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Stimulation by Nitric Oxide Synthase Inhibitors of Gastric and Duodenal HCO_3^- Secretion in Rats

KOJI TAKEUCHI, TOMOHISA OHUCHI, HIROKI MIYAKE and SUSUMU OKABE
Department of Applied Pharmacology, Kyoto Pharmaceutical University, Misasagi, Yamashina, Japan
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ABSTRACT

The role of nitric oxide (NO) in the regulation of gastroduodenal HCO_3^- secretion was investigated in anesthetized rats using the NO biosynthesis inhibitor N^G-nitro-L-arginine methyl ester (L-NAME). HCO_3^- secretion was measured at pH 7.0 using a pH-stat method in the chambered stomach in the presence of omeprazole or in the proximal duodenum. Intravenous administration of L-NAME (1–5 mg/kg) increased HCO_3^- secretion in a dose-dependent manner in both the stomach and duodenum, with a concomitant elevation of arterial blood pressure. The stimulatory effect of L-NAME on HCO_3^- secretion was mimicked by another NO synthase inhibitor, N^G-monomethyl-L-arginine (50 mg/kg), but not by the enantiomer N^G-nitro-D-arginine methyl ester, and was significantly antagonized by concurrent administration of L-arginine, but not D-arginine, at 200 mg/kg. The exogenous NO donor nitroprusside (4 mg/kg) by itself decreased the rate of HCO_3^- secretion and significantly antagonized the HCO_3^- stimulatory action of L-NAME. Furthermore, the increased HCO_3^- secretion caused by L-NAME was significantly attenuated by prior administration of atropine (1 mg/kg, s.c.) or indomethacin (5 mg/kg, s.c.) and by bilateral vagotomy but was not influenced by sensory deafferentation after capsaicin pretreatment, though none of the treatments had any effect on the changes in blood pressure induced by L-NAME. These results suggest that L-NAME stimulates HCO_3^- secretion in the gastroduodenal mucosa. This action is associated with the inhibition of NO biosynthesis and may be partly dependent on vagal-cholinergic innervation and mediated by endogenous prostaglandins.

HCO_3^- secretion from the surface epithelial cells is considered one of the defensive factors in the gastroduodenal mucosa (Flemstrom and Turnberg, 1984; Garner *et al.*, 1984). PGs and their derivatives, which confer protection of the mucosa against acid, increase HCO_3^- secretion as well as mucosal blood flow (Robert, 1981; Garner *et al.*, 1984). On the other hand, nitric oxide (NO), which accounts for the biological actions of endothelium-derived relaxing factor and is the endogenous stimulator of the soluble guanylate cyclase (Furchgott, 1984), is now known to be generated in various other cells, including the epithelium, mast cells, macrophages and enteric neurons (Moncada *et al.*, 1991), and mimics the protective action of PGs in the gastric mucosa (Pique *et al.*, 1989; Whittle *et al.*, 1990; Lippe *et al.*, 1992). Unexpectedly, we found in a preliminary study that one of the NO synthase inhibitors, L-NAME, increased HCO_3^- secretion in the stomach, suggesting that NO formed endogenously may play a role in modulation of the mucosa's ability to secrete HCO_3^- under pathophysiological conditions (Takeuchi *et al.*, 1992b). However, the mechanism underlying the increase of HCO_3^- secretion brought about by the inhibition of NO production remains unknown.

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In the present study, we examined the effects of L-NAME on HCO_3^- secretion from the gastroduodenal mucosa of anesthetized rats and characterized these effects with regard to inhibition of NO biosynthesis and relation to such HCO_3^- stimulatory pathways as endogenous PGs and the vagal-cholinergic mechanism.

Materials and Methods

Animals. Male Sprague-Dawley rats weighing 230 to 250 g (Charles River, Shizuoka, Japan) were used. The animals were kept in individual cages with raised mesh bottoms to prevent coprophagia, and they were deprived of food but were allowed free access to tap water for 18 hr before the experiments. All studies were carried out using 5 to 6 animals per group under anesthetized conditions induced by i.p. administration of urethane (1.25 g/kg).

Determination of gastric and duodenal HCO_3^- secretion. Gastric and duodenal HCO_3^- secretion was determined in the chambered stomach and in the duodenal loop according to previously published methods (Takeuchi *et al.*, 1986; 1992a). Briefly, the abdomen was incised, and the stomach was exposed, mounted on a chamber (exposed area: 3.1 cm^2) and perfused with saline that was gassed with 100% O_2 and kept in a reservoir. Gastric alkaline secretion was measured at pH 7.0 by using a pH-stat method (Hiranuma Comtite-7, Mito, Japan) and by adding 10 mM HCl to the reservoir. To unmask HCO_3^- in the

ABBREVIATIONS: PGs, prostaglandins; PGE_2, prostaglandin E_2; D-NAME, N^G-nitro-D-arginine methyl ester; L-NAME, N^G-nitro-L-arginine methyl ester; L-NMMA, N^G-monomethyl-L-arginine; cyclic AMP, adenosine-3′,5′-cyclic monophosphate; cyclic GMP, guanosine-3′,5′-cyclic monophosphate.

stomach acid, secretion was completely inhibited by omeprazole given i.p. in a dose of 60 mg/kg. On the other hand, the duodenal loop (1.7 cm) was made between the pyloric ring and the area just above the outlet of the common bile duct in order to exclude the influences of bile and pancreatic juice. Then the loop was perfused with saline as described for the gastric preparation, and HCO_3^- secretion was measured at pH 7.0 by using the pH-stat system and by adding 10 mM HCl to the reservoir. In some animals, arterial blood pressure (BP) was monitored through a femoral artery by a pressure transducer and amplifier system (San-ei, type 45277, Tokyo, Japan). After basal HCO_3^- secretion had stabilized, the following agents were given i.v. as a bolus injection: PGE_2 (0.3 mg/kg), L-NAME (1, 2.5 and 5 mg/kg), D-NAME (5 mg/kg) and L-NMMA (50 mg/kg). In both gastric and duodenal preparations, HCO_3^- secretion was measured for 90 min after administration of these agents. In some cases, the effects of the NO precursor L-arginine, the exogenous NO donor nitroprusside, indomethacin and atropine on the HCO_3^- stimulatory action of L-NAME were examined. L- or D-arginine (200 mg/kg) or nitroprusside (4 mg/kg) was given i.v. 5 min before injection of L-NAME, whereas indomethacin (5 mg/kg) or atropine (1 mg/kg) was given s.c. 30 min before treatment with L-NAME. In addition, the influences of vagotomy and sensory deafferentation on the action of L-NAME were examined. Vagotomy was performed bilaterally at the subdiaphragmatic portion 60 min before administration of L-NAME. Sensory deafferentation was performed by s.c. injection of capsaicin for 3 days (20, 30 and 50 mg/kg) 2 weeks before the experiment (Yonei *et al.*, 1990). In a separate experiment, the effect of endothelin-1 (ET-1: 0.3–1 nmol/kg) on gastric HCO_3^- secretion was also examined. ET-1 was given i.v. as a bolus injection after basal HCO_3^- secretion had stabilized.

