Biochemical Pharmacology, Vol. 31, No. 21, pp. 3475–3481, 1982.
Printed in Great Britain.

0006-2952/82/213475–07 $03.00/0

EFFECTS OF PENTAGASTRIN, HISTAMINE, CARBAMYLCHOLINE AND CATECHOLAMINES ON GASTRIC SECRETION, MOTILITY AND EMPTYING IN THE RAT

SATORU TANI* and NORIO MUTO
Faculty of Pharmaceutical Sciences, Josai University, Saitama-ken 350-02, Japan

(*Received* 23 *September* 1981; *accepted* 5 *April* 1982)

Abstract—We developed a simplified method for the simultaneous measurement of gastric secretion and gastric motility in the rat. By using this method, we investigated the actions of pentagastrin, histamine, carbamylcholine and catecholamines on the stomach. Carbamylcholine stimulated acid secretion and induced contraction of the stomach, but norepinephrine tended to inhibit acid secretion and induced relaxation of the stomach. Histamine induced relaxation first and then contraction after 2–5 min. Pentagastrin induced a relaxation that did not show dose dependence, but tachyphylaxis. Isoproterenol, which stimulated acid secretion in our experiments, induced relaxation of the stomach in a dose-dependent manner. We also investigated the relationship between gastric motility and gastric emptying. Carbamylcholine caused an enhancement of gastric emptying, but isoproterenol caused its suppression. Pentagastrin, histamine and norepinephrine did not affect gastric emptying. As shown in the results of our experiments, carbamylcholine caused stimulation of acid secretion, contraction of the stomach, and enhancement of gastric emptying. However, other secretagogues did not always induce contraction of the stomach or increase gastric emptying.

Gastric acid secretion is dually controlled by hormones; gastrin stimulates, and secretin and cholecystokinin inhibit, autacoids; histamine stimulates and prostaglandins inhibit; and acetylcholine stimulates and norepinephrine inhibits [1]. Most of these substances which affect gastric secretion are also related to smooth muscle motility [2–4]. Many reports describe gastric motility [5–9], but few of them [9] mention the relation of gastric secretion to motility and emptying.

In this study, at first we developed a simplified method which could measure gastric secretion and gastric motility simultaneously. By using this method, we investigated the actions of pentagastrin, histamine, carbamylcholine and catecholamines on the stomach. Furthermore, the effects of these substances on gastric emptying of test meal were examined. The roles of gastrin, histamine, and neurotransmitters in the function of the stomach are discussed.

METHODS AND MATERIALS

Measurements of gastric acid secretion and gastric motility. Male Wistar rats weighing about 200 g were starved for 1 day and used for measurements of gastric acid secretion and gastric motility. The perfused rat stomach [10] was modified in the following ways: the stomach was filled with about 9 ml of neutralized saline, which made the load to the stomach about 1 ml of saline, and the saline was changed every 20 min instead of perfused, as shown in Fig. 1. Gastric motility was directly converted to the change of water level and recorded on a kymograph. The acidity of the 20-min saline that was exchanged was estimated by titration with 0.02 N NaOH to pH 7.0.

Measurement of gastric emptying. Gastric emptying was measured by a modification of the method described by Droppleman *et al.* [11]; male Wistar rats weighing about 200 g were starved for 1 day and, then, were given 1 ml of test meal containing 0.01% quinaldine red by stomach tube; a test compound was administered simultaneously by subcutaneous injection while the animal was conscious. The rats were killed after a period of time. A laparotomy was performed; the stomach was quickly ligated at the pylorus and the cardia, and removed. The contents of each stomach were washed with ethanol (10 ml final volume) and centrifuged. A clear, red ethanol extract was obtained by this procedure and was measured by a colorimeter at 530 nm. The results from coprophagic rats were omitted, since coprophagy vitiated the colorimetry and gastric emptying. The percentage of test meal that remained after gastric emptying was calculated according to the following formula:

$$\text{Test meal remaining (\%)} = \frac{\text{amount of quinaldine red recovered from the stomach} \times 100}{\text{amount of quinaldine red recovered from 1 ml of test meal}}$$

* Author to whom correspondence should be addressed.

