Relationship between intraduodenal 5-hydroxytryptamine release and interdigestive contractions in dogs

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Abstract

Background: 5-hydroxytryptamine (5-HT) is released into the intestinal lumen during the fasting state. However, the relationship between the intraduodenal 5-HT and the interdigestive cyclic motor activity in conscious dogs is unclear. Aim: To correlate intraduodenal 5-HT concentrations with the interdigestive gastroduodenal migrating motor complex (MMC). Methods: 6 dogs were implanted with 2 force transducers for recording gastroduodenal contractions and 2 catheters for measuring duodenal volume by a non-absorbable marker perfusion technique. Intraduodenal 5-HT concentrations were determined by high performance liquid chromatography at 5-min intervals. Results: During fasting, gastroduodenal motor activity cycled as the MMC; luminal 5-HT concentrations and total outputs varied cyclically in temporal association with the MMC. Mean 5-HT concentrations peaked during phase II (P<0.05 vs. phase I and III), and 5-HT outputs during phases II or III were greater than during phase I (P<0.05). Exogenous motilin (0.3 μg/kg-hr, IV) stimulated 5-HT release into the duodenal lumen with peak values (P<0.05) during motilin-induced phase II and III. Gastroduodenal motor activity was not altered, however, during exogenous intraduodenal administration of 5-HT (300 ng/mL-min). Conclusions: 5-HT is released cyclically into the duodenal lumen in close temporal association with the MMC, but its physiologic significance in regulation of gastroduodenal motility is unknown.

Key words: gastrointestinal motility, motilin, IMC, MMC, intraluminal 5-HT

Introduction

Gastrointestinal motor activity shows two distinctly different patterns before and after ingestion of a meal. During the interdigestive or fasting state, a cyclic motor pattern termed the interdigestive migrating contractions (IMC) or the migrating motor complex (MMC) is observed. These motor patterns have been divided into four phases (Code et al., 1975). Phase I is a quiescent period with virtually no contractions. Phase II consists of intermittent, low-
amplitude, irregular contractions. Phase III, also called the activity front (AF), consists of a short burst of regular, high amplitude contractions that begin in the stomach and migrate aborally to the terminal ileum in a very orderly manner; these phases have been classically used as the characteristic motor patterns during the fasting state. The period of this cyclic motor pattern during fasting can be observed about every 2 hours. Numerous studies have examined control and initiation of the MMC, and two factors (vagal nerves or motilin) stand out as possible candidates.

5-hydroxytryptamine (5-HT) is also considered to be an important mediator of interdigestive motility, though its exact role in the genesis of MMC is controversial. Ormsbee et al. (1984) showed that parachlorophenylalanine (pCPA), a 5-HT synthesis inhibitor, disrupted the period of MMC and reduced the number and amplitude of phase II and III contractions in the canine small intestine. Moreover, Piñeiro-Carrero et al. (1991) showed that 5,6- and 5,7-dihydroxytryptamine (5,6-DHT and 5,7-DHT), indoleamine neurotoxin affecting enteric serotonergic neurons disrupted the MMC in rats, suggesting that 5-HT neurons play an important role in the regulation of interdigestive contractions in the canine stomach.

5-HT is also released into the intestinal lumen (Bülbring et al., 1958; Ahlman et al., 1981; Ferrara et al., 1987) and colon (Kojima, 1999) in several species, and one group has suggested that luminal 5-HT plays an important role in regulating gastrointestinal motor activity, such as peristalsis (Bülbring and Lin, 1958). In addition, in vitro work showed that 5-HT evoked a peristaltic reflex (Craig et al., 1991). As reported previously (Kellum et al., 1986), 5-HT is released into the intestinal lumen after the local intraarterial infusion of motilin in a canine model with an isolated gut, and intraluminal administration of 5-HT induces phasic contractions similar to the motilin-induced contractions, suggesting that luminal 5-HT may initiate the motilin-induced contractions. In contrast, Ferrara et al. (1986) reported that the release of 5-HT in an intestinal Thiry-Vella loop did not vary significantly with the MMC, suggesting that intraluminal 5-HT is not important in the local regulation of the canine MMC in the small intestine.

The aims of this study, therefore, were two fold; first to determine whether 5-HT is released into the intact duodenum in temporal association with the MMC, and second to examine the effect of exogenous intraduodenal 5-HT on the initiation of phase III activity in conscious dogs. Our hypothesis was that intraduodenal 5-HT is important in the genesis of fasting motor activity.