Measurement of vascular permeability. To examine the effects of L-NAME and ET-1 on gastric mucosal vascular permeability, we used a dye method to measure the extravasated amount of dye as described in a previous paper (Takeuchi *et al.*, 1987). The animals were given L-NAME (5 mg/kg) or ET-1 (1 nmol/kg) i.v., and were sacrificed 1 hr later. In each case, 1 ml of 1% Evans blue (w/w) was injected i.v. 30 min before killing. The animals were killed by bleeding from the descending aorta, the stomachs were removed and the amount of dye that had accumulated in the mucosa in 30 min was measured. The stomach was opened along the greater curvature, and the mucosa was scraped off (using two glass slides), weighed and put into a tube containing 5 ml distilled water. The extraction of dye was performed according to the modified method described by Katayama *et al.* (1978). Briefly, mucosal scrapings were soaked overnight in stoppered glass tubes containing 2 ml of 3.5 N KOH at 37°C. Then 18 ml of a mixed solution of 4 N H_3PO_4 and acetone (1.75:16.25) was added to each tube to make up a total volume of 25 ml. The tube was shaken vigorously for a few seconds and centrifuged at 3000 rpm for 15 min. Absorbance of supernatant was measured at 620 nm on a Hitachi spectrophotometer (Mito, Ibaraki, Model 200–100). The amount of dye recovered from the mucosa was expressed in micrograms per 100 mg of tissue (μg/100 mg tissue).

Preparation of drugs. Drugs used were urethane (Tokyo Kasei, Tokyo, Japan), L-NAME, L-NMMA, nitroprusside, indomethacin, Evans blue (Sigma, St. Louis, MO. U.S.A.), D-NAME (Bachem, Bubendorf, Switzerland), PGE_2 (Funakoshi, Tokyo, Japan), L-arginine, D-arginine, atropine, capsaicin (Wako, Osaka, Japan), endothelin-1 (Peptide Institute, Osaka, Japan) and omeprazole (Hessle, Mondale, Sweden). Urethane, L-NAME, D-NAME, L-NMMA, L-arginine, D-arginine, nitroprusside, ET-1 and atropine were dissolved in saline. PGE_2 was first dissolved in absolute ethanol and then diluted with saline to a desired concentration. Capsaicin was dissolved in a solution consisting of 10% ethanol, 10% Tween 80 (Wako) and 80% saline (v/v). Indomethacin was suspended in saline with a drop of Tween 80, whereas omeprazole was suspended with 0.5% carboxymethylcellulose solution. Each agent was prepared immediately before use and was given in a volume of 0.1 ml per 100 g body wt in case of i.v. administration or in a volume of 0.5 ml per 100 g body wt in cases of i.p. and s.c. administration. Control animals received saline as the vehicle.

Statistics. Data are presented as the mean ± S.E. from 4 to 6 rats per group. Statistical analyses were performed using a two-tailed Dunnett's multiple comparison test (Dunnett, 1955), and values of $P < .05$ were regarded as significant.

Results

Effects of L-NAME on gastric and duodenal HCO_3^- secretion. Under our experimental conditions, the rat stomach spontaneously secreted HCO_3^- at a steady rate of 0.25 ± 0.03~ 0.35 ± 0.02 μEq every 5 min in the animals given omeprazole to inhibit acid secretion. Intravenous administration of L-NAME (1, 2.5 and 5 mg/kg) produced a significant increase of gastric HCO_3^- secretion in a dose-dependent manner, and at 5 mg/kg the rate of secretion increased from 0.23 ± 0.03 μEq/5 min to the maximal values of 0.85 ± 0.16 μEq/5 min within 20 min and remained elevated for 90 min thereafter (fig. 1). The increase in HCO_3^- output caused by L-NAME (5 mg/kg) was 5.9 ± 1.1 μEq/hr, which is equivalent to that (4.8 ± 1.0 μEq/hr) induced by PGE_2 (0.3 mg/kg).

On the other hand, the proximal duodenum (1.7 cm) secreted HCO_3^- at a steady basal rate of 0.44 ± 0.13~ 0.55 ± 0.12 μEq/5 min during a 90 min test period. As observed in the stomach, L-NAME administered i.v. as a single injection produced a dose-dependent increase of HCO_3^- secretion in the duodenum. After administration of this agent at 5 mg/kg, the duodenal HCO_3^- secretion was increased from 0.53 ± 0.07 μEq/5 min to the maximal values of 1.45 ± 0.32 μEq/5 min, about 3 times greater than basal levels, and remained elevated even 90 min later (fig. 2). ΔHCO_3^- output induced by L-NAME was 5.2 ± 1.2 μEq/hr at 2.5 mg/kg and 8.2 ± 1.5 μEq/hr at 5 mg/kg, and

