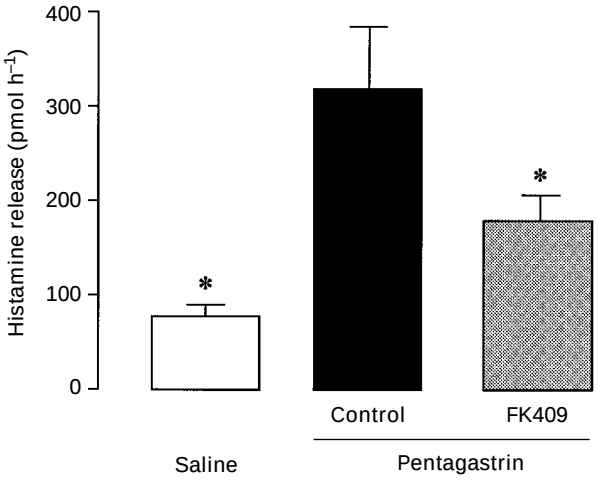


Figure 7 Effect of FK409 on the luminal release of histamine in response to pentagastrin in the anaesthetized rat. Pentagastrin (60 μg kg^{-1} h^{-1}) was continuously infused i.v. from a tail vein, while FK409 (6 mg ml^{-1}; 2 ml) was applied intragastrically for 15 min, 1 h after the onset of pentagastrin infusion. Values indicate histamine output for 1 h and are expressed as pmol h^{-1}. Data are presented as the means±s.e. from 5 rats. *Statistically significant difference from the control, at $P<0.05$.

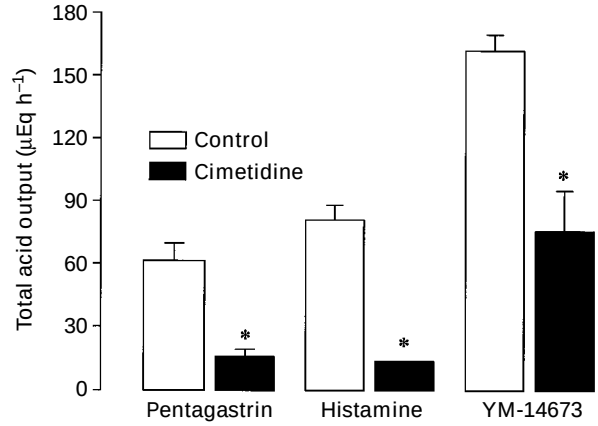


Figure 8 Effects of cimetidine on acid secretion induced by pentagastrin and YM-14673 in the anaesthetized rat. Pentagastrin (60 μg kg^{-1} h^{-1}) was continuously infused i.v. from a tail vein, while YM-14673 (0.3 mg kg^{-1}) was administered i.v. as a bolus injection. Cimetidine (60 mg kg^{-1}) was administered i.p. 30 min before these treatments. Values indicate total acid output for 1 h after pentagastrin infusion or YM-14673 injection and are expressed as μEq h^{-1}. Data are presented as the means±s.e. from 5 rats. *Statistically significant difference from the control, at $P<0.05$.

damage was completely antagonized by L-NAME, suggesting an inhibitory role of NO in regulation of gastric acid secretion (Takeuchi *et al*., 1994). In contrast, Bilski *et al*. (1994) showed that an NO synthase inhibitor failed to affect basal acid secretion but reduced the acid secretion in response to meat feeding or pentagastrin in dogs.

In the present study, we found that the NO donors FK409 and SNP, applied topically to the stomach, decreased both basal and stimulated acid secretion by pentagastrin or YM-14673, but had no effect on the acid secretory response to histamine. These results are partly consistent with the previous observation by Barrachina *et al*. (1994), who showed that i.v. infusion of S-nitroso-glutathione, another NO donor, reduced vagally-mediated acid secretion by 2-deoxy-D-glucose and gastric distension. Since NO containing neurones have been identified in the central nervous system as well as in the gastrointestinal mucosa (Knowles *et al*., 1989; Bredt *et al*., 1990) and as NO plays a role as a neuromodulator in some non-adrenargic and non-cholinergic neurones in the gut (Sanders & Ward, 1992; Lowenstein *et al*., 1994), it is possible that NO donors reduce vagally-mediated acid secretion by suppressing neuronal activity of the vagus nerves. In addition, the present study demonstrated that NO donors reduced the acid secretion induced peripherally by pentagastrin but not histamine. Brown *et al*. (1993) found that high concentrations of NO donors inhibit acid secretion in rat isolated parietal cells, suggesting a direct inhibitory action at the parietal cell level. Although SNP at a relatively high dose (6 mg kg^{-1}, i.v.) reduced acid secretion in response to histamine (Takeuchi *et al*., 1994), this effect may be largely due to hypotension and hypoperfusion caused by the NO donor. FK409, an orally active NO donor, administered p.o., was rapidly absorbed and subsequently a marked reduction of systemic blood pressure was observed (Kita *et al*., 1994a). We confirmed that intragastric FK409 was immediately absorbed from the surface of gastric wall and produced a potent reduction of systemic blood pressure, as the present study was performed in the chambered stomach with pylorus ligation. In the present study, the NO donors caused significant inhibition of basal and stimulated acid secretion in response to pentagastrin or YM-14673, but had no effect on histamine-induced acid secretion. If the hypotensive effect itself caused a profound reduction in acid secretion, then the inhibitory effect would be similarly observed in the acid secretory response, irrespective of whether the secretion is stimulated by pentagastrin or histamine. Furthermore, SNP applied intragastrically caused a negligible change in systemic blood pressure, but did suppress significantly the acid secretory response to pentagastrin. Thus, it is unlikely that the inhibitory effect of intragastric NO