A part of the results reported here was presented as an abstract (Tanaka et al., Neurogastroenterol. Mot. 14: 595, 2002) and also presented at the meeting of the XIth European Symposium on Neurogastroenterology and Motility in Tübingen, Germany (October 2–4, 2002).

**Materials and Methods**

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

**Preparation of animal model**

Six mongrel dogs were anesthetized by a single intravenous (IV) injection of thiopental
sodium (20 mg/kg; Ravonal, Tanabe Pharmaceutical Co., Osaka, Japan). General anesthesia was maintained by intratracheal isoflurane (Halothane, Takeda Pharmaceutical Co., Osaka, Japan) and oxygen. After celiotomy, one strain gauge force transducer was implanted chronically on the serosal surface in the gastric antrum 3 cm proximal to the pyloric ring and the other on the duodenum opposite the main pancreatic duct, to monitor circular gastrointestinal-contractions. A silicone tube (Silascon Medical Tube SH No. 1, Kaneka Medix Co., Osaka, Japan) was inserted into the proximal duodenum 6 cm distal to the pyloric ring for infusion of the nonabsorbable marker polyethylene glycol (PEG). A catheter (Top extension tube X2-50; Meditop, Tokyo, Japan) was also inserted into the distal duodenum 20 cm distal to the proximal duodenal tube for aspiration of luminal contents. The catheter was led out through a stainless steel cannula implanted on the right lateral abdominal wall.

The lead wires of the transducers and the proximal duodenal tube exited the abdominal cavity through a skin incision between the scapulae. The outer ends of the lead wires were attached to a small connector. A silicone tube (Silascon Medical Tube SH No. 1, Kaneka Medix Co.) was implanted in the superior vena cava via a branch of the right external jugular vein as a route for the injection of test agents. The dogs fitted with a canvas jacket to protect the connector and the tube, were housed in individual cages and fed dry-pellet type dog food (Gaines Meal, Ajinomoto-General Foods Co., Tokyo, Japan) once a day with free access to water.

Conduct of experiments

Experiments were started two weeks later after the operation. After an overnight fast, dogs lay quietly in a Pavlov sling. The connector for the transducers was connected to cable lead wires from an amplifier (UG-5, Nihon Kohden Kohgyo Co., Tokyo, Japan). Signals from the amplifier were recorded on a multichannel pen-writing recorder (WI-681G, Nihon Kohden Kohgyo) to measure the gastroduodenal contractile activity. PEG (3350; Sigma Chemical, St. Louis, MO) solution (37\(^{\circ}\)C) diluted with 154 mM NaCl, at a concentration of 5 mg/mL, was constantly infused at a rate of 1.0 mL/min through the proximal duodenal tube with a peristaltic pump (PST-100, Iwaki Glass, Tokyo, Japan) for measuring duodenal volume. After a 30-min equilibration, duodenal contents were collected through the distal duodenal catheter at 5-min intervals. Experiments were continued over at least 3 gastroduodenal MMC cycles. The luminal contents were frozen at \(-80^{\circ}\)C for further analysis of PEG and 5-HT concentrations.

Exogenous canine motilin (Peptide institute, Osaka, Japan) at 0.3 \(\mu\)g/kg-hr was administered intravenously during phase I beginning 20 min after the end of phase III activity in the gastric antrum and infused continuously for 30 min to examine whether exogenous motilin stimulated luminal 5-HT release. During this period, spontaneous phase III activity would not be expected to be initiated in normal dogs during fasting.

Exogenous 5-HT (300 ng/mL-min; Sigma Chemical, St. Louis, MO) was administered intraduodenally during phase I at 10 min after the end of phase III activity, in which intraduodenal concentration/output of 5-HT was low values in comparison with the phase II/III activity in the gastric antrum, and infused constantly during at least 2 MMC cycles to examine whether exogenous 5-HT stimulated gastroduodenal motility.
Measurement for PEG concentrations

PEG concentrations were measured by a spectrophotometer (DU-650, Beckman Coulter, Fullerton, CA) according to the method of Hydén (Hydén, 1955). In brief, 0.25 mL of duodenal sample was added 4.75 mL of 154 mM NaCl, 1.0 mL 10% BaCl₂, 2.0 mL 0.3 N Ba(OH)₂, and 2.0 mL 5% ZnSO₄·7H₂O. The mixture was vortexed and filtered using Whatman 44 filter paper (Whatman International, Maidstone, UK). The PEG concentration of each filtrate was determined at 650 nm by the spectrophotometer after the addition of 30% trichloroacetic acid (Wako Pure Chemical, Osaka, Japan).