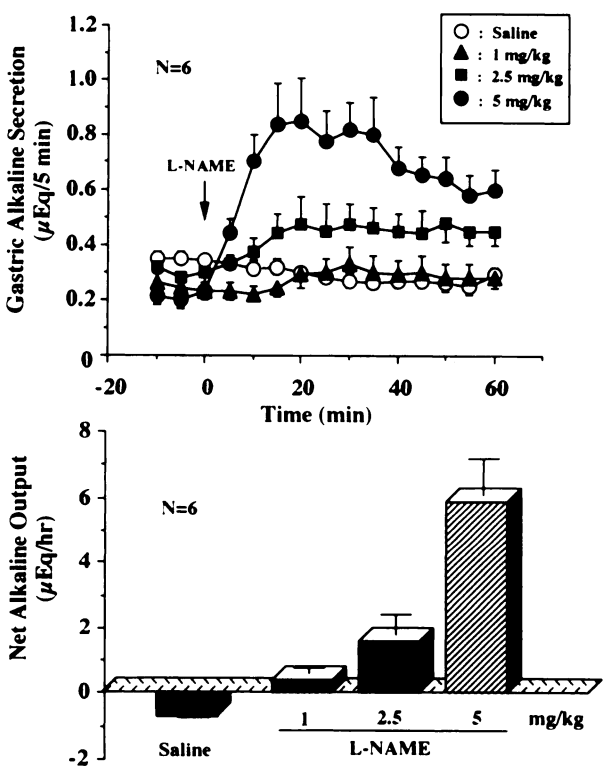

Fig. 1. Dose-response effects of L-NAME on gastric HCO_3^- secretion in anesthetized rats. The animals were given omeprazole (60 mg/kg, i.p.) to inhibit acid secretion. L-NAME (1, 2.5 and 5 mg/kg) was administered i.v. as a single injection. Data are presented as the means ± S.E. of values determined every 5 min from six rats. Lower panel shows ΔHCO_3^- output obtained for 1 hr in each group.

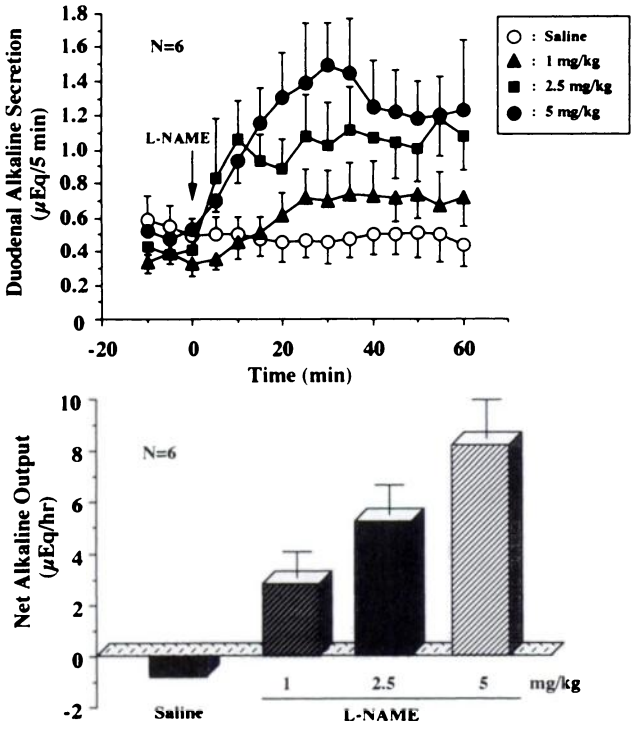

Fig. 2. Dose-response effects of L-NAME on duodenal HCO_3^- secretion in anesthetized rats. L-NAME (1, 2.5 and 5 mg/kg) was administered i.v. as a single injection. Data are presented as the means ± S.E. of values determined every 5 min from six rats. Lower panel shows ΔHCO_3^- output obtained for 1 hr in each group.

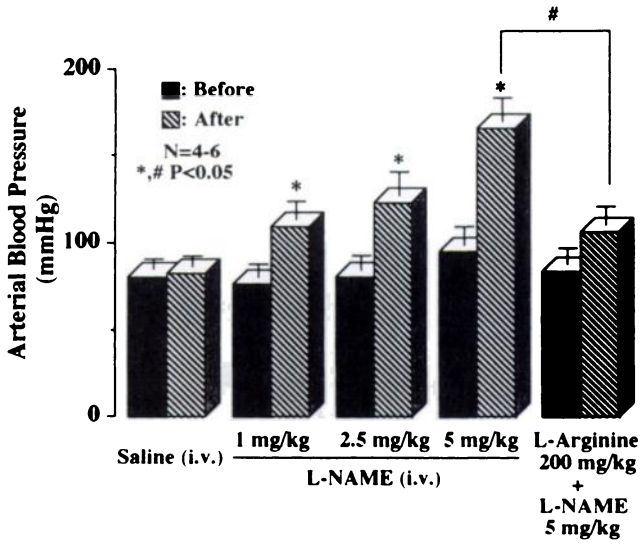

Fig. 3. Effects of L-NAME, either alone or in combination with L-arginine, on arterial blood pressure in anesthetized rats. L-NAME (1, 2.5 and 5 mg/kg) was administered i.v. as a single injection. L-arginine (200 mg/kg) was given i.v. 5 min before administration of L-NAME. Data are presented as the means ± S.E. from 4 to 5 rats per group. Statistically significant difference at $P < .05$; * from saline control, # from the group given 5 mg/kg of L-NAME.

the latter was even greater when compared to that (4.1 ± 0.7 μEq/hr) induced by PGE_2 (0.3 mg/kg).

Intravenous administration of L-NAME (1, 2.5 and 5 mg/kg) caused a marked elevation in arterial blood pressure in a dose-dependent manner (fig. 3). This pressor response was already significant at 1 mg/kg, and the mean blood pressure was increased in response to 5 mg/kg of L-NAME from 94.3 ± 10.5 mm Hg to 165.8 ± 13.5 mm Hg within 5 min, Δ increase being 75.8 ± 8.4%. The rise in blood pressure caused by L-NAME was significantly mitigated when the animals were pretreated with L-arginine (200 mg/kg) given i.v. 5 min before administration of L-NAME; Δ increase in blood pressure was 26.4 ± 4.6%.

In addition, both gastric and duodenal HCO_3^- secretions were stimulated by another NO synthase inhibitor, L-NMMA. As shown in figures 4 and 5, L-NMMA (50 mg/kg) administered i.v. caused a significant increase of HCO_3^- secretion, though the potency at this dose was modest and equivalent to 1/3 of that induced by L-NAME at 5 mg/kg; ΔHCO_3^- output was 2.4 ± 0.7 μEq/hr in the stomach and 4.5 ± 1.0 μEq/hr in the duodenum. In contrast, administration of D-NAME, the enantiomer of L-NAME, did not significantly affect the mucosa's ability to secrete HCO_3^- in either the stomach or the duodenum, and ΔHCO_3^- output induced by D-NAME was not significantly different from that observed in the animals given saline.

