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EFFECTS OF GASTROINTESTINAL HORMONES AND THEIR RELATED COMPOUNDS ON GASTRIC MOTILITY IN THE RAT

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We examined the effects of certain gastrointestinal hormones on gastric motility using rat stomach preparatios *in vivo*. Changes of water level caused by the movement of the stomach which was filled with saline were recorded. Single injections of cholecystokinin (1, 2 and 4 μg/kg) induced relaxation of the stomach. Single injections of bombesin in low doses (below 0.2 μg/kg) induced relaxation and in high doses (over 0.2 μg/kg) contraction after brief relaxation. Single injections of neurotensin (1, 2, 4 and 8 μg/kg), somatostatin (5, 10 and 20 μg/kg) and substance P (1, 2, 4 and 8 μg/kg) induced relaxation followed by contraction, but their dose-response relations were obscure. Infusions of neurotensin (1, 5 and 25 μg/kg/h) and somatostatin (2.5 and 5 μg/kg/h) enhanced the stomach tension, whereas substance P (1, 5 and 25 μg/kg/h) reduced it. Single injections and infusions of neurotensin, somatostatin or substance P showed different effects on gastric motility. On the other hand, Met-enkephalin (1, 10 and 100 μg/kg) and porcine motilin (1, 10 and 100 μg/kg) did not affect gastric motility in our rat stomach preparations. These results suggest that some gastrointestinal hormones take part in stomach movements.

Keywords —gastric motility; cholecystokinin; bombesin; somatostatin; neurotensin; substance P; perfused rat stomach

As ingested food is mixed with secreted gastric juice and passed to the duodenum, the functions of the stomach might be conceived as having three subdivisions: gastric secretion, including acid, pepsin and mucus; motility, including contraction and relaxation, and gastric emptying. From this viewpoint, we intend to totally evaluate the effects of chemicals on the stomach. There are many reports which deal with gastric motility, but few are concerned with the motility caused by gastrointestinal hormones *in vivo*. Many factors are involved in gastrointestinal diseases, one of which must be the disorder of the gastric motility. However, how the disorder of stomach motility causes diseases is still obscure. For this reason, it is very important to clarify the action of chemicals which are related to gastrointestinal hormones on gastric motility.

Several kinds of hormones are found in the gastrointestinal tract. These hormones may affect secretion, motility or other functions. Moreover, they are found in the brain, which suggests that they also have the properties of neurotransmitters. Roles of some hormones in the gastrointestinal movement were not clearly understood, even though they were known to exist in the smooth muscle layer in high concentrations.

In our previous paper, we reported the development of a simultaneous measurement of gastric motility and acid secretion, in which we demonstrated the effects of pentagastrin, histamine, carbamylcholine and catecholamines on gastric motility and secretion. However, it is difficult to explain all functions of the stomach by studying these chemicals only, although they must play very important roles. In as much as the actions of chemicals which are given *in vitro* are often different from those *in vivo*, in the present work we examined the effects of some gastrointestinal hormones on gastric motility using

in vivo rat stomach preparation.

METHOD AND MATERIALS

Measurement of Gastric Motility — A method of simultaneous measurement of gastric motility and acid secretion was described previously.[1] Briefly, we used the method which was a modification of the perfused rat stomach: Wistar male rats weighing about 200 g were fasted for a day and anesthetized under urethane. A glass tube (5 mm, diameter) joined to a drain inserted into the stomach through the pylorus stood vertically and a small float which was made of a plastic tube was placed into the glass tube. After stopping the outlet, the stomach was filled with saline through the esophagus, and the saline allowed to flow into the glass tube. Usually, about 9 ml of saline was filled in so as to make a load to the stomach, but sometimes it was adjusted depending upon the size of the stomach. Motility of the stomach was recorded as the change of the float level by an isotonic transducer and calculated to the volume change of the stomach. Saline was exchanged every 20 min, and chemicals were administered through the femoral vein within 0.5 ml in a single injection and 2 ml/kg/h in infusion.

