Mechanisms Underlying Stimulation of Gastroduodenal HCO$_3^-$ Secretion by N$^G$-Nitro-l-Arginine Methyl Ester, an Inhibitor of Nitric Oxide Synthase, in Rats

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ABSTRACT—We investigated the mechanism underlying stimulation of HCO$_3^-$ secretion by the nitric oxide (NO) synthase inhibitor N$^G$-nitro-l-arginine methyl ester (L-NAME) in the gastroduodenal mucosa of anesthetized rats. A chambered stomach (in the presence of omeprazole) or a duodenal loop was perfused with saline, and HCO$_3^-$ secretion was measured at pH 7.0 by a pH-stat method. Intravenous administration of L-NAME increased gastroduodenal HCO$_3^-$ secretion with a concomitant rise in arterial blood pressure and a decrease in heart rate, and the changes were all antagonized by simultaneous administration of l-arginine. Vagotomy had no effect on the increased blood pressure response, but significantly inhibited the decrease of heart rate and increase of HCO$_3^-$ secretion caused by L-NAME. The HCO$_3^-$ stimulatory action of L-NAME was also inhibited by prior administration of yohimbine or prazosin. These agents alone lowered blood pressure and reduced the magnitude of the blood pressure response caused by L-NAME, leading to inhibition of heart rate changes. When ΔHCO$_3^-$ output induced by L-NAME was plotted against Δblood pressure change (from basal values) under various conditions, a significant relationship was found between these two factors. These results suggest that L-NAME stimulates gastroduodenal HCO$_3^-$ secretion in association with the inhibition of endogenous NO production, and this mechanism may be in part mediated by a neural reflex through the vagal efferent nerve, resulting from the pressor response to L-NAME.

Keywords: Nitric oxide, N$^G$-Nitro-l-arginine methyl ester, Gastroduodenal HCO$_3^-$ secretion

Nitric oxide (NO), which accounts for the biological actions of endothelium-derived relaxing factor (1), is now known to be generated in various other cells including the epithelium, macrophages and enteric neurons (2) and mimics the protective action of endogenous prostaglandins (PGs) in the gastric mucosa (3, 4). However, we found recently that the NO synthase inhibitor N$^G$-nitro-l-arginine (L-NAME) markedly stimulated HCO$_3^-$ secretion in the gastroduodenal mucosa (5, 6). Since the HCO$_3^-$ stimulatory effect of L-NAME was antagonized by simultaneous administration of l-arginine, it may be assumed that this action is associated with the inhibition of endogenous NO production. Yet, the mechanism underlying the stimulation of HCO$_3^-$ secretion by L-NAME still remains unclear.

L-NAME causes an elevation of arterial blood pressure at the doses that stimulate HCO$_3^-$ secretion (6). In general, the rise in blood pressure is accompanied by a reflex activation of the vagus nerve or by suppression of the sympathetic neuronal tone. Furthermore, since HCO$_3^-$ secretion is increased by vagal electrical stimulation and inhibited by electrical stimulation of splanchnic nerves (7, 8), it may be possible to speculate that the HCO$_3^-$ stimulatory action of L-NAME is related to changes in such neuronal activity resulting from elevation of blood pressure.

In this study, we thus investigated the mechanism underlying stimulation of gastroduodenal HCO$_3^-$ secretion by L-NAME in rats, mainly in relation to the blood pressure changes caused by this drug.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats, weighing 230−250 g (Charles River, Shizuoka), were used. The animals were kept in individual cages with raised mesh bottoms to prevent coprophagia, and they were deprived of food but al-
allowed free access to tap water for 18 hr before the experiments. All studies were carried out using 4–6 animals per group under anesthetized conditions induced by i.p. administration of urethane (1.25 g/kg).