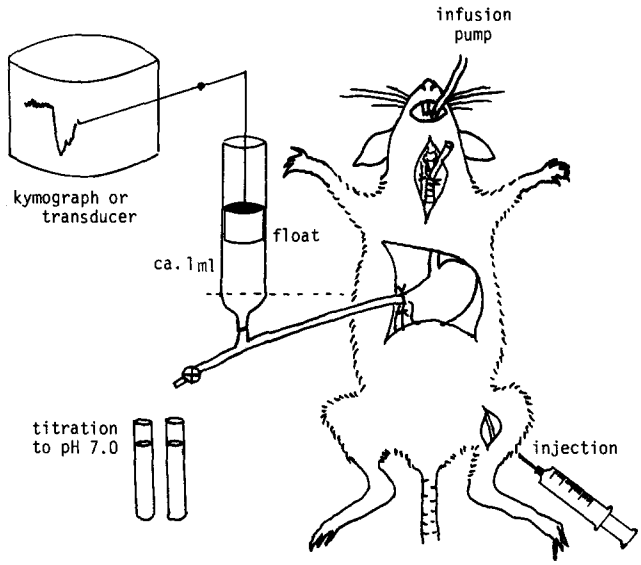


Fig. 1. Schematic diagram of the measurement of gastric secretion and gastric motility. A glass tube (about 5 mm diameter), joined to a drain which was inserted in the stomach from the pylorus, stood vertically, and a small float that was made of a plastic tube was put into the glass tube. Saline was placed in the stomach, by an infusion pump, and flowed into the glass tube. The volume of saline in the stomach was set at about 9 ml, which made the load to the stomach 1 ml.

Student's *t*-test was used to determine the significance of differences.

Materials. *t*-Butyloxycarbonyl-β-Ala-Trp-Met-Asp-Phe-NH_2 (pentagastrin) was purchased from Sumitomo Chemical, Osaka. *l*-Norepinephrine bitartrate, *dl*-isoproterenol hydrochloride, carbamylcholine chloride (Sigma Chemical Co., Saint Louis, MO), histamine dihydrochloride (Wako Pure Chemical Industries, Osaka), quinaldine red (for indicator) and diphenhydramine hydrochloride (Tokyo Kasei, Tokyo) were used. Fluid test meal [12] which contains all nutrients was purchased from Oriental Yeast, Tokyo. Metiamide was a gift from Smith, Kline & Fujisawa, Tokyo.