Materials— Chemically synthesized sulfated octapeptide, cholecystokinin; cyclic tetradecapeptide somatostatin; undecapeptide, substance P; tridecapeptide, neurotensin; tetradecapeptide, bombesin and pentapeptide, Met-enkephalin were purchased from Peptide Institute, Inc. (Osaka, Japan). Porcine motilin was purchased

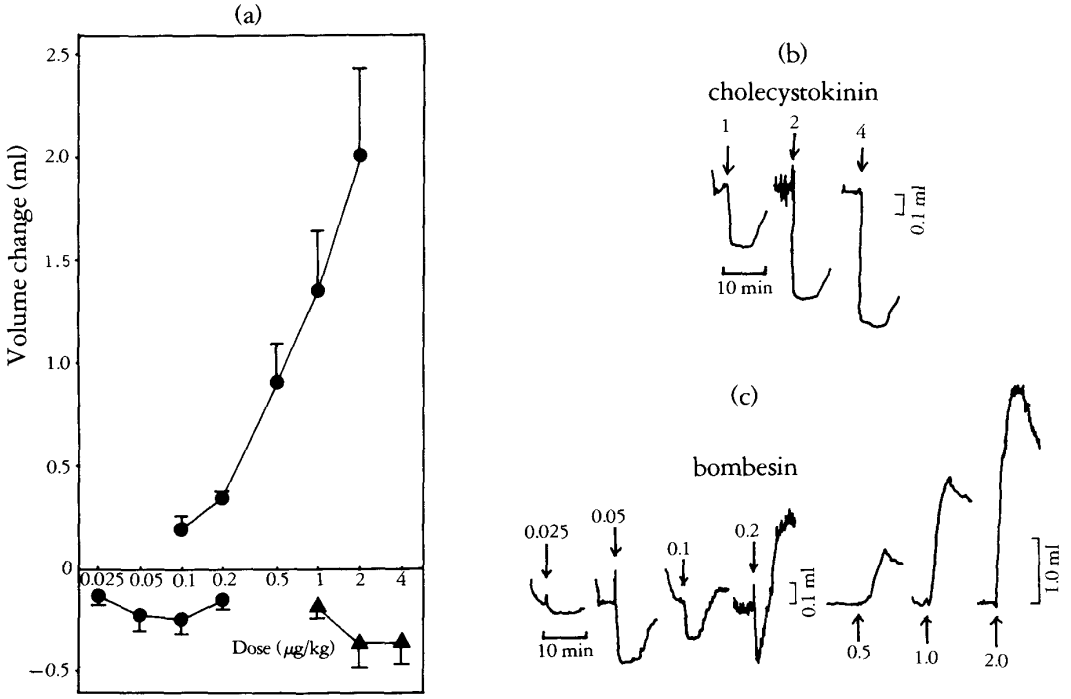

FIG. 1. *Dose-Response Curves of Cholecystokinin- and Bombesin-Induced Relaxations and Contractions (a), and Typical Profiles of Gastric Motility Induced by Cholecystokinin (b) and Bombesin (C)*

Cholecystokinin, low doses of bombesin (0.025, 0.05, 0.1 and 0.2 μg/kg) and high doses of it (0.5, 1 and 2 μg/kg) were injected through the femoral vein every 80 min, respectively. A plus value in the volume change represents a contraction, and a minus value represents a relaxation. Symbols are cholecystokinin-induced relaxation; (▲) and bombesin-induced relaxation and contraction; (●). Values are mean ± S.E. from 6 rats in cholecystokinin and 5 rats in bombesin administration, respectively.

from Peninsula Laboratories (San Carlos, U.S.A.). Freshly desolved chemicals were used for injections.

RESULTS

Effects of Single Injections of Cholecystokinin and Bombesin on Gastric Motility

Peptides were administratered by *i.v.* injections at 80 min intervals. Gastric motility was recorded for about 15 min; for 5 min before injection as a basal level and for 10 min after injection. Cholecystokinin (1, 2 and 4 μg/kg) induced relaxation of the stomach. As shown in Fig. 1b, cholecystokinin strongly relaxed the stomach for more than 10 min. Bombesin showed a biphasic effect on the stomach motility; low doses of bombesin (below 0.2 μg/kg) induced relaxation, but higher doses of it (over 0.2 μg/kg) induced contractions in a dose-dependent manner. The induced volume changes in relaxation below 0.2 μg/kg and over 0.1 μg/kg were shown in Fig. 1a. Sometimes the bombesin-induced contractions appeared after brief relaxation, and higher doses shortened the time to contractions. Higher doses of bombesin often caused urination at the same time.

