Effects of Substance P on Gastric Motility Differ Depending on the Sites and Vagal Innervation in Conscious Dogs

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SHIBATA, C., SASAKI, I., NAITO, H., OHTANI, N., MATSUNO, S., MIZUMOTO, A., IWANAGA, Y. and ITOH, Z. Effects of Substance P on Gastric Motility Differ Depending on the Sites and Vagal Innervation in Conscious Dogs. Tohoku J. Exp. Med., 1994, 174 (2), 119-128 — The effect of substance P on gastric motility was studied in conscious dogs by means of strain gauge force transducers chronically implanted on the gastric body, antrum, and a vagally-denervated fundic pouch. Intravenous infusion of substance P in the interdigestive state induced phasic contractions in the pouch and antrum. Atropine inhibited these contractions in the pouch and antrum. Hexamethonium enhanced substance P-induced contractions in the gastric antrum, but reduced those in the pouch. Pretreatment with phentolamine, propranolol, or naloxone did not affect substance P-induced contractions in the pouch and antrum. The intact gastric body scarcely reacted to substance P. Mean systemic blood pressure was lowered by substance P-infusion, but there was no dose-dependency in the reduction of the blood pressure, nor was it affected by the pretreatment with atropine or hexamethonium. These results suggest that 1) the vagal innervation influences the effect of substance P on motility in the gastric body, and that 2) substance P may stimulate postsynaptic excitatory cholinergic and presynaptic inhibitory neurons simultaneously in the gastric antrum. —— strain gauge force transducer; substance P; vagal nerve; vagally-denervated fundic pouch

According to the results of immunohistochemical studies on the canine stomach, substance P-like immunoreactivity exists in all layers of the stomach and forms a dense network, especially in the myenteric plexus and circular muscle layer (Nilsson and Brodin 1977; Brodin and Nilsson 1981; Tange 1983). Another
study has proposed the possibility that the substance P-containing spinal afferents that project to the gastric submucosa are an important component of the gastric sensory innervation (Sharkey et al. 1984).

In studies in vitro, substance P induced tetrodotoxin-insensitive contractions in muscle strips from the stomach, and sensitivity to substance P decreased in the sequence of fundus, corpus, and antrum (Milenov and Golenhofen 1983). It has also been reported that substance P stimulates smooth muscles and excitatory cholinergic neurons in canine gastric antrum in vitro (Mayer et al. 1986). Fox et al. (1983) have shown that, in anesthetized dogs, intra-arterial injection of substance P induced contractions in the gastric body by stimulating cholinergic nerves, and tetrodotoxin- and atropine-insensitive nerves. Others suggested that the contractile response in the gastric antrum evoked by substance P was completely abolished by atropine in anesthetized cats (Edin et al. 1980). Furthermore, Delbro et al. (1983) reported that the gastric contractions induced by electrical stimulation of the feline vagal nerve after blockade of the ganglionic transmission by hexamethonium was reduced by specific substance P antagonist. Thus, the effect and action mechanism of substance P on gastric motility differ depending on the experimental designs or species.

However, the influence of the gastric sites and vagal innervation on the effect of substance P is not well understood. In the present study, we have studied the effect of substance P on motility in the gastric body, vagally-denervated fundic pouch, and gastric antrum with or without antagonists in order to clarify this issue in conscious dogs.

