Modulation by Endogenous Nitric Oxide of Acid Secretion Induced by Gastric Distention in Rats: Enhancement by Nitric Oxide Synthase Inhibitor

MOTOHIRO KITAMURA, SHINICHI SUGAMOTO, SHOJI KAWAUCHI, SHINICHI KATO, and KOJI TAKEUCHI

Department of Pharmacology and Experimental Therapeutics, Kyoto Pharmaceutical University, Misasagi, Yamashina, Kyoto, Japan

Accepted for publication June 18, 1999 This paper is available online at http://www.jpet.org

ABSTRACT

The mechanism underlying acid hypersecretion induced by gastric distention was investigated in rats, especially in relation to endogenous nitric oxide (NO). Under urethane anesthesia, rat stomach was distended by instillation of saline (1–10 ml) through the acute fistula that was provided through a pylorus. Gastric samples were collected every 1 h, and the acid secretion was measured by titration with 100 mM NaOH. Gastric acid secretion was increased by distention, and the degree of stimulation was dependent on the volume of saline instillation; a maximal response occurred with 6-ml instillation, which maintained the intraluminal pressure of about 20 cm H$_2$O. The increased acid secretory response induced by distention was completely blocked by omeprazole and significantly mitigated by vagotomy, sensory deafferentation, atropine, or famotidine but markedly enhanced by L-NAME methyl ester (L-NAME). On the other hand, the enhanced acid response in the presence of L-NAME occurred in an L-arginine-sensitive manner and was almost totally abolished by vagotomy and sensory deafferentation as well as by atropine. Gastric distention increased the release of NO metabolites and histamine into the gastric lumen. The NO metabolite release in the distended stomach was significantly decreased by vagotomy or L-NAME, whereas the histamine output was decreased by vagotomy but increased by L-NAME in an L-arginine-sensitive manner, respectively. These results suggest that 1) gastric distention increases acid secretion, initially through the perception by sensory neurons of the mechanical stimulation and mainly through the efferent vagal-cholinergic pathway, with the process being modified by endogenous NO, and 2) this molecule, released in a vagal-dependent manner, exerts a negative influence on acid secretion, at least in part by suppressing histamine release from the histamine-containing cells.

The gastric phase in the regulation of acid secretion consists mainly of distention of the stomach and bathing of the gastric mucosa with certain chemicals of food, primarily amino acids and peptides (Johnson, 1977). Distention of the stomach activates mechanoreceptors in the mucosa of the oxyntic gland area, leading to activation of long or short neuronal reflexes, which are all mediated by the cholinergic system (Grossman, 1962; Johnson, 1977; Hakanson et al., 1980; Noto et al., 1997). It has been shown that the mechanism for the distention-induced acid secretion also involves the peripheral capsaicin-sensitive sensory neurons, located both in the gastric mucosa and in the celiac ganglion, because sensory deafferentation attenuated the acid response to distention (Esplugues et al., 1990). Moreover, the distention of an antral portion stimulates gastrin release, and the response is also mediated by the vagovagal reflexes and local reflexes (Jonson, 1977; Esplugues et al., 1990), although the participation of gastrin in the acid secretory response to gastric distention is controversial.

On the other hand, a growing body of evidence suggests that nitric oxide (NO) acts as a transmitter in some nonadrenergic and noncholinergic nerves in the gastrointestinal tissue and modulates various functions, including acid secretion (Breder et al., 1990; Sanders and Ward, 1992; Moncada et al., 1993; Barrachina et al., 1994, 1995; Takeuchi et al., 1994; Esplugues et al., 1996; Kato et al., 1998). Barrachina et al. (1995) reported that acute inhibition by endotoxin of the distention-induced acid secretion requires the release of synthesis of NO and the integrity of the peripheral nervous system. They also showed that the NO donor preferentially inhibited acid secretion neuronally induced by gastric distention or 2-deoxy-D-glucose (Barrachina et al., 1994). More recently, Esplugues et al. (1996) showed that physiological inhibition of acid secretion observed during stress is mediated by a nervous reflex involving a neuronal pathway that includes NO synthesis in the brain, specifically in the dorsal motor nucleus of the vagus. These findings suggest the existence of a regulatory mechanism for acid production triggered by a nervous reflex involving NO. However, the role of

ABBREVIATIONS: NO, nitric oxide; L-NAME, N$_2$-nitro-L-arginine methyl ester; NOx, nitric oxide metabolites; CCK, cholecystokinin.
endogenous NO in the acid secretory response induced by gastric distention has been little studied.