**Determination of gastroduodenal $\text{HCO}_3^-$ secretion**

$\text{HCO}_3^-$ secretion was measured in a chambered stomach or a duodenal loop according to the previously published papers (6, 9). The stomach mounted in an ex-vivo chamber or the duodenal loop made between the pyloric ring and the area proximal to the outlet of the common bile duct was perfused with saline that was gassed with 100% $\text{O}_2$, heated at 37°C and kept in a reservoir. $\text{HCO}_3^-$ secretion was measured at pH 7.0 by a pH-stat method (Hiranuma Comitie-7, Mito) and by adding 10 mM HCl to the reservoir. To unmask $\text{HCO}_3^-$ in the stomach, acid secretion was inhibited by omeprazole (60 mg/kg, i.p.). The femoral artery was cannulated, and both arterial blood pressure and heart rate were monitored with a pressure transducer and polygraph (CASE-7903; San-ei, Tokyo). After basal $\text{HCO}_3^-$ secretion had stabilized, L-NAME (5 mg/kg) was administered i.v. as a single injection. L-Arginine (200 mg/kg) was given i.v. 5 min before L-NAME, whereas yohimbine (5 mg/kg) and prazosin (0.5 mg/kg) were given s.c. 30 min before administration of L-NAME. Vagotomy was performed at subdiaphragmatic portion 60 min before administration of L-NAME. In some animals, L-NAME (5 mg/ml) was applied topically to the stomach (2 ml) or the duodenum (0.5 ml) for 15 min, and $\text{HCO}_3^-$ secretion was measured before and after the exposure.

**Measurement of vascular permeability**

To examine the effects of L-NAME on gastric and duodenal mucosal vascular permeability, we used a dye method to measure the extravasated amount of dye as described in a previous paper (10). The animals were given L-NAME (5 mg/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, both the stomach and duodenum (2 cm proximal to the pylorus) were removed, and the amount of dye that had accumulated in the mucosa in 30 min was measured. The stomach and the duodenum were opened along the greater curvature and along the mesenteric artery, respectively. Then, the mucosa was scraped off using glass slides and weighed. The extraction of dye was performed according to the modified method described by Katayama et al. (11). The amount of dye recovered from the mucosa was expressed in $\mu$g/100 mg tissue.

**Preparation of drugs**

Drugs used were urethane (Tokyo Kasei, Tokyo); $N^G$-nitro-L-arginine methyl ester, yohimbine, prazosin, Evans blue (Sigma Chemicals, St. Louis, MO, USA); L-arginine (Wako, Osaka); and omeprazole (Hessle, Mondale, Sweden). Urethane, L-NAME and L-arginine were dissolved in saline, while yohimbine and prazosin were suspended in saline with a trace of Tween 80 (Wako). 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 the 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 four to six rats per group. Statistical analyses were performed by a two-tailed Dunnett's multiple comparison test, and values of $P<0.05$ were regarded as significant. The correlation between alkaline secretion and blood pressure responses was assessed by linear-regression analysis.
Nitric Oxide and HCO$_3^-$ Secretion

RESULTS

Effects of L-NAME on gastroduodenal HCO$_3^-$ secretion

Under our experimental conditions, the animals spontaneously secreted HCO$_3^-$ at steady rates of 0.2–0.4 μEq/5 min in the gastric mucosa (in the absence of acid secretion) and 0.3–0.5 μEq/5 min in the duodenal mucosa, respectively. Intravenous administration of L-NAME (5 mg/kg) caused an increase of HCO$_3^-$ secretion in both the stomach and duodenum (Fig. 1, A and B). This dose of L-NAME also caused a marked elevation in arterial blood pressure; the mean blood pressure elevated from $84.3 \pm 10.5$ mmHg to $146.8 \pm 13.5$ mmHg, and the heart rate decreased from $382.4 \pm 12.2$ beats/min to $321.6 \pm 18.1$ beats/min within 5 min. These changes caused by L-NAME were all attenuated when the animals were pretreated with L-arginine (200 mg/kg) (Fig. 2). Co-administration of L-arginine reduced $\Delta$HCO$_3^-$ output from $5.9 \pm 1.1$ μEq/hr to $1.3 \pm 0.6$ μEq/hr in the stomach and antagonized the increase in blood pressure and decrease in heart rate caused by L-NAME. In addition, the HCO$_3^-$ stimulatory action of L-NAME was also significantly mitigated by vagotomy; $\Delta$HCO$_3^-$ output was $2.2 \pm 0.7$ μEq/hr in the stomach and $2.2 \pm 0.7$ μEq/hr in the duodenum, the values being equivalent to 35.6% and 26.8% of those induced by L-NAME in the control animals. Of particular interest is the finding that vagotomy did not affect the increased blood pressure response, but totally inhibited the decrease in heart rate caused by L-NAME.