Effects of Single Injections of Neurotensin, Somatostatin, Substance P, Met-Enkephalin and Porcine Motilin on Gastric Motility

Neurotensin (1, 2, 4 and 8 μg/kg), somatostatin (2.5, 5, 10 and 20 μg/kg) and substance P (1, 2, 4 and 8 μg/kg) induced relaxation followed by contractions of the stomach. These peptides caused both contraction and relaxation, but sometimes only contraction occured after substance P or somatostatin. The peak values of contraction and/or relaxation from the basal level within 10 min are summarized in Table I. Typical profiles of gastric motility induced by neurotensin, somatostatin and substance P are shown in Fig. 2a–c. The change from relaxation to contraction took a few minutes to occur after *i.v.* administrations of the peptides. Met-enkephalin (1, 10 and 100 μg/kg) and porcine motilin (1, 10 and 100 μg/kg) did not affect gastric motility in our rat preparations.

Effects of Infusions of Somatostatin, Neurotinsin and Substance P on Gastric Motility

Peptides were infused for 60 min at 100 min intervals. When the infusion of chemicals was started or stopped, gastric motility was recorded for about 15 min; for 5 min before infusion as

TABLE I. *Effects of Single Injections of Neurotensin, Substance P, Somatostatin, Met-Enkephalin and Porcine Motilin on Rat Gastric Motility in Vivo*

	Neurotensin (μg/kg)				Substance P (μg/kg)			
	1	2	4	8	1	2	4	8
Contraction[a)]	0.09 ±0.03	0.08 ±0.05	0.08 ±0.04	0.08 ±0.04	0.10 ±0.03	0.21 ±0.04	0.19 ±0.05	0.18 ±0.06
Relaxation[b)]	−0.13 ±0.05	−0.10 ±0.04	−0.15 ±0.06	−0.17 ±0.10	−0.08 ±0.03	−0.22 ±0.07	−0.22 ±0.04	−0.18 ±0.04

	Somatostatin (μg/kg)				Met-Enkephalin (μg/kg)			Porcine motilin (μg/kg)		
	2.5	5	10	20	1	10	100	1	10	100
Contraction[a)]	0.09 ±0.03	0.24 ±0.06	0.26 ±0.05	0.28 ±0.07				0	0	0
Relaxation[b)]					0	0	0			

Values are mean ± S.E. from 6 rats in neurotensin, 5 rats in substance P and somatostatin, and 3 rats in Met-enkephalin and porcine motilin, respectively. a) The volume of saline (ml) which was overflowed from the stomach. b) The volume of saline (ml) which was flowed into the stomach.

basal level and for 10 min after starting infusion, or for 5 min under infusion as induced level and for 10 min after stopping infusion. Somatostatin infusion (2.5 and 5 μg/kg/h) enhanced the tension of the stomach. After starting infusion of somatostatin, contractions of the stomach appeared immediately, and they disappeared after termination of infusion (Fig. 3b). Dose-dependent contractions were observed in a small range around 5 μg/kg/h (Fig. 3a).

Higher doses of neurotensin (5 and 25 μg/kg/h) enhanced the tension of the stomach, but a low dose (1 μg/kg/h) induced weak relaxation (Fig. 4a, b). Responses of the stomach to neurotensin were slow and weak, but dose-dependent.

Infusion of substance P (1, 5 and 25 μg/kg/h) induced relaxation of the stomach in a dose-dependent manner (Fig. 5a). As shown in Fig. 5b, responses of the stomach to substance P were rapid. Substance P-induced relaxation became gradually greater during infusion, and returned to the basal level by stopping infusion. Changes to the basal level by stopping infusion were dose-dependent.