Measurement for 5-HT concentrations

One mL of duodenal aspirate was immediately placed into test tubes containing 100 µL of 10% EDTA and 5% L-ascorbic acid. Then, 1.0 mL perchloric acid (HClO₄) and NaHCO₃ were added and centrifuged at 3,000 rpm for 20 min at 4°C. The supernatant was filtered through a 0.45-µm nylon filter (Ultrafree-MC, Millipore Co., Bedford, MA), and the samples were subjected to high-performance liquid chromatography (HPLC; Eicom, Kyoto, Japan).

HPLC system and procedure

The mobile phase for HPLC contained the following components in 1 liter of deionized water with a conductivity of 17 MΩ (Milli-Q System, Japan Millipore, Tokyo, Japan) and was run at a flow rate of 1 mL/min; 84% of 1 M citric acid, 0.1 M sodium acetate, 16% methanol, 160 mg/L of sodium 1-octanesulfonate and 5 mg/L EDTA. The HPLC system consisted of degasser, liquid chromatography, circulation type handy cooler and electrochemical detector. The detector was set at +650 mV vs. Ag-AgCl at 25°C. The chromatography column, MA-50DS (4.6 mm × 150 mm), was obtained from Eicom, Kyoto, Japan.

Analysis of data

Gastroduodenal contractile activity was identified by visual inspection. Criteria of each phase of the gastric and duodenal MMC was described by Code and Marlett (Code and Marlett, 1975). Phase I period with no contractile activity was easily identified. Phase II activity showed an intermittent irregular contractions. Phase III was defined as a burst of contractions with an amplitude >75% of the maximum amplitude of contractions observed during the interdigestive state. It is difficult to differentiate phase II from phase III in the duodenum; therefore, the MMC activity in the antrum was used instead of the duodenal MMC. Output of 5-HT for 5 min was calculated in each dog by multiplying the 5-HT concentration by duodenal volume at 5-min intervals.

\[
\text{5-HT output into the duodenum (ng)} = \text{5-HT concentration (ng/mL) } \times \text{duodenal volume (mL)}
\]

Statistical methods

Experiments were repeated at least twice in each dog. Mean values in each dog were obtained, and grand means were calculated across all six dogs. All results were expressed as
mean ± SEM, and were analyzed by analysis of variance followed by Fisher’s protected least-squares difference test. Differences between groups were considered significant at P<0.05.

**Results**

Figure 1 shows spontaneous changes in intraluminal 5-HT concentrations and output in the duodenum during the interdigestive state in a conscious dog. Luminal 5-HT concentrations in the duodenum varied cyclically in close temporal association with gastroduodenal motor activity and peaked during phase II activity in the stomach and duodenum. This finding was consistent in all six dogs. The mean 5-HT concentrations during phase II activity were significantly higher than those in phase I or III activity as shown in Table 1. The mean total output of 5-HT into the duodenum for 5 min also changed cyclically as shown in Fig. 1. Duodenal volume during phase III activity was significantly higher than that during phase I or II, the mean 5-HT output during phase III activity, therefore, became a similar value to that during phase II (Table 1).
After the administration of exogenous plasma motilin, phase III was initiated prematurely in the stomach and duodenum. Intraluminal concentrations of 5-HT in the duodenum also increased, according to the increase of the amplitude of gastroduodenal contractions (Fig. 2). The mean 5-HT concentrations during motilin-induced phase II activity were significantly higher than that during phase I. Duodenal volume during motilin-induced phase III activity was significantly higher than that during phase I or II, the mean 5-HT output during phase II or III activity, therefore, was significantly higher in comparison with that during phase I period (Table 1).

Fig. 2. Effect of exogenous motilin on intraduodenal 5-HT concentrations and output in a fasted dog. Intravenous administration of canine motilin (0.3 µg/kg-hr) induced phase III contractions in the stomach and duodenum and enhanced intraduodenal 5-HT concentrations and output. Time intervals of the upper and lower figures are same.