Effects of L-arginine and nitroprusside on HCO_3^- stimulatory action of L-NAME. To investigate whether the HCO_3^- stimulatory action of L-NAME is related to inhibition of NO biosynthesis, we examined the effects of L- and D-arginine on the HCO_3^- responses induced by L-NAME. Intravenous administration of L- or D-arginine (200 mg/kg) by itself did not affect the luminal alkalinization in either the stomach or the duodenum (not shown). However, pretreatment of the animals with L-arginine significantly reduced the increase of

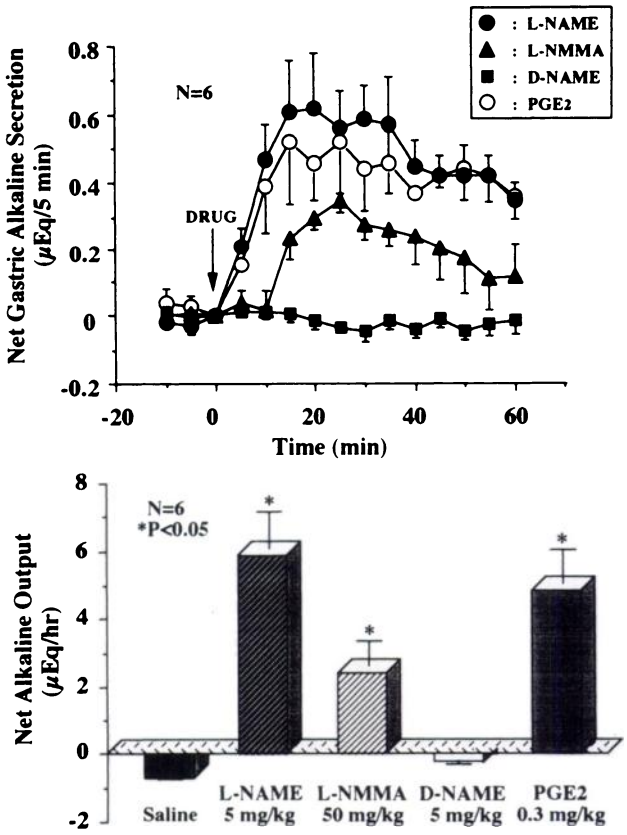

Fig. 4. Effects of various arginine analogs on HCO_3^- secretion in the stomach of anesthetized rats. Acid secretion was inhibited by omeprazole. L-NAME (5 mg/kg), D-NAME (5 mg/kg), L-NMMA (50 mg/kg) and PGE_2 (0.3 mg/kg) were administered i.v. as a single injection. Data are expressed as the net HCO_3^- output and represent the means ± S.E. of values determined every 5 min from six rats. Lower panel shows ΔHCO_3^- output obtained for 1 hr in each group. * Statistically significant difference from saline control (lower panel), at $P < .05$.

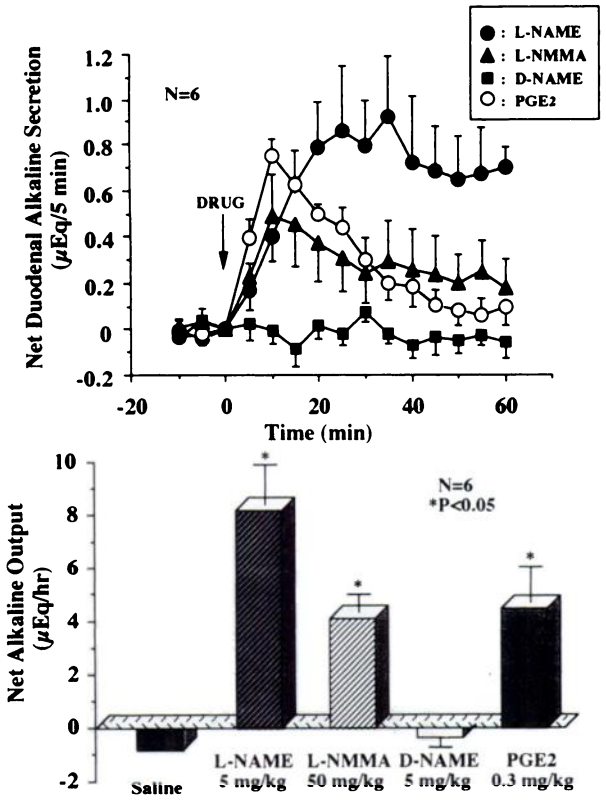

Fig. 5. Effects of various arginine analogs on HCO_3^- secretion in the duodenum of anesthetized rats. L-NAME (5 mg/kg), D-NAME (5 mg/kg), L-NMMA (50 mg/kg) and PGE_2 (0.3 mg/kg) were administered i.v. as a single injection. Data are expressed as the net HCO_3^- output and represent the means ± S.E. of values determined every 5 min from six rats. Lower panel shows ΔHCO_3^- output obtained for 1 hr in each group. * Statistically significant difference from saline control (lower panel), at $P < .05$.

