Effects of Nitric Oxide Synthase Inhibitors on Gastric Alkaline Secretion in Rats

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ABSTRACT—The effects of N^G -nitro-L-arginine methyl ester (L-NAME), the nitric oxide (NO) synthase inhibitor, on gastric HCO_3^- secretion were examined in anesthetized rats. Intravenous administration of L-NAME (1, 2.5, 5 mg/kg) increased HCO_3^- secretion in a dose-related manner. This effect of L-NAME was mimicked by N^G -mono-methyl-L-arginine (50 mg/kg, i.v.) and was antagonized significantly by concurrent administration of L-arginine but not D-arginine (200 mg/kg, i.v.). These results indicate that gastric HCO_3^- secretion is stimulated by inhibition of NO biosynthesis.

Keywords: Nitric oxide synthase inhibitor, HCO_3^- secretion, Stomach

Nitric oxide (NO), synthesized from the semi-essential amino acid L-arginine by NO synthase in the vascular endothelium is now established to mediate various biological actions under physiological conditions (1). Recent studies have shown that NO also plays an important role in the modulation of the gastric mucosal integrity by interacting with other protective mediators (2, 3). Regulation of gastric mucosal blood flow is considered to be the major mechanism responsible for the mucosal protective action of NO (2, 4), yet the influences of the inhibition of NO biosynthesis on other defensive factors such as HCO_3^- secretion has not been studied. In the present study, we thus examined the effects of the NO synthase inhibitor on HCO_3^- secretion in the rat stomach using N^G -nitro-L-arginine as the methyl ester (L-NAME) and characterized these effects in relation to endogenous NO.

Male Sprague Dawley rats (230–250 g), kept in individual cages with raised mesh bottoms, were deprived of food but allowed free access to tap water for 18 hr before the experiments. The animals were anesthetized with urethane (1.25 g/kg, i.p.; Tokyo Kasei); and then the stomach was exposed, mounted on a chamber (exposed area: 3.14 cm²), and perfused with saline that was gassed with 100% O₂. HCO_3^- secretion was determined at pH 7.0 by continuous titration of the perfusate with 10 mM HCl under acid inhibition by omeprazole (60 mg/kg, i.p.). In some cases, the stomach was perfused with acidified saline (pH 4.5) at the rate of 1

ml/min, and the pH of the perfusate and transmucosal potential difference (PD) were monitored simultaneously with arterial blood pressure (BP). The pH was measured by a flow type glass electrode (Horiba, Model 6901-25T), while PD was determined by using two agar bridges, one positioned in the chamber and the other in the abdominal cavity (5). BP was monitored via the femoral artery by a pressure transducer and amplifier system (Nihon Kohden). N^G -nitro-L-arginine methyl ester (L-NAME: Sigma), N^G -monomethyl-L-arginine (L-NMMA: Sigma) and prostaglandin E₂ (PGE₂, Funakoshi) were given i.v. after basal secretion had stabilized. In some cases, L- or D-arginine (Wako) was given i.v. 5 min before administration of L-NAME. Data are presented as the mean $\pm$ S.E. from 5–6 rats. Statistical analyses were performed by the two-tailed Dunnett's multiple comparison test, and values of $P < 0.05$ were regarded to indicate a significant difference.

In the animals pretreated with omeprazole, the gastric mucosa mounted on a chamber and perfused with acidified saline (pH 4.5) generated a PD of -40 – -45 mV (mucosa negative), secreted alkali to keep the luminal pH at 4.8–5.2, and BP was maintained around 90–100 mmHg. Intravenous administration of L-NAME at 5 mg/kg produced a marked increase of pH with a slight decrease in PD and persistent rise in BP (Fig. 1). Titration of the perfusate with 10 mM HCl at pH 7.0 revealed the production of about 0.2–0.4 μEq of HCO_3^- every 5 min, and this rate was well-

Fig. 1. Representative recordings of changes in PD and pH of the chambered stomach simultaneously with systemic BP in an anesthetized rat after i.v.-administration of L-NAME (5 mg/kg). The mucosa was perfused with acidified saline (pH 4.5) under acid inhibition by omeprazole (60 mg/kg, i.p.). Note that the luminal pH was increased markedly after administration of L-NAME with a slight decrease in PD and persistent elevation of BP.