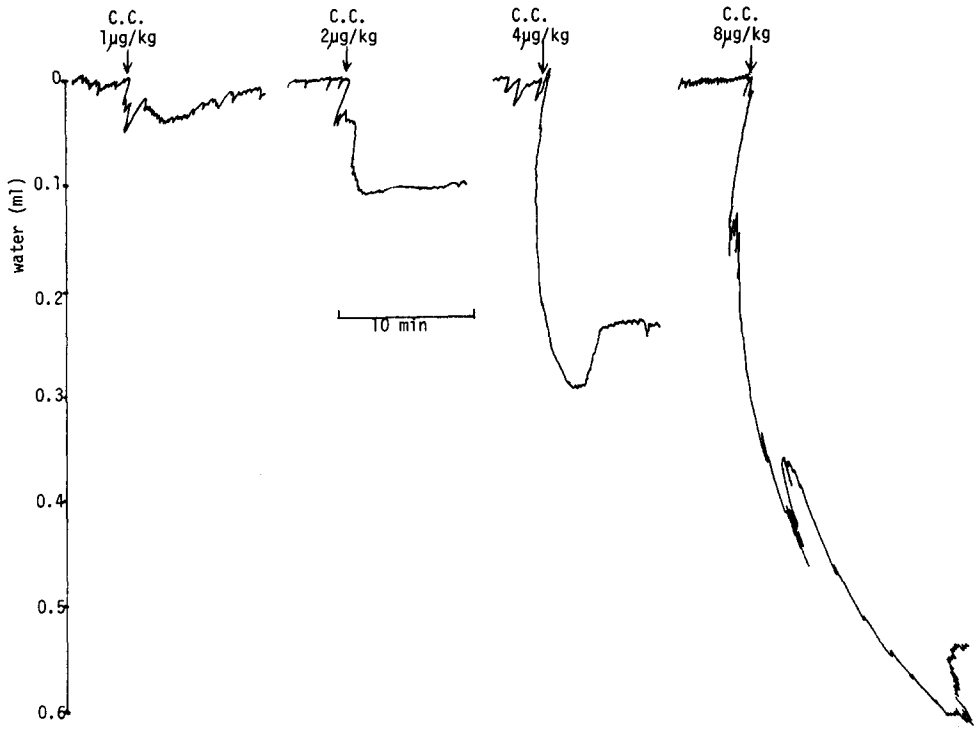
RESULTS

Contraction of the stomach by carbamylcholine. Carbamylcholine induced strong and immediate contraction of the stomach as well as stimulation of gastric acid secretion. Figure 2a shows a typical profile of gastric motility induced by carbamylcholine. Contraction of the stomach was shown as a downward movement on a kymograph, because saline in the stomach flowed out by contraction, as is shown in Fig. 1. Contraction of the stomach, which was expressed as ml of outflow, and secretion of gastric acid were stimulated by carbamylcholine in a dose-dependent manner, although carbamylcholine at a dose of 8 μg/kg induced strong contraction (Fig. 2b).

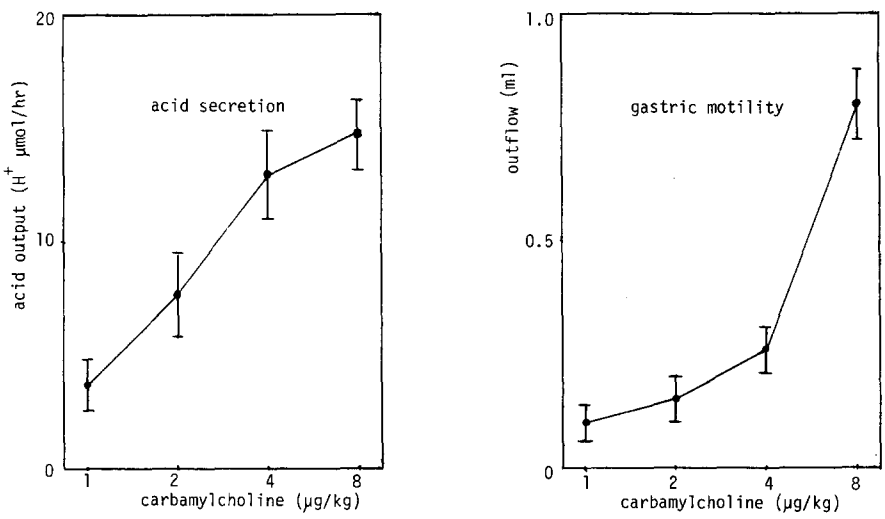
Relaxation of the stomach by catecholamines. Isoproterenol induced relaxation of the stomach immediately. The relaxation reached a maximum response within 1 min after the injection and lasted more than 10 min (Fig. 3a). Isoproterenol stimulated gastric acid secretion and induced relaxation of the stomach in a dose-dependent manner, although norepinephrine tended to inhibit gastric acid secretion at a high dose and induced relaxation of the stomach (Fig. 3b). Relaxation of the stomach caused the influx of saline into the stomach so that it was expressed as ml of water lowered from the control level in our experiments. Relaxation induced by isoproterenol was greater than that induced by norepinephrine.