Clear relaxation was not observed with infu-

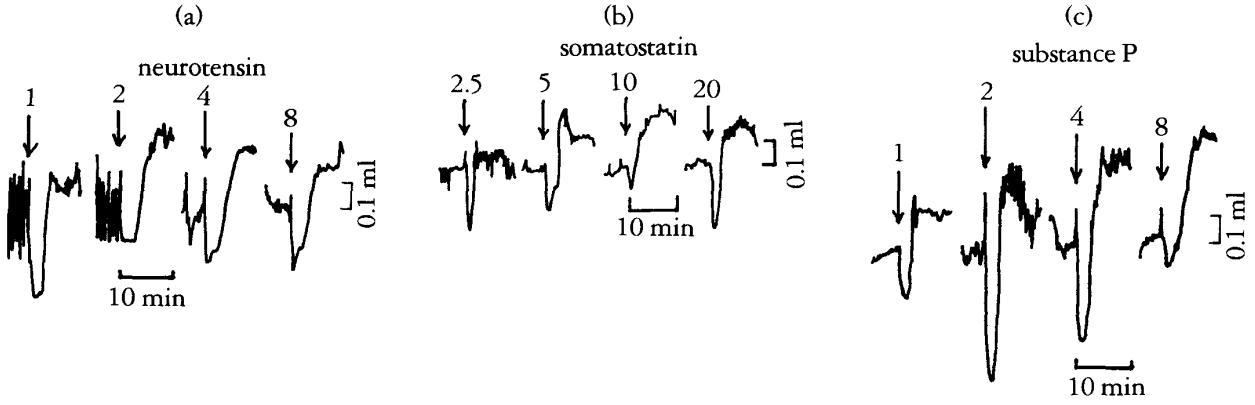

FIG. 2. *Typical Profiles of Gastric Motility Induced by Single Injections of Neurotensin (a), Somatostatin (b) and Substance P (c)*
The chemicals were administered through the femoral vein every 80 min.

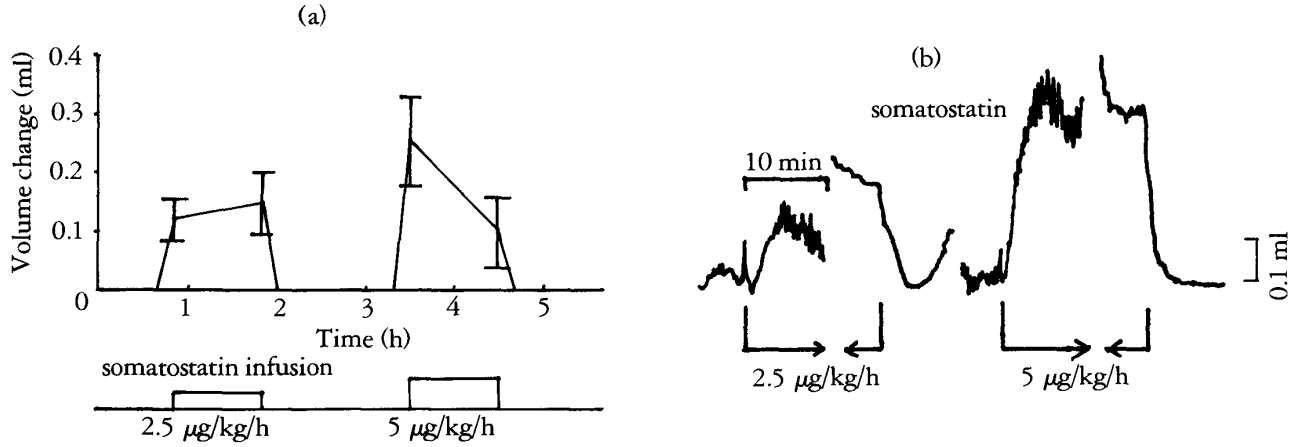

FIG. 3. *Somatostatin-Induced Gastric Motility (a) and Typical Profile of Gastric Motility (b)*
Somatostatin (2.5 and 5 μg/kg/h) was infused through the femoral vein during indicated periods. Values are mean ± S.E. from 6 rats.

sion of somatostatin or neurotensin.