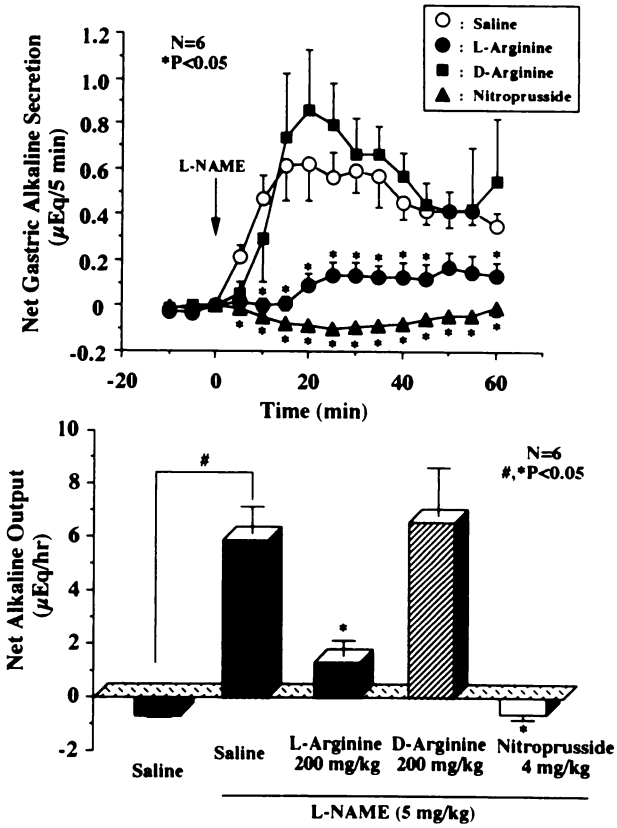

Fig. 6. Effects of L-arginine, D-arginine and nitroprusside on the HCO_3^- stimulatory effect of L-NAME in the stomach of anesthetized rats. L- and D-arginine (200 mg/kg) and nitroprusside (4 mg/kg) were administered i.v. 5 min before administration of L-NAME (5 mg/kg). Data are expressed as the net HCO_3^- secretion and represent the means ± S.E. of values determined every 5 min from six rats. Lower panel shows ΔHCO_3^- output obtained for 1 hr in each group. Statistically significant difference at $P < .05$; * from the group given saline and L-NAME (upper and lower panels), # from saline control (lower panel).

HCO_3^- secretion induced by L-NAME (5 mg/kg); ΔHCO_3^- output was 1.3 ± 0.6 μEq/hr in the stomach, which was about 22.0% of that (5.9 ± 1.1 μEq/hr) induced by L-NAME in control rats (figs. 6 and 7). On the other hand, prior administration of D-arginine (200 mg/kg) did not significantly affect the HCO_3^- responses to L-NAME in either the stomach or the duodenum; ΔHCO_3^- output induced by 5 mg/kg of L-NAME in the duodenum was 9.3 ± 1.4 μEq/hr, which is not significantly different from that (8.2 ± 1.5 μEq/hr) obtained in control animals. Even in the presence of D-arginine, L-NAME (5 mg/kg) increased HCO_3^- output to the maximal values of 4 times and 3 times over the basal levels in the stomach and the duodenum, respectively. We further examined whether the exogenous NO donor nitroprusside antagonized the HCO_3^- stimulatory action of L-NAME. Intravenous administration of nitroprusside (4 mg/kg) by itself slightly suppressed the basal rate of HCO_3^- secretion and significantly inhibited the increase of HCO_3^- secretion induced by subsequent injection of L-NAME. Especially in the stomach, the increased HCO_3^- secretion by L-NAME (5 mg/kg) was completely inhibited by nitroprusside; ΔHCO_3^- output was −0.6 ± 0.1 μEq/hr. Although HCO_3^- secretion in the duodenum was increased by L-NAME from 0.37 ± 0.08 μEq/5 min to the maximal values of 0.76 ± 0.26 μEq/5 min (198.9 ± 36.1% of basal values) in the presence of nitroprusside, the ΔHCO_3^- output (2.6 ± 1.0 μEq/hr) induced by L-NAME was significantly lower than that observed in the absence of nitroprusside. On the other hand, both L-arginine and nitroprusside significantly antagonized the increased blood pressure response caused by L-NAME (data not shown).

Effects of various treatments on HCO_3^- stimulatory action of L-NAME. Subcutaneous administration of indomethacin (5 mg/kg) or atropine (1 mg/kg) alone did not significantly affect the spontaneous rate of alkaline secretion in both the stomach and the duodenum (data not shown). However, pretreatment of the animals with either of these agents significantly reduced the HCO_3^- secretion in response to L-NAME, though the effectiveness differed somewhat between stomach and duodenum (figs. 8 and 9). In the stomach, ΔHCO_3^- output induced by L-NAME was reduced much more potently by indomethacin (85%) than by atropine (35.4%). In contrast, the HCO_3^- secretion induced by L-NAME in the duodenum was inhibited similarly by these agents, and ΔHCO_3^- output was reduced from 8.2 ± 1.5 μEq/hr to 3.7 ± 0.8 μEq/hr in the presence of indomethacin and to 4.2 ± 1.7 μEq/hr in the presence of atropine, reductions of 54.5% and 48.8%, respectively. The stimulatory effect of L-NAME on HCO_3^- secretion was also significantly mitigated by vagotomy. L-NAME given intravenously increased HCO_3^- secretion even in the vagotomized animals, but this effect was significantly less potent when compared with control animals. In these rats, ΔHCO_3^- output in response to L-NAME was 2.2 ± 0.7 μEq/hr in the stomach and 1.8 ± 0.8 μEq/hr in the duodenum, values equiv-

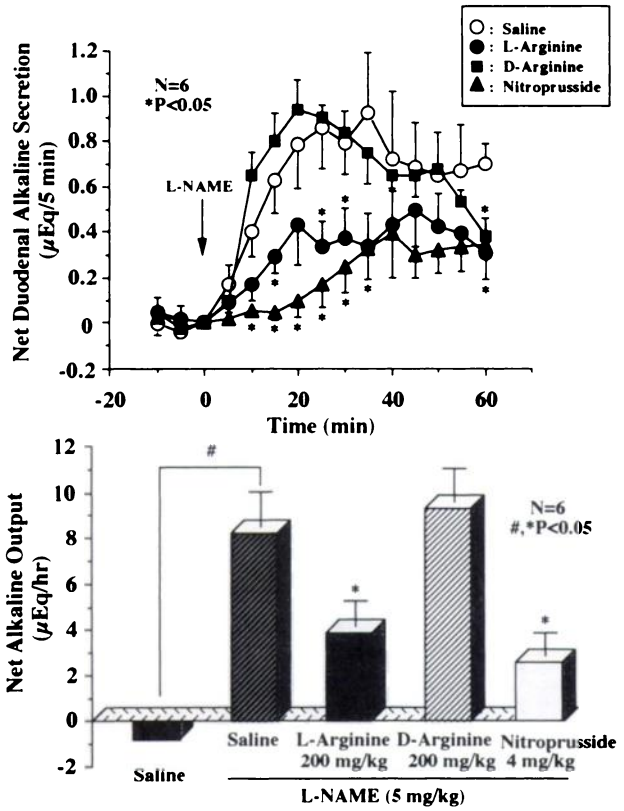

Fig. 7. Effects of L-arginine, D-arginine and nitroprusside on the HCO_3^- stimulatory effect of L-NAME in the duodenum of anesthetized rats. L- and D-arginine (200 mg/kg) and nitroprusside (4 mg/kg) were administered i.v. 5 min before administration of L-NAME (5 mg/kg). Data are expressed as the net HCO_3^- secretion and represent the means ± S.E. of values determined every 5 min from six rats. Lower panel shows ΔHCO_3^- output obtained for 1 hr in each group. Statistically significant difference at $P < .05$; * from the group given saline and L-NAME (upper and lower panels), # from saline control (lower panel).