maintained during the 90 min-test period. Administration of L-NAME (1, 2.5 and 5 mg/kg) increased gastric HCO_3^- output significantly in a dose-related manner, and this secretion at 5 mg/kg reached maximal levels ($0.85 \pm 0.16 \mu\text{Eq}/5 \text{ min}$) of 4 times greater than the basal values ($0.23 \pm 0.03 \mu\text{Eq}/5 \text{ min}$) and remained elevated for 1 hr; ΔHCO_3^- output was $5.6 \pm 1.0 \mu\text{Eq}/\text{hr}$, which is almost equivalent to that ($4.8 \pm 1.0 \mu\text{Eq}/\text{hr}$) induced by PGE_2 (0.3 mg/kg) (Fig. 2, A and B). Gastric HCO_3^- secretion was also stimulated significantly by another NO synthase inhibitor, L-NMMA (50 mg/kg), ΔHCO_3^- output being $2.4 \pm 0.7 \mu\text{Eq}/\text{hr}$. On the other hand, the stimulatory effect of L-NAME (5 mg/kg) on gastric HCO_3^- secretion was significantly antagonized by pretreatment with L-arginine (200 mg/kg, i.v.) at the dose which partially mitigated the rise in BP seen after administration of L-NAME (%increase in BP at 5 min-post treatment: $41.7 \pm 2.6\%$ vs. $26.4 \pm 4.6\%$, $P < 0.05$). In the rats pretreated with L-arginine, the ΔHCO_3^- output induced by L-NAME was $1.3 \pm 0.6 \mu\text{Eq}/\text{hr}$, which is about 23.2% of the ΔHCO_3^- output obtained in control animals given L-NAME alone. D-Arginine did not affect the increased HCO_3^- response to L-NAME.

The present study showed that gastric HCO_3^- secre-

Fig. 2. Effects of L-NAME (5 mg/kg), L-NMMA (50 mg/kg), PGE_2 (0.3 mg/kg) and L- or D-arginine (200 mg/kg) plus L-NAME (5 mg/kg) on HCO_3^- secretion in the stomachs of anesthetized rats [A] and the net HCO_3^- output induced by these treatments [B]. Each agent was given i.v. after basal secretion had stabilized. L-Arginine or D-arginine was given 5 min before administration of L-NAME. [A]: Data are expressed as a % of the basal values (time 0) and represent the means $\pm$ S.E. of values determined every 5 min from 5–6 rats ($\circ$ saline, $\bullet$ PGE_2 , $\blacksquare$ L-NAME, $\triangle$ L-arginine plus L-NAME, $\square$ D-arginine plus L-NAME). [B]: Values indicate the net HCO_3^- output obtained for 1 hr after the treatments and represent the means $\pm$ S.E. from 5–6 rats. *Statistically significant difference from the group given L-NAME alone, at $P < 0.05$.

tion was significantly stimulated by the NO synthase inhibitor L-NAME in the anesthetized rats. It is unlikely that the increase of luminal alkalization by L-NAME is brought about by an inhibition of acid secretion, because in this study, HCO_3^- secretion was measured in the animals pretreated with omeprazole, by which acid secretion had been completely blocked. On the other hand, the effect of L-NAME on HCO_3^- secretion may appear through inhibition of NO biosynthesis but is not attributable to the non-specific action of this agent. This contention is supported by the following findings: (a) another NO synthase inhibitor, L-NMMA, increased gastric HCO_3^- secretion similar to L-NAME and (b) the effect of L-NAME was significantly antagonized by concurrent administration of L-arginine but not by D-arginine. In a preliminary study, we also found

that L-NAME increased HCO_3^- secretion in the rat duodenum and that this effect was significantly antagonized by L-arginine or the exogenous NO donor nitroprusside (unpublished data, K. Takeuchi et al.). These results suggest that NO may be involved as the endogenous inhibitor in the regulation of HCO_3^- secretion. The increased HCO_3^- responses induced by L-NAME were accompanied by the decrease in gastric PD and the persistent rise in systemic BP. Kubes and Granger (6) reported that inhibition by L-NAME of NO production by vascular endothelium leads to a reversible rise in microvascular protein efflux in the cat ileum. Although the mechanism by which the inhibition of NO biosynthesis increased HCO_3^- secretion remains unknown, it might be assumed that these phenomena are causally-related to the increased HCO_3^- responses to L-NAME. Certainly, since other vasopressor agents such as norepinephrine and vasopressin are known to inhibit HCO_3^- secretion (7, 8), it is unlikely that the elevation of BP simply leads to an increase of HCO_3^- secretion.

In general, HCO_3^- secretion is considered as one of the defensive factors in the stomach as well as in the duodenum (9, 10). The present results seem to be inconsistent with the protective role of NO in the gastric mucosa. The HCO_3^- secretion in the stomach is known to increase in response to vagal excitation or agents that stimulate acid secretion (11, 12). Barrachina et al. (13) recently reported that L-NAME significantly reversed the antisecretory effect of endotoxin on distention-stimulated acid output and suggested the involvement of NO in the regulation of acid secretion. If HCO_3^- secretion is physiologically important as an acid-neutralizing factor, it would be understandable that NO suppresses the "protective HCO_3^- " as well as the "aggressive H^+ " secretions.

The present study showed for the first time that the administration of L-NAME stimulates gastric HCO_3^- secretion in anesthetized rats, although the mechanisms responsible for this phenomenon have not been fully evaluated yet. Further studies to elucidate the role of NO in the regulation of HCO_3^- secretion are warranted.

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