In the present study, we investigated factors involved in the modulation of acid secretion in response to gastric distention in rats, particularly the role of vagus nerves and endogenous NO.

Materials and Methods

Male Sprague-Dawley GS 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 using five or six rats per group. All experimental procedures described here were approved by the Experimental Animal Research Committee of the Kyoto Pharmaceutical University.

Measurement of Acid Secretion. Animals were anesthetized with urethane (1.25 g/kg i.p.), and the trachea was cannulated to ensure a patent airway. Body temperature was maintained at 36 ± 1°C using a heating lamp. Acid secretion was measured in the acute fistula rat, according to a previously published method (Nuida et al., 1991). In brief, the abdomen was incised, both the stomach and duodenum were exposed, and the cardiac portion was ligated without interfering with vagus nerves. An acute fistula (inside diameter, 3 mm) made with a polyethylene tube was inserted into the stomach from a small incision made in the duodenum and held in place by a ligature around the pylorus. At the beginning of each experiment, the stomach was rinsed several times with physiological saline and filled with 1 ml of saline for 1 h for determination of the basal secretion. Then, the stomach was distended to various degrees by filling with 2 to 10 ml of saline (154 mM NaCl) through the fistula, and the solution was changed every 1 h. These volumes were selected on the basis of a preliminary study showing that the gastric volume in rats fed ad libitum was 4 to 9 ml (Noto et al., 1997). The intragastric pressure was about 20 cm H2O when the stomach was distended by 6 ml of saline. The collected samples were centrifuged at 3000 rpm for 15 min and titrated to pH 7.0 against 100 mM NaOH using an autoburette (Comitite-8; Hiranuma, Tokyo, Japan). The effects of the following drugs were examined on the acid hypersecretion in response to stomach distention induced by 6 ml of saline: omeprazole (60 mg/kg), famotidine (100 mg/kg), atropine (1 mg/kg), and YM-022 (cholecystokinin (CCK)-B/gastrin receptor antagonist, 3 mg/kg; Nishida et al., 1994; Saita et al., 1994) were administered i.p. 30 min before the first instillation of 6 ml of saline, whereas Nω-nitro-L-arginine methyl ester (L-NAME; 10 mg/kg) was administered i.v. 10 min before the onset of distention. In the case of L-NAME, half the animals were administered L- or D-arginine (300 mg/kg) i.p. 20 min before L-NAME. In addition, the effects of bilateral vagotomy and sensory deafferentation on the acid secretory response induced by stomach distention were examined. Bilateral vagotomy was performed acutely in the cervical portion 3 h before the onset of stomach distention. Chemical ablation of sensory nerves was performed according to a previously described method (Matsumoto et al., 1992). Briefly, the animals were administered capsaicin s.c. once daily for three consecutive days (20, 30, and 50 mg/kg) 2 weeks before the experiments. All capsaicin injections were made with the animals under ether anesthesia, and the rats were pretreated with terbutaline (0.1 mg/kg i.m.) and aminophylline (10 mg/kg i.m.) to prevent respiratory impairment. The effectiveness of the treatment was tested by examining the protective wiping movements of the eye.

Measurement of Nitrite/Nitrate (NOx) in Gastric Lumen. Luminal NOx levels were measured indirectly as nitrite/nitrate (NO2⁻ and NO3⁻) before and after distention of the stomach by instillation of 6 ml of saline. The stomach was filled with 1 ml of saline for 1 h for the determination of the basal secretion. Then, it was distended by filling with 6 ml of saline, and the solution was changed every 1 h. NOx concentrations in gastric contents were measured according to the Griess method (Green et al., 1982) after reduction from nitrate to nitrite using 0.05 U/ml of nitrate reductase (from Aspergillus; Sigma Chemical Co., St. Louis, MO) in the presence of 5 mM NADPH for 1 h at 37°C. Nitrites were incubated with Griess reagent (0.1% naphthalenediamine dihydrochloride and 1% sulfanilamide in 2.5% H3PO4) for 10 min at room temperature, and the absorbance at 550 nm was measured. For the standard curve, sodium nitrate was used.