Effects of diphenhydramine and metiamide on histamine-induced motility of the stomach. Histamine induced both relaxation and contraction of the stomach; histamine induced relaxation of the stomach immediately after the injection, or did not show any change, and then induced contraction after 2–5 min (Fig. 4a). Relaxation by histamine did not appear in all cases but in regard to contraction of the stomach, histamine induced it in a dose-dependent manner. Figure 4b shows the dose–response curves in gastric acid secretion and contraction of the stomach by histamine. Table 1 shows the effects of diphenhydramine and metiamide on histamine-induced gastric motility. The responses to the same dose of histamine before and after the infusion of an antagonist were compared. Diphenhydramine (5 mg per kg per hr) inhibited both histamine-induced relaxation and contraction, but did not inhibit acid secretion. Metiamide (2 mg per kg per hr), which inhibited acid secretion, reduced histamine-induced relaxation of the stomach to 89.2%, but there were some cases in which metiamide enhanced, or did not affect, it.

Tachyphylaxis in pentagastrin-induced relaxation of the stomach. Pentagastrin induced relaxation of the stomach, but tachyphylaxis developed. When the same dose of pentagastrin (5 μg/kg) was administered three times at 80-min intervals, the relaxation response of the stomach was smaller after the third



(a)



(b)

Fig. 2. (a) Typical profile of the stomach contraction induced by carbamylcholine. Carbamylcholine (C.C.) (1–8 μg/kg) was administered intravenously every 80 min at arrows. Each response of the stomach was recorded from 5 min before to 10 min after the administration of carbamylcholine. (b) Dose–response curves of the effects of carbamylcholine on gastric acid secretion and gastric motility. The contraction of the stomach is expressed as the volume (ml) of outflow at the maximum response to the indicated dose. Values are means ± S.E. of five experiments.