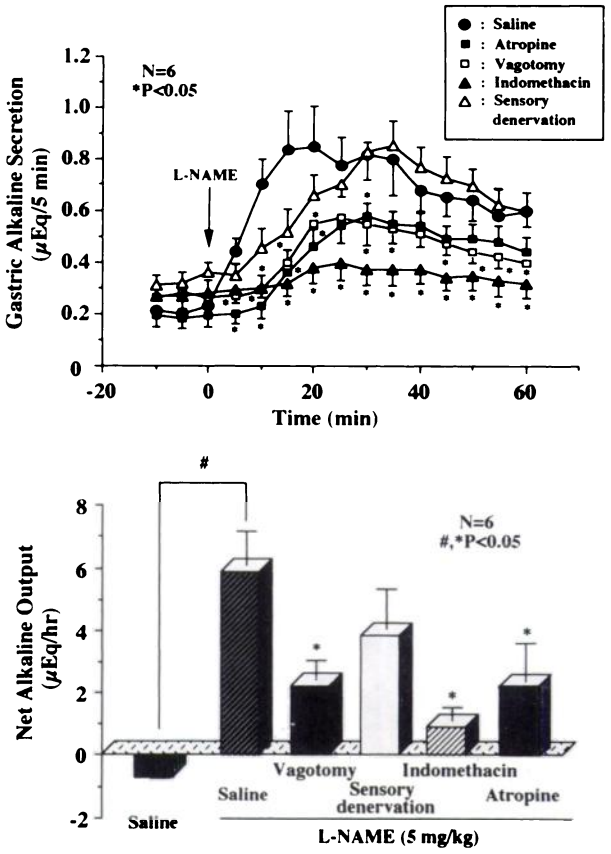

Fig. 8. Effects of indomethacin, atropine, vagotomy and sensory denervation on gastric HCO_3^- stimulatory action of L-NAME in anesthetized rats. Indomethacin (5 mg/kg) or atropine (1 mg/kg) was given s.c. 30 min before i.v. administration of L-NAME (5 mg/kg). Vagotomy was performed at the neck portion 1 hr before i.v. administration of L-NAME; while sensory deafferentation was performed by consecutive s.c. injections of capsaicin 2 weeks before the experiment. Data are expressed as the net HCO_3^- output and represent the means ± S.E. of values determined every 5 min from six rats. Lower panel shows ΔHCO_3^- output obtained for 1 hr in each group. Statistically significant difference at $P < .05$; * from the group given saline and L-NAME (upper and lower panels), # from saline control (lower panel).

alent to 35.6% and 22.0% of that induced by L-NAME in control animals, respectively. On the other hand, sensory deafferentation did not significantly affect the HCO_3^- stimulatory action of L-NAME. In these animals, ΔHCO_3^- output induced by L-NAME was slightly decreased in both the stomach (3.9 ± 0.7 μEq/hr) and the duodenum (6.7 ± 2.1 μEq/hr), but these values are not significantly different from those in control animals. None of the treatments had any effect on the increase of arterial blood pressure after administration of L-NAME (not shown).

Effect of ET-1 on gastric HCO_3^- secretion. Because L-NAME increased HCO_3^- secretion with a marked elevation in arterial blood pressure, we examined whether such phenomena were observed with another vasoactive agent, ET-1, in the stomach. Intravenous administration of ET-1 (0.3–1 nmol/kg) significantly increased gastric HCO_3^- secretion in a dose-related manner, and at 1 nmol/kg the rate of HCO_3^- secretion was increased from 0.26 ± 0.02 μEq/5 min to the maximal values of 1.13 ± 0.19 μEq/5 min, which is approximately 4 times greater than control values (fig. 10). This dose of ET-1 also produced a marked elevation in arterial blood pressure, which rose from 84.3 ± 8.7 mm Hg to the plateau values of 156.2 ± 11.8 mm Hg within 5 min. The increased HCO_3^- response caused by ET-1 (1 nmol/kg) was almost completely blocked by vagotomy without any effect on the elevation in blood pressure (it rose from 78.4 ± 13.5 mm Hg to 148.6 ± 10.2 mm Hg).

Effects of L-NAME and ET-1 on mucosal vascular permeability. To investigate the mucosal vascular permeability response to L-NAME or ET-1, we measured the amount of dye trapped in the mucosa (extravascular sites) for 30 min after intravenous injection of 1% Evans blue. In control stomachs, the amount of extravasated dye was minimal, the values being 2.05 ± 0.19 μg/100 mg tissue. These values were not significantly altered by intravenous administration of either L-NAME or ET-1; the amounts of extravasated dye were 1.88 ± 0.08 μg/100 mg tissue and 2.23 ± 0.19 μg/100 mg tissue, respectively.

