Effects of Nitric Oxide Synthase Inhibitor on the Digestive System Measured by Simultaneous Monitoring of Gastric Motility, Gastric Emptying Activity and Postprandial Pancreaticobiliary Secretion in Dogs

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Abstract: Relationships between the NO synthase inhibitor and gastric and pancreaticobiliary functions measured simultaneously in the digestive state have been little studied. The aim of this study was to estimate the effect of NO synthase inhibitor on integrated digestive function in conscious dogs. A strain gauge force transducer was implanted on the gastric antrum of 6 mongrel dogs to measure gastric contractile activity and two duodenal cannulas were inserted into the proximal and distal sites to measure the gastric emptying rate and the pancreaticobiliary output into the duodenum using our novel method. Postprandial pancreatic and biliary secretion were presented as amylase and bile acid activity, respectively. Furthermore, a cervical cannula was placed into the superior vena cava as a route for the administration of NO synthase inhibitor, Nω-nitro-L-arginine (L-NNA), at a dose of 2.5 mg/kg-h. In a group given L-NNA, gastric contractile activity after ingestion was significantly enhanced, but the emptying rates of gastric solids and liquids were significantly suppressed in comparison with the control. The mean 0–1 h amylase integrated output was significantly (P<0.05) decreased in comparison with the control, and the mean bile acid integration of 0–1 h output was also significantly (P<0.01) decreased. A possible explanation for this observation is that smaller volumes of nutrient are delivered into the duodenum; however, it could also be that postprandial pancreaticobiliary secretion is inhibited by an alteration of blood flow or by a change in contractions of the sphincter of Oddi after the administration of L-NNA.

Key words: gastric contractile activity, gastric emptying, nitric oxide, nitric oxide synthase inhibitor, postprandial amylase, postprandial bile acid

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Introduction

It is known that sympathetic and parasympathetic nerve fibers provide both inhibitory and excitatory regulation of the gastrointestinal smooth muscle, and their neurotransmitters are noradrenaline (NA) and acetylcholine (ACh), respectively. Moreover, in the enteric nervous system (ENS; intrinsic nerves), substance P (SP) and ACh are excitatory neurotransmitters, and adenosine triphosphate (ATP) [17], vasoactive intestinal polypeptide (VIP) [9], carbon monoxide (CO) [30] and nitric oxide (NO) are inhibitory neurotransmitters. These compounds regulate gastrointestinal motility both in the digestive state as well as the interdigestive state.

Since Furchgott et al. [8] described the existence of endothelium derived vascular relaxing factor (EDRF), which regulates the smooth muscle of the vascular system, in 1980, and Palmer et al. [25] and Ignarro et al. [11] confirmed that EDRF and NO are identical in 1987, numerous physiologists have investigated the role of NO. Since then, it has been found that NO plays an important role not only in the cardiovascular system but also in the gastrointestinal tract, i.e. esophagus [22], esophageal sphincter [19], stomach [7], small bowel [32, 33], ileocolonic junction [3], large bowel [28], internal anal sphincter [29], liver [34], pancreas [14, 16], gallbladder [20], and sphincter of Oddi [21].

As NO, which is one of the nonadrenergic non-cholinergic (NANC) neurons, participates in postprandial gastric motility as an inhibitory neuron, gastric motor activity is enhanced after the administration of NO synthase inhibitor. Furthermore, gastric emptying is suppressed after the inhibition of activity of the NO neuron. For instance, Sarna [31] showed that postprandial gastric contractions were enhanced by the administration of NO synthase inhibitor, L-NNA. Moreover, it was shown that the NO synthase inhibitor suppressed the emptying of gastric solids [23] and liquids [27]. The effect of NO on pancreaticobiliary secretion has also been reported. Konturek et al. [14, 15] demonstrated that the NO inhibits exocrine pancreatic secretion in both fasting and fed states in dogs. Mourelle et al. suggested that the NO modulates gallbladder contractions [20] and the sphincter of Oddi [21] in both fasting and CCK-induced contractile states in guinea pigs and rabbits. However, the NO synthase inhibitor of integrated digestive function (simultaneous monitoring of gastric contractility, gastric emptying and exocrine pancreatic and bile acid secretion) is still unknown.

The aim of this study was to investigate the effects of a specific NO synthase inhibitor (L-NNA) on postprandial pancreaticobiliary secretion, gastric contractility, and gastric emptying in an in vivo large animal model. Our hypothesis was that L-NNA would increase gastric contractility, but delay gastric emptying and pancreaticobiliary secretion.