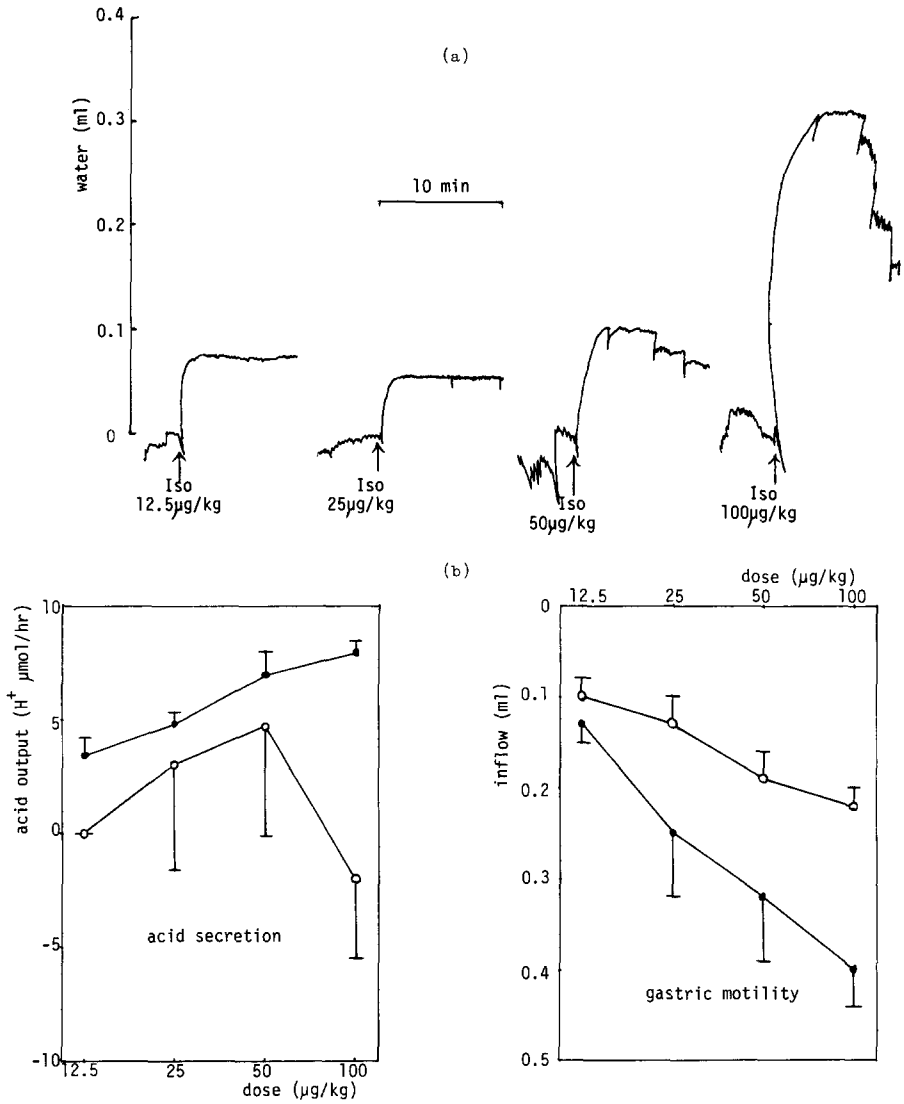


Fig. 3. (a) Typical profile of stomach relaxation induced by isoproterenol. Isoproterenol (Iso) (12.5–100 μg/kg) was administered intravenously every 80 min at arrows. (b) Dose–response curves of the effects of isoproterenol (●) and norepinephrine (○) on gastric acid secretion and gastric motility. The relaxation of the stomach is expressed as the volume (ml) of inflow at the maximum response to the indicated dose. Values are means ± S.E. of five experiments for isoproterenol and three experiments for norepinephrine.

Table 1. Effects of diphenhydramine and metiamide on gastric response to histamine*

Treatment	% of control		
	Acid	Contraction	Relaxation
Diphenhydramine (5 mg/kg/hr) (5)	137.8 ± 22.1	23.4 ± 8.0	10.9 ± 5.2
Metiamide (2 mg/kg/hr) (8)	36.3 ± 5.4	148.3 ± 45.1	89.2 ± 43.0

* Histamine (0.8 mg/kg) was injected before (control) and after infusion of diphenhydramine or metiamide. The cases in which histamine did not induce relaxation of the stomach were omitted. Values are means ± S.E. of percent of control responses to histamine; the numbers of experiments are given in parentheses.

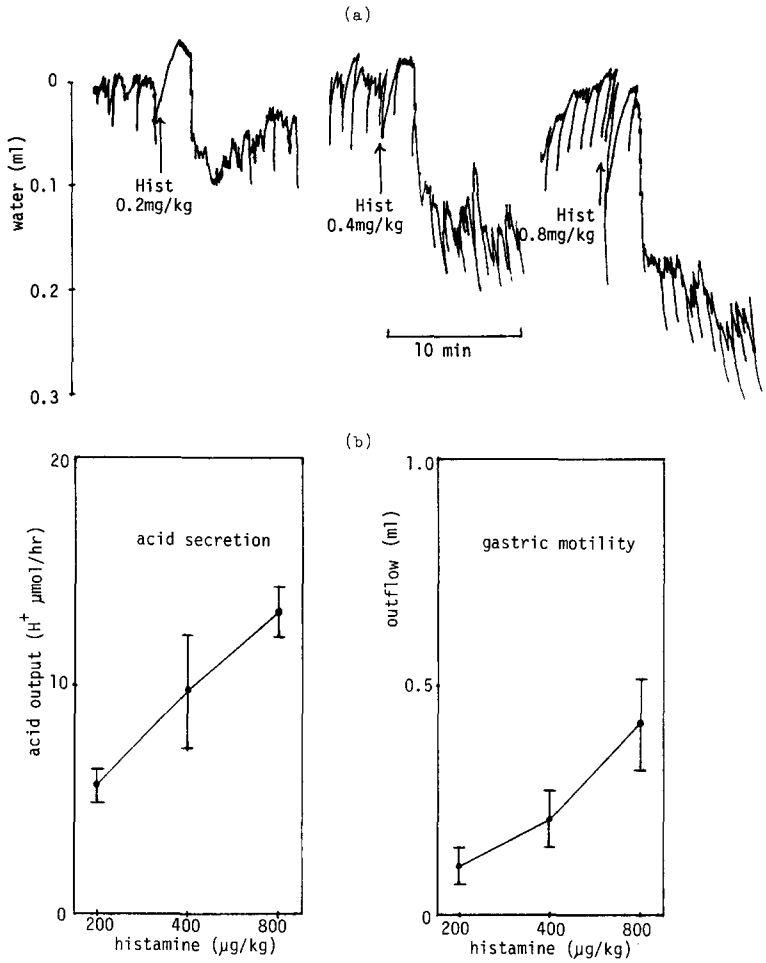


Fig. 4 (a) Typical profile of gastric motility induced by histamine. (b) Dose–response curves of the effects of histamine on gastric acid secretion and gastric motility. Values are means ± S.E. of five experiments.