DISCUSSION

Gastrointestinal hormones were found to be distributed not only in the gastrointestinal tract but also in some organs, such as the brain,[2,3] the lung[4,5] and other tissues.[6] Concentrations of the hormones varied in different parts and tissues of the gastrointestinal tract. A high concentration of immunoreactive cholecystokinin was found in the mucosa of the duodenum and the jejunum, but the concentration was low in the smooth muscle of the same organs.[7] Immunoreactive bombesin was detected in longitudinal and circular smooth muscle extracts throughout the small intestine.[7] Substance P containing nerve fibers was found in smooth muscle of the guinea-pig taenia coli.[8] The existence of gastrointestinal hormones in the muscle layer suggests that they may participate in the movement of the gut.

Marked effects were seen in single injections of cholecystokinin and bombesin in our experiments. Cholecystokinin is known to induce contraction of not only the gallbladder but also the small intestine of guinea-pigs and cats *in vitro,*[9-11] and relaxation of the antrum of cats *in vivo.*[12] In our previous paper, we reported that pentagastrin induced relaxation of the stomach *in vivo,*[1] which was accompanied by tachyphylaxis. Due to the similar peptide suquences to pentagastrin, cholecystokinin also induced relaxation. Bombesin which was isolated from the skin of an

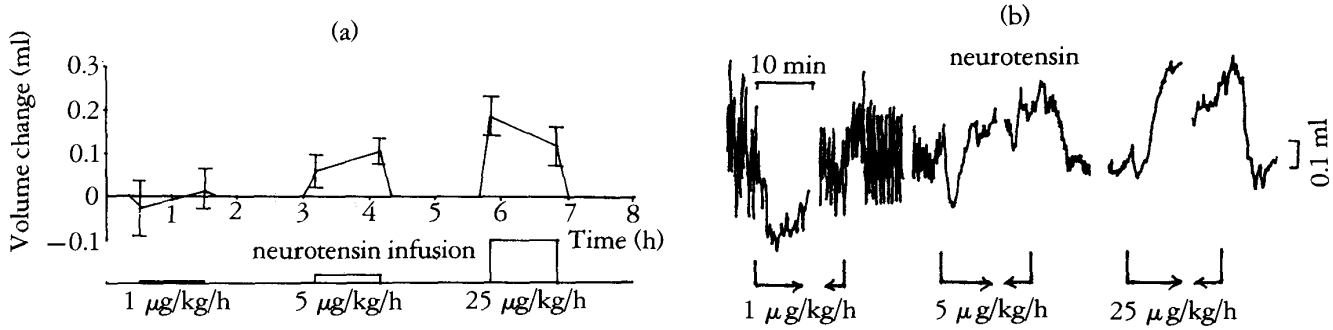

FIG. 4. *Neurotensin-Induced Gastric Motility (a) and Typical Profile of Gastric Motility (b) Neurotensin (1, 5 and 25 μg/kg/h) was infused during indicated periods. Values are mean ± S.E. from 6 rats.*