On the other hand, the mucosal application of L-NAME (5 mg/ml for 15 min) did not cause any effect on basal rates of HCO$_3^-$ secretion in both the stomach and the duodenum; the HCO$_3^-$ output in the stomach was $1.4 \pm 0.6$ μEq/30 min and $1.2 \pm 0.7$ μEq/30 min (N=4) before and after exposure to L-NAME, respectively. Even in such tissues, the subsequent i.v. administration of L-NAME (5 mg/kg) produced an increase of alkaline secretion as observed in the normal tissues without exposure to L-NAME. In addition, L-NAME applied topically to the...
gastric or duodenal mucosa did not cause any change in either blood pressure or heart rate (data not shown).

**Effects of yohimbine and prazosin on HCO₃⁻ stimulatory and blood pressure responses induced by L-NAME**

Subcutaneous administration of yohimbine (5 mg/kg) or prazosin (0.5 mg/kg) alone did not affect the rates of alkaline secretion in either the stomach or the duodenum. However, the HCO₃⁻ secretory response to L-NAME (5 mg/kg) was significantly reduced by pretreatment with either of these agents in both the stomach and duodenum (Fig. 3). In the stomach, ∆HCO₃⁻ output caused by L-NAME was reduced to 0.3±0.2 μEq/hr and 0.7±0.2 μEq/hr in the case of yohimbine and prazosin, respectively, and the inhibition was even greater when compared to that by L-arginine (Fig. 4A). Similar results were obtained in duodenal HCO₃⁻ secretion; ∆HCO₃⁻ output was 3.4±1.2 μEq/hr and 3.2±0.8 μEq/hr, respectively (Fig. 4B). Both yohimbine and prazosin alone caused a profound decrease in blood pressure (~20 mmHg) with minimal change in heart rate. In the animals pretreated with these alpha-blockers, the subsequent administration of L-NAME caused an increase of blood pressure but did not produce any change in heart rate (Fig. 5). These agents did not affect the magnitude of the blood pressure response induced by L-NAME, yet the maximal values of blood pressure remained in significantly lower ranges than those in the control animals (146.5±4.6 mmHg); values were 102.3±10.1 mmHg and 110.3±6.8 mmHg for yohimbine and prazosin, respectively.

**Relationship between HCO₃⁻ secretory responses and blood pressure changes**

When ∆blood pressure was calculated from the difference between the maximal values obtained after administration of L-NAME and those observed before any treatment, it is evident that ∆blood pressure change was significantly reduced by either prazosin or yohimbine as well as L-arginine (Fig. 6A). Vagotomy did not affect ∆blood pressure induced by L-NAME, yet completely prevented the decrease of heart rate seen after administration of L-NAME (Fig. 6B). When ∆HCO₃⁻ output induced in the stomach and the duodenum by L-NAME was plotted against changes in blood pressure (∆blood pressure from basal values) observed under various treatments, a significant relationship was obtained between these two parameters (Fig. 7), the correlation coefficient (r) being...
0.94 in the stomach and 0.98 in the duodenum.

**Effect of L-NAME on mucosal vascular permeability**

To investigate the mucosal vascular permeability response to L-NAME, we measured the amount of dye trapped in the gastric and duodenal mucosa (extravascular sites) for 30 min after i.v. administration of 1% Evans blue. In the control animals, the amount of extravasated dye was minimal, the values being 2.05 ± 0.19 μg/100 mg tissue in the stomach and 1.95 ± 0.21 μg/100 mg tissue in the duodenum (Fig. 8A). These values in the stomach were not altered by i.v. administration of L-NAME (5 mg/kg, 1.88 ± 0.08 μg/100 mg tissue), while those in the duodenum were significantly increased to 2.82 ± 0.24 μg/100 mg tissue. The increased vascular permeability caused in the duodenum by L-NAME was significantly antagonized by co-administration of L-arginine, but was not affected by vagotomy or pretreatment with either prazosin or yohimbine (Fig. 8B). Concurrent administration of L-arginine with L-NAME reduced the amount of extravasated dye to 2.21 ± 0.19 μg/100 mg tissue, which is not significantly different from that (1.95 ± 0.21 μg/100 mg tissue) observed in the control rats that did not receive L-NAME treatment.