Materials and Methods

The Review Committee on Animal Use approved the procedures used in the present animal experiments at Gunma University, Maebashi, Japan.

Preparation of Animal Model

Six healthy mongrel dogs weighing 8–13 kg were used. Dogs were anesthetized using thiopental sodium (Ravonal 20 mg/kg, IV) and maintained during the operation under general anesthesia using inhalation of halothane and oxygen. A cervical venous catheter (SH No.1, Silascon Medical Tube; Kaneka Medix, Osaka, Japan) was placed as described previously [12] as a route to give an electrolyte solution containing antibiotics against several infections for the first three postoperative days and to administer normal saline to the control group or the nitric oxide synthase inhibitor, Nω-nitro-L-arginine (L-NNA) to the experimental group. Through a mid-ventral celiotomy, a strain gauge force transducer [13] was chronically implanted on the gastric antrum 3 cm proximal to the pyloric ring to measure circular muscle contractions, and two indwelling duodenal tubes were implanted: one silicone tube (SH No.1, Silascon Medical Tube) was inserted into the proximal duodenum 6 cm distal to the pyloric ring to measure the duodenal volume by means of continuous infusion of a non-absorbable marker, phenolsulfonphthalein (PSP); the other tube (X2-50, Top Extension Tube; Meditop, Tokyo, Japan) was inserted into the distal duodenum 3 cm proximal to the pyloric ring to measure circular muscle contractions, and two indwelling duodenal tubes were implanted: one silicone tube (SH No.1, Silascon Medical Tube) was inserted into the proximal duodenum 6 cm distal to the pyloric ring to measure the duodenal volume by means of continuous infusion of a non-absorbable marker, phenolsulfonphthalein (PSP); the other tube (X2-50, Top Extension Tube; Meditop, Tokyo, Japan) was inserted into the distal duodenum 20 cm distal to the proximal duodenal tube to collect duodenal luminal contents containing ingested meals delivered into the duodenum and pancreaticobiliary juice. The proximal duodenal tube and the lead wires of the strain gauge force transducer were brought out together to the flank through a subcu-
taneous tunnel. The distal duodenal tube was cemented within an exteriorized stainless steel cannula, which was embedded in the right lateral abdominal wall.

Conduct of Experiments

After surgery, the lead wires and the proximal duodenal tube were protected with jacket protectors. Dogs were housed in individual cages and fed once daily at 10:00 after returning to normal food, with drinking water supplied ad libitum. During the recovery, the dogs were trained to become accustomed to standing for 6 to 8 h per day and eating test meals comfortably in a Pavlov sling. Thereafter, the experiments were started. Dogs were placed in the sling at 10:00 after an overnight fast. The lead wires of the transducer were connected to the cable leads of the amplifiers (UG-6, Nihon Kohden Kohgyo, Tokyo, Japan), and gastric antral motor activity was monitored on a pen-writing recorder (WT685G, Nihon Kohden Kohgyo). Normal saline containing PSP at a concentration of 375 µg/ml was constantly infused at a rate of 3.0 ml/min through the proximal duodenal tube by means of a peristaltic pump (PST-100, Iwaki Glass, Tokyo, Japan) and a hot stirrer (SR-350, Advantec Toyo Kaisya, Tokyo, Japan) (37°C) to determine duodenal volume as reported previously [35]. After a 30-min equilibration period, 1.5-ml samples of duodenal contents were collected through the distal duodenal tube at 5-min intervals. Three consecutive samples were combined to produce a 15-min sample. After two more successive samples, all dogs ate their test meals within 5 min during phase I of the interdigestive period, and the collection of samples at 5-min intervals was continued for 6 h after ingestion. The dog food (4 kcal/g, 26% protein, 41% carbohydrate, and 12% fat, Adult Dog Formula, Natural Life Petproducts, Frontenac, KS) which was freeze-dried by a freeze dryer (VD-41, Taitec, Saitama, Japan) and a vacuum pump (DW-120, Sato Vacuum Machinery Industrial, Saitama, Japan) was used to make a solid meal. One hundred grams of freeze-dried dog food mixed with 100 ml of normal saline containing 15 g polyethylene glycol (PEG 3350, Sigma Chemical, St. Louis, MO) as a liquid meal were used as a test meal. Duodenal samples were diluted to 5.0 ml with normal saline and then 1.0 ml 10% BaCl₂, 2.0 ml 0.3 N Ba(OH)₂ and 2.0 ml 5% ZnSO₄·7H₂O were added (the diluted duodenal sample included meals delivered into the duodenum, normal saline, PSP, PEG, bilirubin, bile acid, amylase and succus entericus), and this sample was filtered with a filter paper. Using the filtrate, the PEG concentration was measured using a spectrophotometer (DU-650, Beckman Coulter, Fullerton, CA) at 650 nm after the addition of 30% trichloroacetic acid (TCA, Wako Pure Chemical Industries, Osaka, Japan) according to the method of Hydén [10] and used for the calculation of the gastric emptying rate of liquids. The concentration of PSP in each aspirated sample was measured using the spectrophotometer at 560 nm after the addition of 10% NaOH, as reported previously [35]. Since bile pigment (bilirubin) is adsorbed by adding Ba(OH)₂ and ZnSO₄ [18] and does not pass through to the filtrate, the measurement of PSP in the filtrate is not disturbed by the presence of bilirubin.