Discussion

The local release of vasodilators such as endothelium-derived relaxing factor, identified as NO, is considered to play an essential role in the modulation of gastric mucosal integrity (Pique *et al.*, 1989; Whittle *et al.*, 1990; Moncada *et al.*, 1991). The suppression of NO biosynthesis by arginine analogs caused damage in the mucosa when they were combined with indomethacin or ablation of sensory neurons (Whittle *et al.*, 1990). The present study, however, demonstrated that the selective inhibitor of NO biosynthesis L-NAME markedly increased

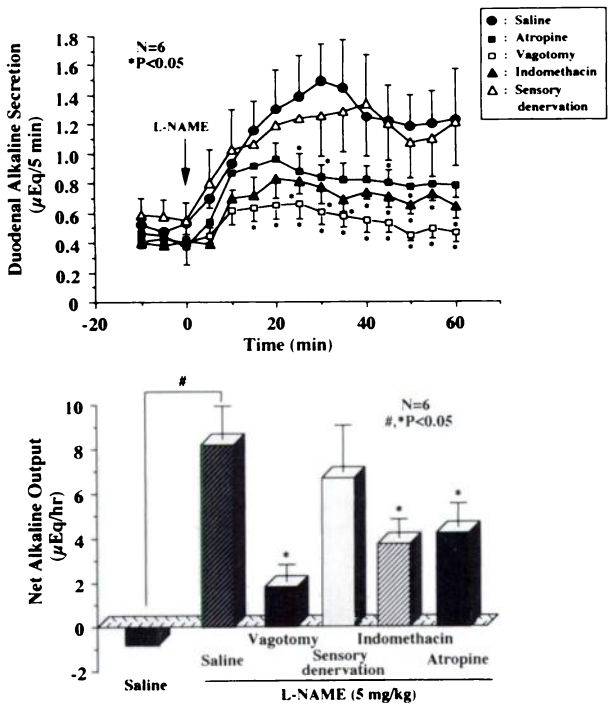

Fig. 9. Effects of indomethacin, atropine, vagotomy and sensory denervation on duodenal HCO_3^- stimulatory action of L-NAME in anesthetized rats. Experimental procedures are referred to figure 8. Data are expressed as the net HCO_3^- output and represent the means ± S.E. of values determined every 5 min from six rats. Lower panel shows ΔHCO_3^- output obtained for 1 hr in each group. Statistically significant difference at $P < .05$; * from the group given saline and L-NAME (upper and lower panels), # from saline control (lower panel).

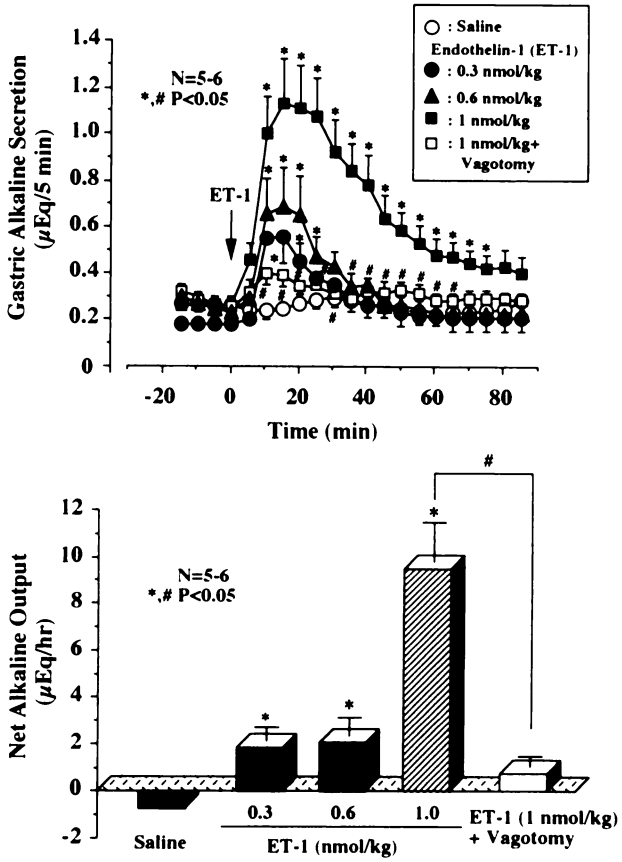

Fig. 10. Effect of endothelin-1 (ET-1) on gastric HCO_3^- secretion in rats with or without bilateral vagotomy. Acid secretion was inhibited by omeprazole. ET-1 (0.3–1 nmol/kg) was administered i.v. as a single injection. Vagotomy was performed 1 hr before administration of ET-1 (1 nmol/kg). Data are presented as the means ± S.E. of values determined every 5 min from five to six rats. Lower panel showed ΔHCO_3^- output obtained for 1 hr in each group. Statistically significant difference at $P < .05$; * from saline control (upper and lower panels), # from the group given 1 nmol/kg of ET-1 (lower panel).

HCO_3^- secretion from the gastroduodenal mucosa and suggested that NO plays a suppressive role in the regulation of HCO_3^- secretion.

It is known that L-NAME inhibits the biosynthesis of NO by suppressing the NO synthase by competing with the precursor L-arginine. In the present study, this NO synthase inhibitor elevated arterial blood pressure dose-dependently, and this effect was partially but significantly reversed by concurrent administration of L-arginine. Under such conditions, intravenously administered L-NAME caused a clear and persistent increase of HCO_3^- secretion in both the stomach and the duodenum, in a dose-dependent manner. Because HCO_3^- secretion was measured in the stomach in the presence of omeprazole, it is unlikely that the enhancement of luminal alkalinization induced by L-NAME is attributable to changes in acid secretion. This contention is substantiated by the finding that L-NAME also augmented the luminal alkalinization in the duodenum with no acid-secreting cells. The HCO_3^- stimulatory action was not observed in the enantiomer D-NAME, but it was mimicked by another NO synthase inhibitor; L-NMMA. In addition, the increased HCO_3^- response to L-NAME was significantly mitigated by prior administration of L-arginine, a substrate of endogenous NO. Such a supplementation of NO from L-arginine was found previously to enhance the endothelium-dependent vasodilation *in vitro* (Palmer *et al.*, 1988), suggesting that the currently observed effect of L-arginine is highly specific. This interpretation is also supported by the fact that the addition of D-arginine under similar experimental conditions was completely ineffective in antagonizing the HCO_3^- responses to L-NAME. These results may suggest that stimulation of HCO_3^- secretion by L-NAME is associated with the inhibition of NO biosynthesis. We might also speculate that NO formed endogenously may play a suppressor role in HCO_3^- secretion under physiological conditions. In fact, the exogenous NO donor nitroprusside alone reduced the rate of HCO_3^- secretion and totally antagonized the HCO_3^- stimulatory effect of L-NAME in both stomach and duodenum. On the other hand, Flemstrom and Garner (1982) reported that cyclic GMP may act as an intracellular mediator in gastric HCO_3^- secretion, whereas duodenal HCO_3^- secretion may be mediated by cyclic AMP. NO is the endogenous stimulator of the soluble guanylate cyclase, leading to accumulation of cyclic GMP (Moncada *et al.*, 1991). Thus it remains unknown whether the effects of L-NAME or nitroprusside on HCO_3^- secretion appeared through changes in the guanylate cyclase/cyclic GMP system in the surface epithelial cells.