Table 2. Effects of pentagastrin, histamine, carbamylcholine and catecholamines on gastric emptying in conscious rats*

Treatment	Dose (s.c.)	Test meal remaining (%)
Control (0.9% NaCl)		38.4 ± 4.7 (16)
Pentagastrin	10 μg/kg	41.4 ± 7.2 (5)
	20 μg/kg	31.7 ± 3.1 (6)
Carbamylcholine	10 μg/kg	34.0 ± 6.1 (7)
	20 μg/kg	21.8 ± 5.1† (8)
Histamine	1 mg/kg	33.3 ± 7.3 (5)
	2 mg/kg	42.4 ± 3.9 (5)
Norepinephrine	200 μg/kg	39.9 ± 6.8 (9)
	400 μg/kg	36.8 ± 9.0 (6)
Isoproterenol	200 μg/kg	64.5 ± 3.9‡ (9)
	400 μg/kg	70.0 ± 3.6‡ (6)

* Gastric contents were measured at 1 hr after giving test meal and administering a chemical. Values are means ± S.E.; the numbers of experiments are given in parentheses.

† Significantly different from control, $P < 0.05$.

‡ Significantly different from control, $P < 0.01$.

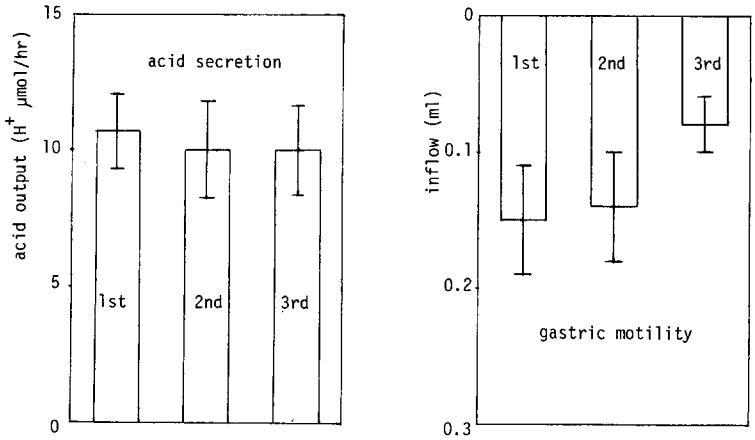


Fig. 5. Tachyphylaxis in relaxation of the stomach induced by pentagastrin. The same dose of pentagastrin (5 μg/kg) was administered three times at 80-min intervals; 1st, 2nd and 3rd in the columns represent the responses the first, second, and third times respectively. Values are means ± S.E. of five experiments.

dose, whereas acid secretion remained the same (Fig. 5). The third relaxation response to pentagastrin was about one-half of the first one. The relaxation induced by pentagastrin was smaller than that induced by catecholamines.

Effects of chemicals on gastric emptying. The time course of gastric emptying of test meal in conscious rats is shown in Fig. 6. The gastric contents were emptied in a time-dependent manner, and the half-life of the given test meal calculated in Fig. 6 was about 45 min. For the purpose of our experiments, we chose 1 hr as the time for the measurement of gastric emptying. Table 2 shows the effects of pentagastrin, carbamylcholine, histamine, and catecholamines on gastric emptying of test meal. Each value represents the percentage of test meal that remained in the stomach. Carbamylcholine enhanced, and isoproterenol suppressed, gastric emptying in a dose-dependent manner, significantly.