Administration of L-NNA

L-NNA at a dose of 2.5 mg/kg-h, as in previous works performed by Orihata and Sarna [23, 24], was administered intravenously through the indwelling cervical tube starting 1 h before feeding and continued for 4 h after feeding. This infusion rate is effective for altering the characteristics of fasting gastrointestinal motor activity for several days [31]. In the L-NNA group, dogs ate meals at the quiescent period during disruption in gastric motility. As a control, normal saline was administered for the same duration as L-NNA.

Measurement of Gastric Antral Contractile and Emptying Activity

Recording of gastric antral contractile activity and measurement of the motor index (MI) were performed in a way similar to that as previously reported [35]. The change in contractile activity of the gastric antrum after ingestion of the test meal was recorded continuously with the pen recorder on a paper chart. The data accumulated on a computer disk were used for the calculation of the area under the curve; MI is represented by the area under the curve.

Measurement of the emptying rates of gastric solids and liquids was also carried out by our newly developed freeze-drying method [35]. Briefly, the gastric emptying rate of solids is represented as the freeze-dried weight of solid components emptied into the duodenum after ingestion of the test meal. The gastric
emptying of liquids is represented as the emptying rate of the PEG into the duodenum.

**Measurement of Postprandial Pancreaticobiliary Secreton**

Measurement of postprandial amylase and bile acid secretion was carried out in a way similar to a previous study [37]. One milliliter of each sample taken from the duodenum at 15-min intervals was collected in separate tubes in an ice box, and immediately stored at -80°C until the assay of amylase and bile acid activity.

**Drugs Used in This Study**

All chemicals were purchased from Wako Pure Chemical, Osaka, Japan. L-NNA was dissolved in normal saline.

**Analysis of Data**

All values were expressed as mean ± SEM. Gastric antral motor activity was represented as the fractional MI at 15-min intervals after feeding and was estimated in terms of the mean integrated MIs for 1, 3 and 6 h. The gastric emptying of solids and liquids were estimated as the mean retention in the stomach at 1, 3 and 6 h, the mean half emptying time (T1/2) and the mean area under the emptying curve (AUC) between 0 and 6 h after feeding. Postprandial amylase and bile acid activity were expressed as the 15-min fractional outputs after feeding and were estimated as the mean integration of 0–1 (early phase), 1–3 (middle phase) and 3–6 (late phase) h outputs into the duodenum.

**Statistical Methods**

The significance of differences between the groups was determined by Student’s paired t test. A P value of <0.05 was considered to represent a significant difference.

**Results**

**Effect of L-NNA on Postprandial Gastric Antral Motor Activity in Conscious Dogs**

Figure 1 shows the effect of L-NNA on gastric antral motor activity after feeding. Contractile activity of the gastric antrum is represented by the fractional MI in Unit/15 min. In the control study, antral contractile activity began to increase immediately after feeding and showed the characteristic digestive motor pattern. In the L-NNA group, the motor activity of the gastric antrum was significantly enhanced immediately after feeding by the administration of L-NNA, at a dose of 2.5 mg/kg-h, and values of the fractional MI continued to be higher than in the control. The mean integrated

![IV Infusion](image)

**Fig. 1.** Effect of L-NNA at a dose of 2.5 mg/kg-h on gastric antral motor activity. Gastric antral motor activity represented by 15-min fractional MI was enhanced by the administration of L-NNA; n=5.
Mls for 1 and 3 h after ingestion of the meal significantly \((P<0.05)\) increased in the L-NNA group (data not shown). In addition, the mean integrated MI for 6 h was greater \((P<0.01)\) than that in the control (control vs L-NNA; 25.8 ± 4.3 vs 47.1 ± 4.7 Unit).