**Materials and Methods**

**Effect of substance P on gastric motility**

Nine healthy mongrel dogs of both sexes (10–15 kg) were used. The dogs were anesthetized by an intravenous injection of pentobarbital sodium (Nembutal, Abbott, North Chicago, IL, USA) at a dose of 30 mg/kg body weight, and access to the abdominal cavity was obtained by a midline incision. In five of the nine dogs, two strain gauge force transducers were sutured onto the serosal surface of the gastric body and antrum in order to measure the contractile activity of the circular muscle. The transducer on the gastric body was implanted on the greater curvature of the stomach opposite the splenic hilus; the antral transducer was placed 3 cm proximal to the gastroduodenal junction. In four of the nine dogs, a vagally denervated fundic pouch (Heidenhain type) was constructed (Itoh et al. 1978). The pouch was cannulated and gastric secretion was drained out spontaneously. In dogs with Heidenhain pouches, another transducer was implanted on the dorsal surface of the pouch, and the transducer on the gastric body was sutured on the ventral surface of the main stomach near the midportion of the cut end from which the gastric fundic pouch was resected. A Silastic tube (602–205; Dow Corning Co., Midland, MI, USA) was chronically placed from the right external
jugular vein into the superior vena cava for the purpose of intravenous infusion of test materials. The tube and the lead wires from the transducers were pulled out from a stab wound made between scapulae through subcutaneous tunnels and the lead wires were connected to a small connector. The tube and the small connector were covered with a jacket to protect them against the dog's scratching. Ten days were allowed as a recovery period. The dogs were housed in individual cages and fed Gaines Meal (Ajinomoto-General Foods Corp., Tokyo) of 20 g dry weight/kg body weight once a day and given water freely. Contractile activity was monitored continuously by connecting the lead cables from an amplifier (UG-5; Nihon Kohden Kohgyo Co., Tokyo) to the small connector under the protective jacket, and was recorded on a multichannel pen-writing recorder (WI-681G; Nihon Kohden Kohgyo Co.). All experiments were conducted during the quiescent period in the interdigestive state. Substance P was first dissolved in distilled water, then diluted with saline to the required concentration (40, 80, 120, 150, 200, 300 ng/kg-min) and was infused intravenously for exactly 10 min through the Silastic tube by means of an infusion pump (STC-520, Terumo Co., Tokyo). Generally, the substance P-induced contractile response disappeared immediately with the cessation of the infusion. At least a 20 min interval separated the two substance P-infusions, and it was found that the contractile responses induced by substance P-infusion at the same dose 20 min apart were similar to each other. To study the effect of antagonists, they were given 5 min prior to the initiation of substance P-infusion (150 ng/kg-min). The following drugs were intravenously injected as antagonists: atropine (0.05 mg/kg+0.05 mg/kg-hr), hexamethonium (3 mg/kg+7 mg/kg-hr), naloxone (0.5 mg/kg+1.0 mg/kg-hr), phentolamine (0.5 mg/kg+0.5 mg/kg-hr), propranolol (0.5 mg/kg+0.5 mg/kg-hr). The same experiments were repeated three times in each dog. Substance P was infused at most three times per day per dog, and if an antagonist was used, the dog was not given any drug on the same day.

Effect of substance P on mean systemic blood pressure

Five mongrel dogs (9-14 kg) were used. They were anesthetized by an infusion of pentobarbital sodium (Nembutal, Abbott) at a dose of 30 mg/kg body weight, and all experiments on systemic blood pressure were carried out under anesthesia. The right femoral artery was cannulated (16 G Venula, Top Co. Ltd., Tokyo), and the proximal end of the catheter was connected to the amplifier (AP-601G; Nihon Kohden Kohgyo Co., Tokyo), and mean systemic blood pressure was recorded on a pen-writing recorder (Desk Top Recorder; Unique Co., Tokyo). After a 30 min equilibration period, substance P at a dose of 40, 150, 300 ng/kg-min, 150 ng/kg-min + atropine (0.05 mg/kg+0.05 mg/kg-hr), or 150 ng/kg-min + hexamethonium (3 mg/kg+7 mg/kg-hr) was infused intravenously for exactly 10 min. At least a 20 min interval separated the two infusions, and if the blood pressure did not return to the pre-administration level after a 20 min
interval, subsequent infusion of substance P was postponed until the blood pressure recovered. The maximum reduction in the blood pressure was measured and analyzed.

**Drugs and peptides**

The following drugs were purchased: substance P and naloxone hydrochloride from Sigma Chemical Co., St. Louis, MO, USA, phentolamine from Ciba-Geigy Ltd., Basal, Switzerland and propranolol from Sumitomo Pharmaceuticals, Osaka. The following drugs were kindly donated: atropine sulfate by Tanabe Pharmaceutical Co. Ltd., Osaka and hexamethonium by Yamanouchi Pharmaceutical Co. Ltd., Tokyo.