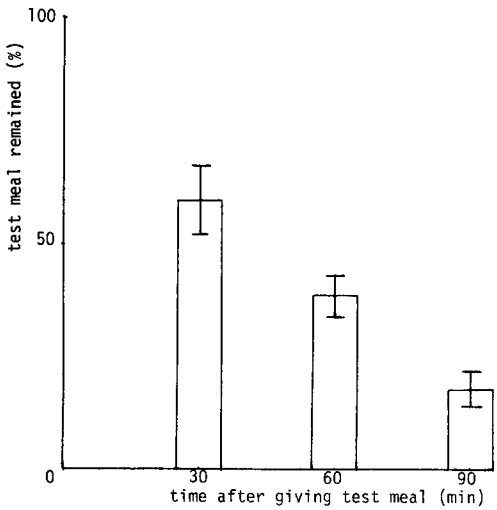


Fig. 6. Time course of gastric emptying of test meal in conscious rats. Values are means ± S.E. of six experiments for 30 and 90 min, and sixteen experiments for 60 min after test meal.

Pentagastrin, histamine and norepinephrine did not affect gastric emptying in our experiments.

DISCUSSION

The method that we used for measuring gastric motility in this work may be used quantitatively, with good control. However, extensibility of the stomach changes with time and conditions, especially when antagonists are administered. The relaxation response reached a limit with high doses of chemicals such as catecholamines. Relaxation of the stomach is restricted by the abdominal space and the weight of a load.

Carbamylcholine-induced contraction and isoproterenol-induced relaxation of the stomach in the rat have been well estimated by other methods [13, 14]; we obtained similar results. We found, however, that the response of the stomach to histamine was complicated. Since histamine induced contraction of the stomach after 2–5 min, it might have acted indirectly on the stomach or induced contraction as a result of an imbalance of relaxation and contraction. It has been said that histamine H_1-agonists induce contraction [15] and that histamine H_2-agonists induce relaxation [16] of stomach smooth muscle, so we examined the effects of diphenhydramine, a histamine H_1-antagonist, and of metiamide, a histamine H_2-antagonist, on histamine-induced gastric motility. Histamine may cause both relaxation and contraction by acting on histamine H_1-receptors, because diphenhydramine inhibited both. The effects of metiamide on relaxation of the stomach, however, will still obscure after our experiments, so we need more experiments to confirm our results. Papers have shown that pentagastrin induces contraction of stomach strips *in vitro* [17], contraction of stomach *in vivo* [18, 19], and relaxation of the stomach [20]. We have shown that pentagastrin induced relaxation of the stomach in our *in vivo* anesthetized rats. Differences in the effects on gastric motility of gastrin or gastrin-like peptides may be caused by differences in the species of animals or the methods used. The relaxation induced by pen-

Table 3. Summary of results obtained from our experiments*

	Acid secretion	Motility	Emptying
Carbamylcholine	++	++	++
Histamine	++	− and ++	±
Pentagastrin	++	−	±
Isoproterenol	+	−−	−−
Norepinephrine	(−)	−	±

* The plus signs (+) under gastric acid secretion, motility and emptying mean stimulation, contraction and enhancement respectively. The minus signs (−) under acid secretion, motility and emptying mean inhibition, relaxation and suppression respectively. The plus or minus sign (±) means no effect or an unclear effect. The minus sign in parentheses represents a result from our previous papers [1, 21].

tagastrin showed tachyphylaxis in our experiments, even when it was injected at 80-min intervals. This suggests that pentagastrin relaxation of the stomach is induced via a second mediator or that pentagastrin causes release of amines or related compounds. In the present paper, we have demonstrated that the effects of each secretagogue on the stomach are different; carbamylcholine induced contraction of the stomach, histamine induced relaxation followed by contraction, and pentagastrin induced relaxation. These results suggest that each secretagogue may stimulate gastric acid secretion by a different mechanism, as we have described in previous papers [1, 21].