**Effect of L-NNA on the Emptying of Gastric Solids and Liquids in Conscious Dogs**

Solid meals significantly \((P<0.01)\) remained in the stomach at 3 h (control vs L-NNA, 62.5 ± 4.7 vs 83.2 ± 3.5%, respectively) and 6 h (30.9 ± 4.3 vs 64.0 ± 6.0%, respectively) after feeding in the L-NNA group. We could not analyze the mean \(T_{1/2}\) of solid emptying in the L-NNA group because less than half of the ingested solid meals were delivered into the duodenum in this group. Table 1 shows the mean AUC between 0 and 6 h after feeding. The mean AUC of solid emptying in the L-NNA group was significantly \((P<0.01)\) higher than that in the saline control. Liquid meals, on the other hand, significantly \((P<0.05)\) remained in the stomach at 3 h (19.3 ± 4.8 vs 36.7 ± 5.6%) after feeding in the L-NNA group. The mean \(T_{1/2}\) of liquid emptying in the L-NNA group was a low value but it was not significantly. The mean AUC of liquid emptying in the L-NNA group was a significant high value (Table 1).

**Effect of L-NNA on Postprandial Pancreaticobiliary Secretion in Conscious Dogs**

Figure 2 shows the effect of L-NNA on postprandial amylase output. In the control study, amylase output into the duodenum rapidly increased after ingestion of meals and reached a peak value at 1 h after feeding. The values gradually decreased thereafter and did not show a significant increase from 3 h after feeding. In the L-NNA group, on the other hand, amylase output did not show a significant increase after feeding in comparison with the control. Figure 3 shows the effect of L-NNA on postprandial bile acid output. Postprandial bile acid output in the control group increased with a similar pattern to the output of amylase in the control. In the L-NNA group, bile acid output into the duodenum did not significantly increase after feeding. Table 2 shows the mean integration of 0–1, 1–3 and 3–6 h outputs of amylase and bile acid into the duodenum.

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**Table 1. Effect of L-NNA on gastric emptying of solids and liquids in conscious dogs**

<table>
<thead>
<tr>
<th></th>
<th>Solids (n=6)</th>
<th>Liquids (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline control</td>
<td>23,168.9 ± 1,294.8</td>
<td>9,136.0 ± 1,490.1</td>
</tr>
<tr>
<td>L-NNA</td>
<td>29,753.6 ± 1,175.5††</td>
<td>14,982.2 ± 2,177.7†</td>
</tr>
</tbody>
</table>

Data are given as mean ± SEM. †\(P<.05\) vs. saline control. ††\(P<.01\) vs. saline control.

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**Fig. 2.** Effect of L-NNA at a dose of 2.5 mg/kg-h on postprandial amylase output. Fifteen-min fractional amylase outputs into the duodenum were suppressed by the administration of L-NNA; n=4.
after feeding. The mean integrated amylase output for 0–1 h after feeding of the L-NNA group was significantly lower than that of the control. The mean integrated bile acid output for 0–1 h in the L-NNA group was significantly lower than that of the control group.

### Discussion

We investigated the effects of the NO synthase inhibitor, L-NNA, on gastric contractile activity and emptying, and postprandial pancreaticobiliary secretion in conscious dogs. L-NNA suppressed the gastric emptying of solids and liquids although gastric antral contractile activity was enhanced. Moreover, L-NNA significantly decreased the output of both amylase and bile acid into the duodenum. There are three possible explanations for these observations. The first is that the nutrient delivery to the duodenum decreases due to the delayed gastric emptying. The second is that the blood flow to the pancreas and gallbladder decreases after the administration of L-NNA. The third is that L-NNA directly affects NO neurons which innervate the gallbladder and the sphincter of Oddi.

It is widely accepted that phasic contractions in the gastric antrum occur to grind, sieve and empty food stuffs into the duodenum after ingestion of the meal, and that gastric emptying is also accelerated when gastric antral motor activity is enhanced by gastrointestinal prokinetic agents during the digestive state. Indeed, cisapride (a 5-HT₄ receptor agonist) and EM574 (a motilin agonist, an erythromycin derivative) enhance the postprandial contractile activity of the gastric antrum and accelerate gastric emptying in dogs [36] and humans [4]. Recently, ABT229, which is a novel motilide and a more potent synthetic derivative of eryth-
romycin without antibiotic activity, has been investigated as a potential prokinetic drug which stimulates gastrointestinal motor activity via activation of motilin receptors [5] and accelerates gastric emptying [6, 41].