Fig. 3. Effect of intraduodenal administration of 5-HT on gastroduodenal motility. Intraduodenal administration of 5-HT (300 ng/mL-min) did not affect the pattern of the interdigestive motor activity. Time intervals of the upper and lower figures are same.
Intraduodenal infusion of exogenous 5-HT, at a dose of 300 ng/mL-min, did not alter the normal pattern of interdigestive motility as shown in Fig. 3. During this infusion, duodenal volume varied cyclically with the MMC with the peak value during the spontaneous phase III activity, and intraluminal concentrations of 5-HT displayed constantly high values. Neither the duration of MMC cycle nor the period of each phase was changed significantly by the intraluminal administration of 5-HT (Table 2).

Table 2  Effect of intraduodenal administration of 5-HT (300 ng/mL-min) on MMC cycle

<table>
<thead>
<tr>
<th></th>
<th>MMC cycle</th>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous (min)</td>
<td>92.5 ± 2.7</td>
<td>62.5 ± 3.2</td>
<td>11.3 ± 1.6</td>
<td>18.7 ± 1.6</td>
</tr>
<tr>
<td>5-HT (min)</td>
<td>88.9 ± 2.2</td>
<td>63.1 ± 3.1</td>
<td>9.2 ± 1.7</td>
<td>16.6 ± 1.9</td>
</tr>
</tbody>
</table>

Values were mean ± SEM, n=6 dogs; no statistical differences noted between groups.

Intraduodenal infusion of exogenous 5-HT, at a dose of 300 ng/mL-min, did not alter the normal pattern of interdigestive motility as shown in Fig. 3. During this infusion, duodenal volume varied cyclically with the MMC with the peak value during the spontaneous phase III activity, and intraluminal concentrations of 5-HT displayed constantly high values. Neither the duration of MMC cycle nor the period of each phase was changed significantly by the intraluminal administration of 5-HT (Table 2).

Discussion

Although the release of 5-HT into the small intestine during the fasting state has been previously described, the relationship of intraduodenal 5-HT with various phases of the gastroduodenal MMC is unclear. In the present study, we verified the release of 5-HT into the intact duodenum during the fasting state in conscious dogs and showed that 5-HT concentrations in the duodenum fluctuated cyclically in close temporal association with the phases of gastroduodenal MMC. Intraduodenal output of 5-HT increased during phases II and III, peaking during phase II. In addition, exogenous plasma motilin (0.3 µg/kg-hr) initiated a premature gastroduodenal phase II/III contractile activity during spontaneous phase I. Similar to the release of the spontaneous intraduodenal 5-HT, intraduodenal 5-HT levels also varied during motilin-induced phases II and III activity, peaking during motilin-induced phase II. Fluctuation of the duodenal volume after the administration of intravenous exogenous motilin or intraduodenal exogenous 5-HT was similar to changes in spontaneous duodenal volume. Moreover, infusion of 5-HT (300 ng/mL-min) into the duodenum did not alter spontaneous motor patterns.

Gastrointestinal tract in most mammals such as humans, dogs and rats has two different motor patterns; interdigestive and digestive contractile patterns. A cyclic motor pattern is observed during fasting state, and two factors stand out as possible candidates. The role of the vagal nerves in initiating and regulating this cyclic contractile activity, especially in the stomach, has been investigated. However, the relationship between vagal nerves and the MMC remains controversial (Hall et al., 1982; Gleysteen et al., 1985). The role of plasma motilin in initiation of the gastric MMC has been also demonstrated. Exogenous plasma motilin induced premature phase III contractions in the stomach (Itoh et al., 1975), plasma motilin concentrations varied cyclically in close association with gastroduodenal motor activity (Itoh et al., 1978) and motilin antiserum inhibited the initiation of spontaneous phase III activity in the stomach (Lee et al., 1983). After removal of the duodenum, which is the primary source of motilin in the gut, no regular, high amplitude contractions were seen (Tanaka et al., 1988). These data suggest that
motilin is necessary for the initiation of phase III contractions in the stomach. 5-HT is also considered as an important mediator of interdigestive motility. 5-HT is present in enterochromaffin (EC) cells in the epithelium (Penttilä, 1967) as well as in nerves of the myenteric and submucosal plexuses (Gershon et al., 1971). Previous studies have evaluated the role of endogenous 5-HT in regulation of the canine gastric MMC. Our laboratory (Itoh et al., 1991; Yoshida et al., 1991; Haga et al., 1996) has reported that intravenous concentrations of 5-HT cycled with phases of the gastric MMC and that endogenous 5-HT played an important role in the initiation of spontaneous phase III activity in the stomach; pCPA, the 5-HT synthesis inhibitor, suppressed initiation of spontaneous phase III activity in the stomach. In addition, the intravenous administration of GR38032F, a 5-HT3 receptor antagonist, abolished phase III contractions in the stomach but not in the small intestine (Yoshida et al., 1991). Exogenous intravenous 5-HT initiated the phasic contractions in the small intestine as shown previously by Ormsbee et al. (1984), suggesting that the endogenous 5-HT may be necessary for initiation of the gastroduodenal MMC. The effect of intraluminal 5-HT on gastrointestinal motor activity, however, is less clear.