Measurement of Histamine Contents in Gastric Lumen. Luminal histamine output was measured before and after distention of the stomach by 6 ml of saline. The stomach was filled with 1 ml of saline for 1 h for determination of the basal secretion. Then, it was distended by filling with 6 ml of saline for 1 h. The amount of histamine in gastric contents was determined by enzyme immunoassay (Histamine EIA kit; Immunotech, Marseilles, France).

Drugs. Urethane (Tokyo Kasei, Tokyo, Japan), atropine, L-NAME, L-arginine, and D-arginine were obtained from Sigma Chemical Co. Capsaicin was obtained from Wako (Osaka, Japan). Famotidine and YM-022 were kindly supplied by Yamanouchi Pharmaceutical Co. Ltd. (Tokyo, Japan). Aminophylline (Neophyllin) was purchased from Eizai (Tokyo, Japan). Terbutaline (Bricanyl) was obtained from Fujisawa (Osaka, Japan). Omeprazole was obtained from Astra (Osaka, Japan). Atropine, L-NAME, L-arginine, or D-arginine was dissolved in saline, whereas omeprazole, famotidine, or YM-022 was suspended in 0.5% carboxymethylcellulose solution (Wako). Capsaicin was dissolved in Tween 80/ethanol solution (10% ethanol, 10% Tween 80, and 80% saline (w/v)). Each drug was prepared immediately before use. Agents were administered i.p. or s.c. in a volume of 5 ml/kg b.wt. or by i.v. in a volume of 1 ml/kg b.wt.

Statistics. Data are presented as the mean ± S.E. from five or six per group. Statistical analyses were performed using a two-tailed Student’s t test and Dunnett’s multiple comparison test. Values of P < .05 were regarded as statistically significant.

Results

Effect of Stomach Distention on Acid Secretion. Anesthetized rats secreted acid at the rate of 10 to 12 μEq/h under basal control conditions when the stomach was instilled with 1 ml of saline, with the total acid output being 29.5 ± 2.8 μEq/3 h. Distention of the stomach by the instillation of more than 2 ml of saline stimulated the acid secretion, in a volume-dependent manner, and in the case of 6-ml instillation, the rate of acid secretion reached 34.5 ± 3.3 μEq/h, with the total acid output being 90.4 ± 7.4 μEq/3 h, 2.7 times greater than control (Fig. 1). Because the instillation of more than 8 ml of saline did not cause any further increase in acid secretion, we performed the experiments when the stomach was distended with 6 ml of saline.

Effect of Various Treatments on Acid Hypersecretion Induced by Stomach Distention. Distention of the stomach by 6 ml of saline caused a marked increase in acid secretion from 9.8 ± 0.9 μEq/h to a peak value of 34.5 ± 3.3 μEq/h, with the total acid output being 90.4 ± 7.4 μEq/3 h. The acid secretory response induced by 6 ml of distention was almost totally attenuated by prior administration of atropine (1 mg/kg i.p.) and famotidine (100 mg/kg i.p.) as well as omeprazole (60 mg/kg i.p.) but was not affected by YM-022 (3 mg/kg i.p.), the specific antagonist of CCKB/gastrin receptor (Fig. 2). In the animals pretreated with omeprazole, the acid output was even lower than that observed in the control stomach instilled with 1 ml of saline. Likewise, the increase of acid secretion in response to 6 ml of distention was also totally inhibited by bilateral vagotomy, similar to the case of atropine, with the inhibition being 95.1% (Fig. 3). On the
other hand, the acid secretory response in the distended stomach was partially but significantly mitigated by sensory deafferentation after capsaicin pretreatment; the acid output was 59.9 ± 2.7 μEq/3 h.