**Data analysis**

In the gastric antrum and the pouch, the mean amplitude was analyzed by visual inspection and expressed as a percentage of the peak amplitude of the interdigestive phase III contractions. Frequency was analyzed by counting the total number of the phasic contractions during substance P-infusion and expressed as /min. All data were expressed as the mean±s.e. One way analysis of

![Diagram](image)

**Fig. 1.** Effect of substance P on gastric motor activity (A), and effect of atropine on substance P-induced contractions (B).

A: When substance P alone (150 ng/kg-min) was given intravenously, phasic contractions were induced in the vagally-denervated fundic (Heidenhain type) pouch and antrum. No apparent response was obtained in the gastric body. Naturally occurring interdigestive phase III contractions can be seen in the left of this figure.

B: Pretreatment with atropine inhibited substance P-induced contractions in the pouch and antrum.
Substance P and Gastric Motility

The effects of Substance P on gastric motility were examined. ANOVA and paired-t test were used to examine dose-dependency and to investigate the effect of antagonists, respectively. Statistical significance was set at the $p<0.05$ level throughout all experiments.

**RESULTS**

*Characteristics of contractile response to substance P in the stomach*

All experiments were done during the quiescent period in the interdigestive state. In the pouch and antrum, phasic contractions were induced by substance P. The intact gastric body scarcely reacted to substance P. The overall contractile pattern induced by substance P was different from that of naturally occurring interdigestive phase III contractions in the pouch and antrum (Fig. 1A). Both in the pouch and antrum, the mean amplitude of substance P-induced contractions increased in a dose-dependent fashion (Fig. 2A). There was no dose-dependency in the frequency of substance P-induced contractions in the pouch and antrum (Fig. 2B).

*Effect of antagonists on substance P-induced gastric contractions*

Pretreatment with atropine inhibited substance P-induced contractions both in the pouch and antrum (Fig. 1B); the effect of atropine in decreasing the mean

![Fig. 2. Dose-amplitude (A) and dose-frequency (B) relationship of substance P-induced contractions in the pouch (●) and the antrum (○).](image)

A: Mean amplitude of substance P-induced contractions increased in a dose-dependent manner in the pouch and antrum. *$p<0.05$, vs. 40 ng/kg-min (ANOVA).*

B: There was no dose-dependency in the frequency of substance P-induced contractions in the pouch or antrum.
amplitude was significant in the two sites (Fig. 3). In the pouch, the frequency of substance P-induced contractions during atropine-infusion could not be measured because phasic components were not clearly identified (Fig. 1B). The frequency of substance P-induced contractions in the antrum was significantly depressed by atropine (Fig. 4). Substance P-induced contractions in the pouch were inhibited by the pretreatment with hexamethonium (Fig. 5); the mean amplitude of substance P-induced contractions in the pouch significantly decreased (Fig. 3). The frequency of substance P-induced contractions in the pouch was not able to be measured because of the abolishment of the phasic contractions (Fig. 5). In contrast, in the antrum, substance P-induced contractions were enhanced by hexamethonium (Fig. 5); the mean amplitude was significantly increased (Fig. 3), but the frequency was not modified (Fig. 4). Naloxone, phentolamine, or propranolol did not affect the amplitude of substance P-induced contractions either in the antrum or the pouch (Fig. 3). Also the frequency of substance P-induced contractions was not affected by these antagonists in the antrum (Fig. 4) or the pouch (data not shown).

![Figure 3. Effect of antagonists on the mean amplitude of substance P-induced contractions in the pouch (■) and antrum (○). Atropine significantly reduced the mean amplitude of the substance P-induced contractions both in the pouch and antrum. Hexamethonium significantly reduced the mean amplitude of the substance P-evoked contractions in the pouch, but significantly enhanced that in the antrum. Neither phentolamine, propranolol, nor naloxone had any effect on the mean amplitude of substance P-induced contractions in the pouch or antrum. *p < 0.05 vs. control (paired-t test). †p < 0.05 vs. control (paired-t test).](image)
Effect of substance P on the mean systemic blood pressure

Rapid and profound decrease in the mean systemic blood pressure was observed within 2 min after the start of the infusion. After this initial profound decrease, the mean systemic blood pressure increased gradually but did not return to the pre-administration level until the end of the infusion. The initial profound reduction in the mean systemic blood pressure did not increase in a dose-dependent manner, nor was it affected by the pretreatment with atropine or hexamethonium (Table 1).