Interestingly, the effect of L-NAME on HCO_3^- secretion was significantly attenuated by indomethacin, a cyclooxygenase inhibitor. Although the HCO_3^- response induced by L-NAME was not affected by sensory deafferentation, this response was also significantly reduced by either surgical vagotomy or pretreatment with atropine. These results may indicate that the mechanism of HCO_3^- secretion in response to L-NAME involves both PG-sensitive and vagal-cholinergic pathways. It is

known that vagal excitation stimulates HCO_3^- as well as acid secretions and increases the release and/or biosynthesis of PGs in the stomach of various species of animals, including rats (Cocearni *et al.*, 1967; Jonson *et al.*, 1986; Tache, 1987). As we mentioned earlier, arterial blood pressure was markedly and persistently elevated after administration of L-NAME. In general, the rise in blood pressure is accompanied by a reflex activation of the vagal nerve activity or by suppression of the sympathetic neuronal tone. Jonson and Fandriks (1989) reported that sympathetic activation *via* direct electrical stimulation of the splanchnic nerves inhibits duodenal HCO_3^- secretion in rats. Furthermore, they showed that reflex sympathetic nerve activation caused by hypotension due to bleeding inhibits duodenal HCO_3^- secretion (Jonson *et al.*, 1989). Thus it is possible to speculate that the HCO_3^- stimulatory action of L-NAME may be related to changes in parasympathetic and/or sympathetic nerve activity resulting from elevation of blood pressure, though the role of these neuronal innervations in the physiological generation of NO has not yet been elucidated. On the other hand, stimulation of capsaicin-sensitive sensory neurons is known to stimulate HCO_3^- secretion in the gastroduodenal mucosa (Takeuchi *et al.*, 1991; 1992a) and is involved in the neural reflex due to the pressor response (Maggi and Meli, 1988). However, sensory deafferentation did not influence the HCO_3^- response induced by L-NAME. Because other vasopressor agents such as norepinephrine and vasopressin are known to inhibit HCO_3^- secretion (Nylander and Flemstrom, 1986; Lenz *et al.*, 1989), it is unlikely that the elevation of blood pressure simply leads to an increase of HCO_3^- secretion. However, ET-1 caused a marked rise in gastric HCO_3^- secretion with a concomitant elevation of arterial blood pressure, similar to that caused by L-NAME, and this effect was significantly blocked by vagotomy. Further studies are needed to investigate these points by measuring HCO_3^- secretory responses to various agents affecting blood pressure and by correlating the changes in both parameters.

On the other hand, the increase of HCO_3^- output by L-NAME might be attributable to leakage of interstitial fluid and plasma into the lumen, because inhibition of NO production by L-NAME has been shown to increase both endothelial and epithelial permeability in the small intestine (Kubes, 1992; Kubes and Granger, 1992). In the present study, however, the vascular permeability in the stomach was not significantly affected by either L-NAME or ET-1. Furthermore, the HCO_3^- responses to these agents were significantly inhibited by vagotomy without any influence on the elevation of systemic blood pressure. These findings may suggest that the luminal alkalinization induced by L-NAME is not simply due to leakage of HCO_3^- from the blood but that it depends partly on the vagus nerves. The explanation for the different effects of L-NAME on vascular permeability is unclear, but they may result from differences in species (feline *vs.* rat), tissue (small intestine *vs.* stomach) and/or methodology (^{51}C-EDTA *vs.* Evans blue).

MacNaughton *et al.* (1989) first demonstrated the protective effect of NO on the gastric mucosa. Later, Whittle *et al.* (1990) showed that inhibition of the biosynthesis of NO alone did not cause any lesion in the gastric mucosa but induced hemorrhagic damage when combined with indomethacin, and they suggested an interaction between NO and PG in the modulation of gastric mucosal integrity. Brown *et al.* (1992) recently reported that NO donors stimulate mucus secretion in the rat stomach. Because HCO_3^- secretion is considered to play an important role in the mucosal defensive mechanism, in collaboration with mucus (Flemstrom and Turnberg, 1984; Garner *et al.*, 1984), it may be expected that NO has a positive effect on HCO_3^- secretion similar to that of PGs. However, the present finding that the inhibition of NO biosynthesis increased HCO_3^- secretion is contradictory to the mucosal protective role of NO, and the finding suggests that NO formed endogenously may exert a deleterious influence on the mucosa by inhibiting HCO_3^- secretion. It might be possible to speculate that endogenous NO has dual effects: that it plays a protective role mainly by increasing mucosal blood flow and exerts a proulcerogenic effect by decreasing HCO_3^- secretion. Miller *et al.* (1993) provided evidence that NO plays an inflammatory/proinflammatory role in the pathogenesis of inflammatory bowel disease of guinea pigs. At present, the physiological significance of the HCO_3^- response induced by the inhibition of NO production remains unknown, yet we observed in a preliminary study that L-NAME protected the duodenal mucosa from ulceration caused by mepirizole (unpublished data).

The current data demonstrate that the selective blockade of NO synthase by L-NAME increased HCO_3^- secretion from the gastroduodenal mucosa of anesthetized rats. This action appears to be associated with inhibition of NO biosynthesis. It may be mediated in part by endogenous PGs and may be dependent on the vagal-cholinergic innervation. Certainly, the possibility remains that NO may cause an inhibitory effect directly on the HCO_3^- transport system in the surface epithelial cells. The present data are not in keeping with the hypothesis that NO is important in the modulation of mucosal integrity (Whittle *et al.*, 1990). They suggest more complicated influences of endogenous NO on the gastroduodenal mucosa and function.

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Send reprint requests to: Dr. Koji Takeuchi, Department of Applied Pharmacology, Kyoto Pharmaceutical University, Misasagi, Yamashina, Kyoto 607, Japan.