Effects of L-NAME on Acid Hypersecretion Induced by Stomach Distention. The acid secretory response induced by distention was markedly enhanced when the animals were administered i.v. with the NO synthase inhibitor L-NAME (10 mg/kg) 10 min before the instillation of 6 ml of saline (Fig. 4, A and B). In these animals, the rate of acid secretion reached 167.3 ± 15.0 μEq/h, with the total acid output being 388.0 ± 41.0 μEq, about 4.3 times greater than that (90.4 ± 7.4 μEq/3 h) observed in the absence of L-NAME. This agent, however, had no effect on the acid secretion in the nondistended stomach instilled with 1 ml of saline (not shown). The potentiation by L-NAME of the distention-induced acid secretion was completely antagonized by coadministration of L-arginine (300 mg/kg i.p.), and the acid output was 73.0 ± 11.9 μEq/3 h, which was not signifi-
cantly different compared with the values in control rats without L-NAME treatment. Simultaneous administration of D-arginine (300 mg/kg i.p.), however, did not affect the increased acid secretory response induced by stomach distention in the presence of L-NAME; the acid output was 321.5 ± 45.2 μEq/3 h, which was not significantly different from control (388.0 ± 41.0 μEq/3 h).

The potentiation by L-NAME of the acid secretory response in the distended stomach was completely blocked by bilateral vagotomy, sensory deafferentation, or prior administration of atropine (1 mg/kg i.p.; Fig. 5). Both vagotomy and atropine reduced the acid secretion to even below the values observed in the distended stomach in the absence of L-NAME, with the acid output being 52.4 ± 4.5 and 48.8 ± 9.4 μEq/3 h, respectively.

**Luminal NOx Release by Stomach Distention.** In control stomachs instilled with 1 ml of saline, the amount of NOx release into the gastric lumen was 128.2 ± 4.6 nmol/h. The luminal release of NOx was increased in the stomach after gastric distention (6 ml of saline), reaching the value of 261.8 ± 27.5 nmol/h 1 h later, about 2.1 times greater than control, which remained elevated during the distention (Fig. 6). The increase in NOx release in response to gastric distention was significantly inhibited at all time points, by bilateral vagotomy or prior administration of L-NAME; at 1 h after distention, the value was 177.5 ± 4.9 and 185.0 ± 23.5 nmol/h, respectively, which were both significantly lower than control (261.8 ± 27.5 nmol/h). Likewise, the luminal NOx output induced by gastric distention was also significantly decreased in sensory deafferented rats, with the value being 198.4 ± 7.2 nmol/h at 1 h after distention.

**Luminal Histamine Release by Stomach Distention.** Under normal conditions, the amount of histamine released into the gastric lumen was 76.9 ± 4.9 pmol/h. The luminal release of histamine was significantly increased in the distended stomach by 6 ml of saline, reaching the value of 114.8 ± 14.8 pmol/h (Fig. 7). The increased release of histamine in the distended stomach was further augmented by prior administration of L-NAME (10 mg/kg i.v.), with the value reaching 172.5 ± 9.5 pmol/h, which was significantly greater than that observed in the absence of L-NAME. The enhancement by L-NAME of the distention-induced histamine release was significantly antagonized by the simultaneous administration of L-arginine (300 mg/kg i.p.); the value was 124.0 ± 7.5 pmol/h. In contrast, the luminal release of histamine in response to stomach distention was completely attenuated by vagotomy, with the value being 79.8 ± 21.6 pmol/h.
pmol/h, which was equal to that in control stomachs without distention.

Discussion

The present study confirmed the recent findings by Noto et al. (1997), who showed that distention of the stomach stimulated acid secretion, mainly mediated by a vagocholinergic mechanism, not by endogenous gastrin. We further showed that the distention-induced acid hypersecretion was markedly enhanced in the presence of L-NAME, with the response being significantly mitigated by bilateral vagotomy as well as by the coadministration of \( \text{L-arginine} \). These findings strongly suggest that endogenous NO plays a modulatory role in the acid secretory response to gastric distention.