donors on acid secretion is attributable to a reduction in blood pressure and gastric hypoperfusion.

It should be noted in the present study that the maximal GMBF response to intragastric application of FK409 and SNP was observed after their removal from the chamber. This may be explained by the following hypothesis: the NO donors might remain in the gastric mucosa even after their removal from the chamber. Indeed, the antisecretory action of these drugs persisted for 60 min and the maximal effect was observed around 30 min after application, the time when the maximal GMBF response was also seen. Alternatively, since NO increases GMBF through activation of a second messenger, guanylate cyclase/guamosine 3′ : 5′-cyclic monophosphate (cyclicGMP) system, it is possible that the activation of this system might persist a little bit longer even after the drug is removed from the chamber. It was reasonable that the maximal GMBF response to SNP appeared later than that caused by FK409, because FK409 is a spontaneous NO releasing drug, which can generate NO, much faster than SNP (Kita *et al*., 1994a).

In the present study, we found that the NO synthase inhibitor L-NAME significantly enhanced the increase of acid secretion in response to vagal stimulation by YM-14673 and slightly to pentagastrin. Intravenous administration of YM-14673, a long-lasting and peripherally active TRH analogue (Fujiwara & Ida, 1989), induced a vagally-mediated acid secretion, similar to i.c. injection of TRH (Takeuchi *et al*., 1990). Tanaka *et al*. (1993) and Saperas *et al*. (1995) found that vagal stimulation by RX 77368, another TRH analogue, caused an increase of GMBF as well as NO generation in the gastric mucosa, and this action was prevented by inhibition of NO synthesis. It was also found in this study that pentagastrin as well as YM-14673 increased luminal release of NO in the stomach, in an L-NAME-sensitive manner. We previously observed that gastric hyperaemic response induced by pentagastrin as well as YM-14673 was significantly attenuated by L-NAME (Kato *et al*., 1997a, b). These results suggest that both YM-14673 and pentagastrin increase the generation of endogenous NO, which in turn may suppress the acid secretion induced by these secretagogues. Potentiation by L-NAME of the acid secretory response might result from removal of the inhibitory influence of NO by inhibition of the NO synthase. Indeed, this effect of L-NAME on YM-14674-induced acid secretion was antagonized by the simultaneous administration of L-arginine (Kato *et al*., 1997b). The present study also showed that the increase in NO_x release induced by YM-14673 was completely attenuated by vagotomy but little affected by omeprazole, while that induced by pentagastrin was almost completely inhibited by omeprazole. Although NO release seems to occur in association with acid secretion, the activation of vagus nerves increases the generation of endogenous NO by a process independent of acid secretion.

On the other hand, pentagastrin-induced acid secretion is mainly part mediated by endogenous histamine released from ECL cells (Richardson 1978; Sandvik *et al*., 1987). Even TRH is known to release histamine in the gastric mucosa by a vagal-cholinergic mechanism (Yanagisawa & Tache, 1990). The present findings that FK409 reduced the acid secretory response to YM-14673 and pentagastrin but not to histamine can be explained by assuming that NO donors inhibit histamine release from ECL cells in response to pentagastrin or YM-14673. Indeed, it has been shown that exogenous NO inhibits the release of histamine in rat mast cells mediated by guanylate-cyclase/cyclicGMP-dependent mechanism (Salvemini *et al*., 1991). A recent study also showed that interleukin-1β (IL-1β) exhibits an antisecretory action against pentagastrin by suppressing histamine release, in an L-NAME-sensitive manner (Wallace *et al*., 1991; Esplugues *et al*., 1993). Also IL-1β has been found to inhibit histamine release from ECL cells via an effect on cyclicGMP (Prinz *et al*., 1997). It has also been shown that FK409 causes a marked increase in the plasma level of cyclicGMP in rats after oral administration (Kita *et al*., 1994b). On the basis of these results, it is assumed that NO is capable of reducing acid secretion by inhibiting histamine release from ECL cells.

The present results taken together suggest that NO, either generated endogenously or administered exogenously, reduced gastric acid secretion under basal and stimulated conditions. Since this effect was observed when the acid secretion was induced by pentagastrin and a vagally-mediated mechanism, but not by histamine, it is likely that NO has an inhibitory action on gastric acid secretion mediated through the suppression of histamine release from ECL cells.

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