**DISCUSSION**

In the present study, we confirmed our previous finding that intravenous administration of L-NAME caused an increase of HCO$_3^-$ secretion in the gastroduodenal mucosa with a concomitant rise in blood pressure (5, 6). Since the increased HCO$_3^-$ response to L-NAME was significantly mitigated by prior administration of L-arginine but not D-arginine, it is considered that stimulation of HCO$_3^-$ secretion by L-NAME is associated with the inhibition of endogenous NO production.

In agreement with the previous finding (5), the effect of L-NAME on HCO$_3^-$ secretion was significantly attenuat-
Fig. 6. Changes in blood pressure (A) and heart rate (B) after administration of L-NAME in anesthetized rats under various treatments. L-NAME (5 mg/kg) was given i.v. as a single injection. L-Arginine (200 mg/kg) was given i.v. 5 min before L-NAME, whereas yohimbine (5 mg/kg) or prazosin (0.5 mg/kg) was given s.c. 30 min before L-NAME. Vagotomy was performed 1 hr before administration of L-NAME. Data are expressed as maximal changes in blood pressure (ΔmmHg) and heart rate (%) from basal values observed before any treatment and represent the means ± S.E. from 4–5 rats. *Statistically significant difference from the controls, at P < 0.05.

ed by vagotomy. We also reported that the stimulatory action of L-NAME on HCO₃⁻ secretion was inhibited by atropine as well as indomethacin (6). Vagal excitation stimulates HCO₃⁻ as well as acid secretions and increases the release and/or biosynthesis of endogenous PGs in the stomach of various species of animals (7, 12, 13). These findings together indicate that the mechanism of HCO₃⁻ secretion in response to L-NAME involves vagal-cholinergic pathways and is partly mediated by endogenous prostaglandins. As observed in this study, the 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 (7, 8). A decrease in heart rate following the increase of blood pressure caused by L-NAME supports a reflex activation of the vagus nerves. In fact, vagotomy mitigated both HCO₃⁻ and heart rate responses induced by L-NAME without affecting the increase of blood pressure. Thus, it may be possible to speculate that the HCO₃⁻ stimulatory action of L-NAME is related to changes in parasympathetic neuronal activity resulting from elevation of blood pressure.

Interestingly, the HCO₃⁻ response caused by L-NAME was also significantly mitigated by prior administration of alpha-adrenoceptor antagonists such as prazosin or yohimbine. Influences of alpha-adrenoceptor agonists on HCO₃⁻ secretion is complex; the alpha₁-agonist phenylephrine stimulates HCO₃⁻ secretion in the rat duodenum, whereas the alpha₂-agonist clonidine inhibits this secretion (8, 14). Norepinephrine inhibits HCO₃⁻ secretion even in the isolated stomach in vitro (15). On the other hand, yohimbine reversed the decrease of HCO₃⁻ secretion caused by clonidine or stimulation of splanchnic...
Fig. 8. Effects of L-NAME on vascular permeability in the stomach and duodenum of anesthetized rats (A) and those of various treatments on the increased vascular permeability caused by L-NAME in the duodenum (B). L-NAME (5 mg/kg) was given i.v. as a single injection. L-Arginine (200 mg/kg) was given i.v. 5 min before L-NAME, whereas yohimbine (5 mg/kg) or prazosin (0.5 mg/kg) was given s.c. 30 min before L-NAME. Vagotomy was performed 1 hr before administration of L-NAME. Data are presented as the amount of extravasated dye (Evans blue) for 30 min and represent the means ± S.E. from 6 (A) or 4-6 (B) rats. *Statistically significant difference from saline, at P < 0.05.