Many studies have demonstrated that stomach distention increases gastric acid secretion via mechanical stimulation (Grossman, 1962; Debas et al., 1974; Johnson, 1977; Hakanson et al., 1980; Esplugues et al., 1990; Barrachina et al., 1994, 1995; Noto et al., 1997). We confirmed that distention of the stomach via saline instillation caused acid hypersecretion, with the degree depending on the volume of saline. The maximal acid response was observed when the stomach was distended by 6 ml of saline, with the intraluminal pressure being 20 cm H_2O. Because the gastric volume in rats fed ad libitum was about 4 to 9 ml (Noto et al., 1997), the acid secretion observed under the present conditions may be a physiological response to mimic the satiety. First, we observed that the distention-induced acid secretion was all but totally attenuated by either vagotomy or prior administration of atropine, confirming that the response is mainly mediated by a vagocholinergic mechanism (Grossman, 1962; Hakanson et al., 1980; Noto et al., 1997). The acid response to stomach distention was also significantly mitigated by sensory deafferentation after capsaicin pretreatment. Raybould and Tache (1989) have shown that capsaicin-sensitive vagal afferent fibers mediate the vagal portion of the secretory response to gastric distention. Esplugues et al. (1990) suggested that these sensory neurons mediating the distention-induced acid secretion are also located in the celiac ganglion, based on the inhibition of the response by local application of capsaicin to the celiac ganglion or acute ganglionectomy. In any case, it is assumed that the perception of the mucosa for mechanical stimulation may require the intact sensory neurons, causing acid hypersecretion through vagal afferent nerves. Certainly, the acid secretory response to gastric distention was completely blocked by omeprazole, the inhibitor of H^+\text{K}^+\text{ATPase}, the enzyme involved in the final step of the acid production (McTavish et al., 1991), as well as famotidine, the histamine H₂ receptor antagonist, suggesting an involvement of endogenous histamine at the parietal cell. On the other hand, the participation of gastrin in the acid response to mechanical stimulation is controversial (Debas et al., 1974; Johnson, 1977; Alumets et al., 1982; Lloyd et al., 1992; Noto et al., 1997), although distention of the stomach is known to stimulate gastrin release. Lloyd et al. (1992) reported that the administration of anti-gastrin monoclonal antibody significantly reduced the acid secretion in response to stomach distention and suggested a crucial role for gastrin in this response. By contrast, Alumets et al. (1982) and Noto et al. (1997) failed to show the increase of serum gastrin levels after mechanical stimulation (i.e., stomach distention).

In the present study, we found that YM-022, the CCK\_\text{B/}
gastrin receptor antagonist, did not significantly affect the increase of acid secretion induced by gastric distention, excluding the role of gastrin in the acid response. On the basis of these results, it is suggested that the acid secretory response to gastric distention involves mainly vagocholinergic efferent nerves as well as sensory afferent nerves, in addition to histamine in the gastric mucosa. It should also be noted that in this study, the distention-induced acid secretion was not completely attenuated by either sensory deafferentation, vagotomy, or administration of atropine, with the effects being less potent compared with omeprazole. Davison and Najafi-Farashah (1985) reported that acid secretion was stimulated by distention in isolated mouse stomach, and this response was affected by neither atropine nor cimetidine. Lloyd et al. (1992) also showed that acid secretion induced by 3 ml of distention was little affected by atropine. Thus, it may be assumed that gastric distention elicits acid secretion, at least in part, by mechanisms independent of the vagocholinergic pathway.

Of most interest in this study is that the acid response to gastric distention was markedly enhanced under the blockade of NO production by L-NAME, the NO synthase inhibitor. Gastric distention (6 ml saline) caused about a 3-fold increase in acid secretion, and this response was enhanced further, being 4 times greater than that observed by distention alone. Potentiation by L-NAME of the acid secretory response might result from removal of the inhibitory influence of endogenous NO by inhibiting the production of endogenous NO. Indeed, this effect of L-NAME was antagonized by the simultaneous administration of L-arginine but not of D-arginine. In addition, we found that gastric distention increased the release of NOx, the metabolites of NO, in the lumen, and this response was attenuated by vagotomy as well as by L-NAME but not by omeprazole (not shown).

It seems that the activation of vagus nerves during gastric distention increases the production of endogenous NO via a process independent of the acid secretion itself. These findings are in agreement with our previous observations that L-NAME caused a significant increase of the acid secretion mediated vagally by YM-14673, an analog of thyrotropin-releasing hormone (Kato et al., 1998). Certainly, it was also shown that YM-14673 increased the luminal release of NO, in an L-NAME-sensitive manner.