In 1958, Bülbring and Lin (Bülbring and Lin, 1958) demonstrated that intraluminal 5-HT stimulated peristalsis, however, the fasting motor pattern of gastrointestinal tract, e.g. the MMC, was as yet undefined. Szurszewski (1969) discovered the MMC, and more recently, four distinct phases during the fasting state were defined by Code and Marlett (1975). In addition, gastrointestinal contractile activity was divided visually into two categories, the interdigestive and digestive states, using a strain gauge force transducer method by Itoh et al. (1975). Since then, numerous investigators have studied hormones and motility in the gastrointestinal tract. Previous work by Ferrara et al. (1986) showed that there was no relationship between luminal 5-HT concentrations and changes in the MMC in small intestine isolated in a Thiry-Vella loop. In contrast, we measured the intraluminal 5-HT concentrations in the intact duodenum, and found that intraduodenal concentrations of 5-HT also cycled with phases of gastric MMC with the peak value in the phase II. Findings by Ferrara and colleagues are in contrast to our current study. Differences from the study by Farrara et al. may depend on the perfused sites and the neural continuity in myenteric and submucosal plexuses of the small intestine. Kellum et al. (1986) and Märtensson et al. (1986) reported that luminal 5-HT infusion initiated the motor activity in the isolated intestine, and they suggested that luminal 5-HT was associated with the initiation of phasic contractions. In contrast, it was also reported that high intraluminal pressure caused the increase in the luminal release of serotonin in duodenum in rats (Fujimiya et al., 1997). In the present study, we could not show that the intraduodenal administration of exogenous 5-HT altered interdigestive contractions in the stomach and duodenum. Our results differ from their results. Their raw chart showed the phasic contractile activity after the administration of intraluminal 5-HT, but this activity may not be the migrating motor complex. Possibilities for these differences may be in the sites perfused (jejunum vs. duodenum) as well as the method of intraluminal 5-HT infusion (isolated vs. intact) and the dosage. In addition, these differences may be associated with the role of 5-HT on gastrointestinal motor activity; Itoh et al. (1991) showed the contractile force of spontaneous phase III activity in duodenum was reduced in 65% after the administration of a 5-HT3 antagonist although gastric motor activity was completely
abolished. We do not know whether intraluminal 5-HT actually reaches structures of the myenteric plexus. Based on our data, however, we are not able to suggest that the intraluminal 5-HT in duodenum is necessary for the regulation in the MMC.

Exogenous plasma motilin intravenously stimulated the luminal release of 5-HT into the duodenum, which is in agreement with the result of Kellum et al. (1986), who reported that 5-HT is released by intraarterial infusion of motilin in an isolated canine intestine. They have also shown that the intraluminal administration of 5-HT induced phasic contractions similar to the motilin-induced contractions, suggesting that luminal 5-HT is responsible for the occurrence of motilin-induced contractions in the dog. Moreover, using the isolated canine perfused stomach, Haga et al. (1996) showed that 5,6-DHT, a specific 5-HT neurotoxin, abolished the occurrence of the contractions induced by intravenous motilin and pCPA inhibited initiation of motilin-induced phase III contractions in the stomach in conscious dogs, suggesting that 5-HT neurons in the myenteric plexus, but not in EC cells in the mucosa, may be responsible for motilin-induced contractions during fasting. In our study, luminal concentrations of 5-HT increase when gastroduodenal phase II and III activity was initiated by exogenous motilin. However, we do not know whether plasma concentrations of motilin vary when exogenous 5-HT is given intraduodenally.

In conclusion, endogenous 5-HT is released cyclically into the duodenal lumen in close temporal association with changes in interdigestive migrating contractions in the stomach and duodenum. Exogenous plasma motilin stimulated the release of 5-HT into the duodenum. However, intraluminal infusion of 5-HT may not play a role in regulating interdigestive contractile activity. Its physiological significance remains unknown, and to elucidate and answer this question, the further study is required.

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References


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