In the present study, however, yohimbine significantly inhibited the HCO₃⁻ secretory response to L-NAME, suggesting that the effect is not related to the HCO₃⁻ transport process at the epithelial cells. As expected, administration of prazosin or yohimbine alone produced a substantial decrease of blood pressure with minimal change in heart rate. Although L-NAME induced a persistent rise in blood pressure even in the animals pretreated with such alpha-blockers, the maximal values in blood pressure were significantly lower than those in control animals, leading to inhibition of the bradycardic response caused by L-NAME. These results strongly support the hypothesis that L-NAME stimulates HCO₃⁻ secretion, at least partly mediated by a neural reflex through vagal efferent nerves, resulting from the marked increase in blood pressure. It also seems that for obtaining such HCO₃⁻ and bradycardic responses induced by L-NAME, the blood pressure not only shows a definite increase but also elevates beyond certain levels. We have recently reported that endothelin-1, a potent vasopressor agent, also increased gastric HCO₃⁻ secretion with a marked elevation of blood pressure (6). On mere speculation, the increase in the vagal nerve tone might lead to changes in acid secretion and motility as well as HCO₃⁻ secretion. Yet, L-NAME does not have any influence on gastric acid secretion, at least in normal stomachs (16). It is assumed that the sensitivity to vagal nerve stimulation might be different depending upon the cell type responsible for each function. Certainly, further studies would be required to clarify this point.

On the other hand, Kubes (17) and Kubes and Granger (18) reported that the inhibition of endogenous NO production by L-NAME enhanced both the endothelial and epithelial permeability in feline small intestine. Therefore, it might be possible that the increase of HCO₃⁻ output by L-NAME is attributable to leakage of interstitial fluid or plasma into the lumen. In this study, the vascular permeability in the gastric mucosa was not significantly altered by L-NAME at the dose that caused a marked increase of HCO₃⁻ output and blood pressure, although the vascular permeability in the duodenum was significantly increased by this agent. However, this increase in the duodenal vascular permeability was not significantly influenced by either prazosin or yohimbine as well as vagotomy, yet such treatments have been demonstrated to inhibit the HCO₃⁻ response to L-NAME in this study. Similar findings were reported by Hallgren et al. (19), who showed that hexamethonium significantly inhibited the HCO₃⁻ response induced by N²-nitro-L-arginine with no effect on the increased vascular permeability change. Thus, these findings indicate that the luminal alkalization induced by L-NAME is not simply due to leakage of HCO₃⁻ from the blood.

Recently, Hallgren and Nylander (20) demonstrated that L-NAME caused an increase of both alkaline secretion and luminal pressure in the rat duodenum, and these effects were prevented by papaverine as well as hexamethonium. They hypothesized that the luminal alkalization by L-NAME in the duodenum may be due to the neural reflex resulting from the increase of luminal pressure through mechano-receptors. In our study, however, the stomach was mounted in a chamber and superfused at a constant flow rate with saline. Under these conditions the pressure of the stomach lumen may be relatively constant, yet L-NAME caused a marked increase of luminal alkalization as observed in the duodenal loop. In addition, we observed that L-NAME applied topically to the mucosa did not have any effect on the basal rates of HCO₃⁻ secretion in both the stomach and duodenum. There-
fore, it seems unlikely that stimulation by L-NAME of HCO$_3^-$ secretion is brought about by an increase of the luminal pressure in these tissues.

The current data demonstrated 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, and this process may be partly mediated by a neural reflex through vagal efferent nerves, resulting from the marked increase of blood pressure. This effect is inhibited by L-arginine at the vascular level and also mitigated by alpha-blockers, probably by decreasing the blood pressure response to L-NAME. Vagotomy blocks the vagal efferent pathway to stimulate HCO$_3^-$ secretion under such conditions. Certainly, since we have reported that the exogenous NO donor nitroprusside not only antagonized the stimulatory effect of L-NAME on HCO$_3^-$ secretion but also caused a dose-dependent reduction in the HCO$_3^-$ response induced by 16,16-dimethyl PGE$_2$ (6, 21), it is possible that the HCO$_3^-$ stimulatory effect of L-NAME may be partly accounted for by removal of the negative influence of endogenous NO on this secretion. Although NO is a stimulator of soluble guanylate cyclase, leading to accumulation of cyclic GMP (2), it remains unknown at present whether L-NAME affects HCO$_3^-$ secretion directly through changes in the guanylate cyclase/cyclic GMP system.

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