DISCUSSION

Although the intact gastric body scarcely reacted to substance P, phasic
contractions were induced by substance P in the vagally-denervated fundic pouch, and the mean amplitude of these contractions was significantly depressed by atropine and hexamethonium. In the gastric antrum, substance P evoked phasic contractions which were inhibited by atropine but enhanced by hexamethonium. These results suggest that the site of action of substance P is different in the gastric body and in the antrum. Because the reactivity of the gastric body to substance P apparently appeared after the construction of the pouch, it is possible that the excitatory and inhibitory effect of substance P may be almost equivalent in the intact gastric body. It is also likely that the sensitivity to the drug is increased in the pouch; this speculation is supported by the fact that the spontaneous phasic contractions occurred in the vagally-denervated fundic pouch in the quiescent period of the interdigestive state (Itoh et al. 1978).

Significant inhibition of substance P-induced antral contractions by atropine, and significant enhancement of these by hexamethonium may indicate that substance P acts on the postsynaptic excitatory cholinergic and the presynaptic inhibitory neurons simultaneously. The excitatory effect must exceed the inhibitory effect in the antrum, because administration of substance P alone induced phasic contractions. Blockade of muscarinic receptors by atropine brings about the reduction in the excitatory effect of substance P. But when ganglionic transmission is blocked by hexamethonium, the effect of substance P on the presynaptic inhibitory neurons disappears. As a result, substance P-induced contractions are inhibited by atropine, and enhanced by hexamethonium. The enhancing effect of hexamethonium on substance P-induced contractions in the canine colon in the conscious state and in the anesthetized state was previously reported by Hou et al. (1989). They postulated the possibility that substance P acts on the presynaptic inhibitory neurons in the colon. The results of the present study suggest the existence of the similar action site of substance P in the canine gastric antrum as in the canine colon.

The effect of substance P in lowering the mean systemic blood pressure was

<table>
<thead>
<tr>
<th>Dose of substance P (ng/kg-min)</th>
<th>40</th>
<th>150</th>
<th>300</th>
<th>150 + Atropine</th>
<th>150 + Hexamethonium</th>
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<tr>
<td></td>
<td>26.7±5.2</td>
<td>30.1±4.8</td>
<td>27.8±3.0</td>
<td>25.6±3.7</td>
<td>32.3±5.3</td>
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Values express the reduction in the mean systemic blood pressure from the pre-administration state. No dose-dependent response was identified, and pretreatment with atropine (0.05 mg/kg + 0.05 mg/kg-hr) or hexamethonium (3 mg/kg + 7 mg/kg-hr) did not affect the reduction in the mean systemic blood pressure.
not dose-dependent, and pretreatment with atropine or hexamethonium had no further effect compared to the effect obtained by the administration of substance P alone. Therefore, the inhibitory or enhancing effect of these antagonists was considered not to be related to the changes in the mean systemic blood pressure.

In the present study, it was confirmed that substance P acts mainly on the neurons, and no myotropic action was observed in conscious dogs. Previous in vitro studies on canine gastric circular muscle strips showed that substance P elicited tetrodotoxin-resistant contractions in the gastric body and the antrum, suggesting the myotropic action of substance P (Milenov and Golenhofen 1983; Mayer et al. 1986). A similar discrepancy between in vivo and in vitro reactions, in the same site of the same species, has already been reported concerning the other peptides, such as motilin and cholecystokinin (Mack and Todd; 1968; Fox et al. 1983; Fisher et al. 1985; Marzio et al. 1985). To solve this problem, an experimental model using the isolated vagally-denervated whole stomach (Mizumoto et al. 1993) may give us key findings. We could not indicate how substance P as a transmitter of thin afferent nerves is involved in the regulation of gastric motility, since we focused on the gastric motor event induced by intravenously injected substance P. Experiments with capsaicin, a sensory neurotoxin, will be required to clarify the role of the afferent nerve fibers that release substance P as a transmitter in substance P-induced gastric contractions.

References