We have developed a technique to simultaneously measure both gastric contractile and solid and liquid emptying activities without a radioisotope in a test meal as a marker in dogs. Using this technique, we showed that the NO synthase inhibitor, L-NNA, suppresses gastric emptying of solids and liquids even though gastric antral motor activity is stimulated. This fact is curious. Nitric oxide is an important NANC inhibitory neurotransmitter that regulates many gut functions. Desai et al. [7] showed the role of NO in receptive relaxation of the stomach, and Toda et al. [38] described NO mediated relaxation in canine duodenal longitudinal smooth muscle. Moreover, Allescher et al. [1] and Vanderwinden et al. [40] reported that NO also regulates contractile motor activity in the pylorus, suggesting that the administration of NO synthase inhibitor markedly induces selective stimulation of contractile activity in the pyloric ring. Pyloric relaxation is a very important factor for explaining gastroduodenal coordination and gastric emptying activity in the digestive state. Erythromycin is considered to increase the force of antral contractions and to improve gastroduodenal coordination [2], and motilides (motilin agonists) accelerate the gastric emptying by modulating postprandial gastroduodenal coordination and by increasing the motility index [6]. Postprandial contractile motor activity of the pylorus that is independent of antral and duodenal contractions, the contractile motor activity without gastropyloroduodenal coordination, appears to suppress gastric emptying [23, 39]. Measurement not only of gastric antral motor activity but also of gastric emptying rate is necessary when evaluating new prokinetic agents to include the effects of pyloric motor activity.

In this study, we also simultaneously measured the pancreaticobiliary secretion in the digestive state. NO synthase inhibitor significantly decreased the outputs of both amylase and bile acid into the duodenum. The first possible explanation for this observation is that because of decreased gastric emptying, smaller volumes of nutrient were delivered into the duodenum in comparison with the control study, and any receptors including CCK or secretin in the duodenum were not fully activated. As a result, outputs of amylase and bile juice were not increased. This idea is drawn from the relationship between the nutrient amount passing into the duodenum and some hormones, CCK and secretin, at the intestinal phase but not the cephalic phase, since amylase output is also increased at the sham-feeding state (cephalic phase) [15]. However, this theory may not fully explain our observations in this study.

A second possible explanation is based on the idea that some receptors of CCK and secretin are activated but these paracrine hormones are not delivered to their target organs. For instance, gastric mucosal blood flow decreases by about 70% after the administration of NO synthase inhibitors [26]. Thus, NO may participate in the regulation of gastrointestinal blood flow. As we did not measure hemodynamic parameters as part of our experimental protocol, this explanation is speculative. Regarding the pancreas, Konturek et al. [15] suggest that the decrease in amylase release after the administration of NO synthase inhibitor is due to a decrease of blood flow to the pancreas. It is considered that NO is an endothelium-derived relaxing factor (EDRF) [25] and mediates the action of some vasodilators. The action of NO on the vascular system is inhibited after the administration of NO synthase inhibitor so that a vasospasm occurs in the vasculature to the pancreas. As a result, activated CCK and secretin may not be able to reach the pancreas. This consideration is drawn from the role of NO in the vascular system. In addition, exocrine pancreatic and bile juice are secreted from the pancreas and gallbladder by activated CCK and secretin, respectively. However, the contractile activity of the sphincter of Oddi may be simultaneously enhanced by NO synthase inhibitor, and as a result, their juices may not be delivered into the duodenum. The work of Mourelle et al. [20] indicated the effect of NO synthase inhibitor on gallbladder strips in vitro. They showed the effect of the NO synthase inhibitor, L-NAME, on the tension of muscle strips in response to CCK-8 and bethanechol. After the administration of L-NAME, CCK-8- or bethanechol-induced contractions were enhanced, suggesting that NO participates in the gallbladder contractions. Moreover, Mourelle et al. [21] also described the effect of L-NAME on the tension of muscle strips of the sphincter of Oddi in response to bethanechol in vitro. They showed that bethanechol-induced contractions were en-
enhanced by L-NAME, suggesting that the sphincter of Oddi is regulated by NO neurons. Thus, inhibition of NO synthase enhances gallbladder contractions and bile juice is delivered into the common bile duct, while simultaneously enhancing the contractions of the sphincter of Oddi, thereby preventing bile and exocrine pancreatic juice from entering the duodenum.

In conclusion, the present results demonstrate that the NO synthase inhibitor, L-NNA, increased gastric antral contractile activity while slowing gastric emptying. L-NNA also suppressed exocrine pancreatic amylase and bile acid output in conscious dogs. The mechanism of the NO effect on the integrated postprandial function of the upper gut is likely multifactorial. Further studies will be required to fully elucidate the complex role of NO.

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References

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