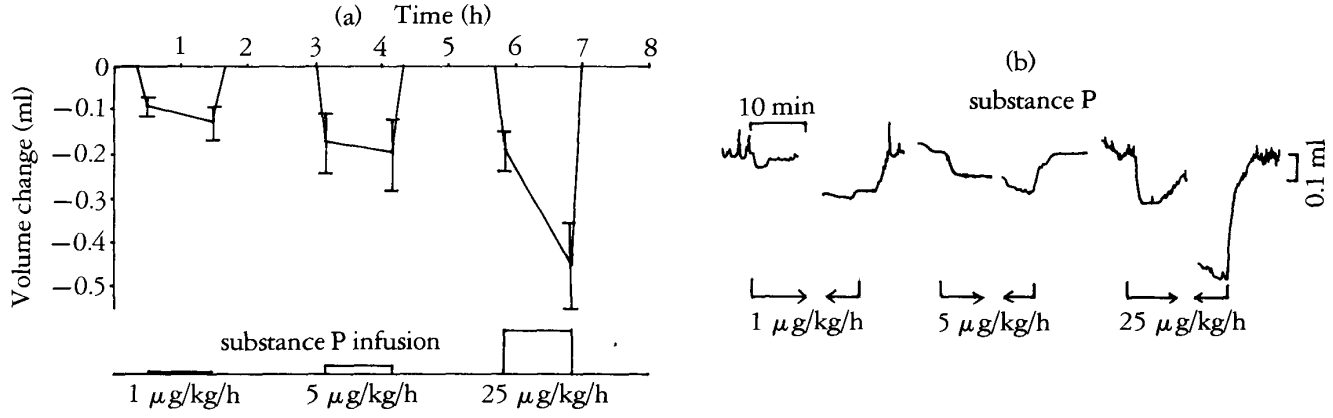

FIG. 5. *Substance P-Induced Gastric Motility (a) and Typical Profile of Gastric Motility (b) Substance P (1, 5 and 25 μg/kg/h) was infused during indicated periods. Values are mean ± S.E. from 5 rats.*

amphibian, *Bombina bombina,* induced relaxation in a low dose and contraction in a high dose. Vagne *et al.*[13] showed that bombesin first induced a low-amplitude contraction followed by a high-amplitude contraction in the cat. We could not compare our results with theirs due to the use of different methods and animals. Such biphasic stomach movement was observed in the response of the lower oesophageal and pyloric spincters and circular smooth muscle strips of the guinea-pig stomach to catecholamines *in vitro.*[14-16] However, we could not determine which muscle contracted or relaxed and what concentration of the chemical reached the stomach from the results of the *in vivo* rat stomach preparation which we used. Since the gastrointestinal tract contracts and relaxes alternatively in a physiological condition, biphasic movement itself may be significant. Bombesin-induced contraction is 150 times as strong as carbamylcholine-induced contraction using the same molar base calculated from our previous work. Common peptide sequences are found in bombesin and gastrin releasing peptide, so that gastrin releasing peptide may physiologically control the movement of the stomach. This is supported by the existence of immunoreactive bombesin in the smooth muscle of the stomach.[17]

Different effects were observed in single injection and infusion experiments neurotensin, substance P and somatostatin. Single injections of neurotensin, substance P or somatostatin induced relaxation followed by contraction after 2—5 min, but infusion of neurotensin or somatostatin induced contraction and substance P induced relaxation. Motor activities of substance P and neurotensin were previously described in other papers. Substance P, which is known to be assayed by the guinea-pig ileum, induced contraction of: the cat stomach,[18] the colon[19] *in vivo,* the rat urinary bladder and the guinea-pig stomach *in vitro,*[20-22] and neurotensin, induced contraction of: the cat colon *in vivo*[19] and the urinary bladder *in vitro*[20] and induced relaxation of the guinea-pig colon *in vitro.*[23] McLean and Fox[24] obtained similar results, in which biphasic canine gastric corpus motility was induced by neurotensin *in vitro.* However, there are few reports that show somatostatin influences a motor function of the gut,[21,25] in which it induced relaxation of the isolated guinea-pig ileum.[25] Somatostatin is known as a growth hormone release inhibitor and an inhibitory peptide of gastric acid secretion. However, we found a remarkable motor effect of somatostatin on the stomach. Additional *in vivo* and *in vitro* experiments are necessary to clarify the mechanism of action of somatostatin. The difference between the effects of infusions and those of single injections, that was also observed for neurotensin, might be due to the concentration of the chemical in the blood reaching to the stomach.

McIntosh and Brown[26] reported the marked effect of motilin on the fundic pouch and Bueno *et al.*[21] showed electrical spiking activity of the antrum of the pig and the dog. However, we could not demonstrate the effect of porcine motilin on the rat stomach motor activity. We also could not demonstrate the effect of Met-enkephalin in our experiments, even if it has a similar activity to morphine which is known as an inhibitor of the intestinal motor activity.

In this paper, we have demonstrated that some gastrointestinal hormones act on the stomach smooth muscle or modify the motor function of the stomach. These results suggest that there may be a regulatory mechanism of the stomach motor activity by a gut peptide hormone other than cholinergic or adrenergic nervous system, as Bauer and Kuriyama[27,28] reported the existence of non-cholinergic, non-adrenergic transmission in the guinea-pig ileum. However, we could not determine whether the peptides used act on the stomach humorally or nervously in a physiological condition. Histochemical determination of these peptides in the gastrointestinal smooth muscle may explain their motor activities.

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