Since disorders of physiologically active substances such as gastrin, histamine and neurotransmitters cause several kinds of diseases, we studied the relationship between these physiologically active substances and stomach function. Then, we examined the effects of these substances on gastric emptying in conscious rats. Subcutaneous administration of pentagastrin, histamine or norepinephrine caused neither an enhancement nor a suppression of gastric emptying, compared with control. On the other hand, carbamylcholine enhanced gastric emptying and isoproterenol suppressed it. It has been said that slow gastric emptying and normal gastric secretion, or rapid gastric emptying and hypersecretion, are commonly encountered in patients with gastric ulcers or duodenal ulcers respectively [22, 23]. This suggests that not only hypersecretion of gastric acid but also disorders of gastric motility, including emptying, should be evaluated as factors in ulcerogenesis.

Table 3 summarizes the effects on gastric secretion, motility and emptying time in our experiments. The effects of these substances on the stomach differ from one another: the substances that stimulated gastric acid secretion did not always induce contraction of the stomach or facilitate gastric emptying. It appears that histamine and pentagastrin take part mainly in stimulating acid secretion. The effects of isoproterenol (which to some extent represents epinephrine in living animals) on gastric secretion and emptying differed from those of norepinephrine. Taking this and other papers into consideration, it seems likely that a high plasma catecholamine concentration under certain conditions such as stress causes gastric ulcers and that excitation of cholinergic nerves causes not only gastric ulcers but also duodenal ulcers. However, the function of the stomach is affected by many factors in living animals, and so it is evident that more work, using different approaches, is necessary.

REFERENCES

1. S. Tani and N. Mutoh, *Biochem. Pharmac.* **28**, 678 (1979).
2. T. Mukai and K. Kubota, *Eur. J. Pharmac.* **65**, 157 (1980).
3. S. Fasth, S. Filipsson, L. Hultén and J. Martinson, *Experientia* **29**, 982 (1973).
4. C. Desvigne, M. L. Gelin, M. Vagne and M. Roche, *Digestion* **20**, 265 (1980).
5. J. Martinson, *Acta physiol. scand.* **65**, 300 (1965).
6. S. E. Vizi, G. Bertaccini, M. Impicciatore and J. Knoli, *Gastroenterology* **64**, 268 (1973).
7. J. M. Van Nueten, *Trends pharmac. Sci.* **1**, 233 (1980).
8. P. L. R. Andrews, D. Grundy and T. Scratcherd, *J. Physiol., Lond.* **298**, 79 (1980).
9. A. Dubois, P. V. Eerdewegh and J. D. Gardner, *J. clin. Invest.* **59**, 255 (1977).
10. M. N. Ghosh and H. O. Schild, *Br. J. Pharmac. Chemother.* **13**, 54 (1958).
11. D. A. Droppleman, R. L. Gregory and R. S. Aliphin, *J. pharmac. Meth.* **4**, 227 (1980).
12. C. S. Lieber and L. M. DeCarli, *Am. J. clin. Nutr.* **23**, 474 (1970).
13. K. Watanabe, *Chem. pharm. Bull., Tokyo* **14**, 101 (1966).
14. S. Ueda, *Folia pharmac. jap.* **67**, 529 (1971).
15. J. Black, W. A. M. Duncan, C. J. Durant, C. R. Ganellin and E. M. Parsons, *Nature, Lond.* **236**, 385 (1972).
16. Z. S. Ercan and R. K. Türker, *Pharmacology* **15**, 118 (1977).
17. E. Mikos and J. R. Vane, *Nature, Lond.* **214**, 105 (1967).
18. A. N. Smith and D. Hogg, *Lancet* **i**, 403 (1966).
19. J. J. Misiewicz, D. J. Holdstock and S. L. Waller, *Gut* **8**, 463 (1967).
20. N. Okike and K. A. Kelly, *Am. J. Physiol.* **232**, E504 (1977).
21. S. Tani, N. Muto and N. Kawata, *J. Pharmacobio-Dynamics* **3**, 507 (1980).
22. S. Harasawa, N. Tani, T. Nomiyama, R. Sakita, M. Miwa, S. Suzuki and T. Miwa, *J. jap. Soc. internal Med.* **68**, 733 (1978).
23. J. D. Deorge, *Gut* **9**, 237 (1968).