A number of studies have investigated the effects of NO synthase inhibitors on gastric acid secretion, although the results remain controversial. Pique et al. (1992) reported that the NO synthase inhibitor \( \text{N}^\text{G}\)-monomethyl-L-arginine did not affect either basal or pentagastrin-stimulated acid secretion in rats. Martinez-Cuesta et al. (1992) showed that the NO synthase inhibitor L-NAME antagonized the inhibitory action of lipopolysaccharide on acid secretion induced by gastric distention or pentagastrin in rats. Brown et al. (1993) reported that a high concentration of NO donor inhibits acid secretion using rat isolated parietal cells, suggesting a direct inhibitory action at the parietal cell. We have also shown that the inhibitory acid response in the stomach after damage was completely antagonized by L-NAME, suggesting an inhibitory role for NO in the regulation of gastric acid secretion (Takeuchi et al., 1994). In contrast, Bilski et al. (1994) reported that the NO synthase inhibitor failed to affect basal acid secretion but reduced the acid secretion in response to
feeding or pentagastrin in dogs, probably because of a decreased mucosal blood flow. More recently, Hasebe et al. (1998) showed with isolated mouse whole stomach that the use of N\textsuperscript{\textomega}-nitro-L-arginine decreased the acid secretion induced by pentagastrin or vagal electrical stimulation. The present results, however, clearly showed that endogenous NO exerts a negative influence on the acid secretory response induced by gastric distention. Because L-NAME is known to cause gastric hyperperfusion (Alumets et al., 1982; Brecht et al., 1990; Esplugues et al., 1996), it is unlikely that the enhanced acid secretion observed under the blockade of NO production is attributable to an increase in the mucosal blood flow. It should also be noted in this study that the enhancement by L-NAME of the distention-induced acid response was almost totally attenuated by vagotomy and capsacain pretreatment, again confirming that gastric distention requires both sensory and vagal nerves for eliciting stimulation of acid secretion. Because NO-containing neurons have been identified in the central nervous system as well as in the gastrointestinal mucosa (Brecht et al., 1990) and because NO plays a role as a neuromodulator in some nonadrenergic noncholinergic neurons in the gut (Sanders and Ward, 1992; Moncada et al., 1993), it is possible that NO decreases vagally mediated acid secretion by suppressing neuronal activity of the vagus nerves.

On the other hand, it is known that vagally induced acid secretion is in part mediated by endogenous histamine release from enterochromaffin-like (ECL) cells (Richardson, 1978; Sandvik et al., 1987). We found that gastric distention also caused a release of histamine in the mucosa via a mechanism that depends on both vagus nerves and NO. Salvenemi et al. (1991) reported that exogenous NO inhibited the release of histamine in rat mast cells, mediated via a guanylate cyclase/cGMP-dependent system. A recent study also showed that interleukin-1\beta (IL-1\beta) exhibits an antisecretory action against pentagastrin by suppressing histamine release, in an L-NAME-sensitive manner (Wallace et al., 1991), and that IL-1\beta causes an inhibition of histamine release from ECL cells mediated by cGMP (Prinz et al., 1997). In the present study, we also found that the luminal release of histamine in response to gastric distention was significantly enhanced by L-NAME, in a L-arginine-sensitive manner. Thus, it may be assumed that NO is capable of reducing acid secretion locally by inhibiting the release of histamine from ECL cells, in addition to modulating the neuroactivity of the vagus nerves.

The present results taken together may suggest that gastric distention increases acid secretion, initially through the perception by sensory neurons of the mechanical stimulation and subsequently through the efferent vagocholinergic pathway, and the latter process is modified by endogenous NO (Fig. 8). It may be assumed that gastric distention releases NO in the mucosa by a vagal-dependent mechanism, which then exerts a negative influence on acid secretion locally by suppressing histamine release from ECL cells.

References


Send reprint requests to: Koji Takeuchi, Ph.D., Department of Pharmacology and Experimental Therapeutics, Kyoto Pharmaceutical University, Misasagi, Yamashina, Kyoto 607-8414, Japan. E-mail: takeuchi@mb.kyoto-phu.ac.jp