*Acta Medica Okayama*

*Volume* 39, *Issue* 6 1985 *Article* 2

DECEMBER 1985

# Effects of caerulein on the gastric motility of rats.

Ze-Zhong Jin* Toshiaki Neya†

Sosogu Nakayama‡

*Okayama University,

†Okayama University,

‡Okayama University,

# Effects of caerulein on the gastric motility of rats.*

Ze-Zhong Jin, Toshiaki Neya, and Sosogu Nakayama

## Abstract

The effects of caerulein on gastric motility in urethane-anesthetized rats were studied. Caerulein administered into the lateral cerebral ventricle (i.c.v.) and jugular vein (i.v.) caused predominantly an inhibitory effect on gastric motility but sometimes an excitatory or a biphasic effect. The inhibitory response was reduced after vagotomy and/or splanchnicotomy, or after guanethidine. The remaining inhibitory response was abolished by tetrodotoxin, but was resistant to atropine and guanethidine. The excitatory response was abolished by atropine. Discharges of the gastric branch of the vagus nerve were decreased by i.v. injection of caerulein but increased by i.c.v. injection, whereas those of the splanchnic nerve were increased by both i.v. and i.c.v. injection. These results suggest that caerulein causes an inhibition of gastric motility by centrally stimulating vagal non-adrenergic inhibitory nerves and splanchnic adrenergic nerves and inhibiting vagal cholinergic nerves, and by peripherally stimulating non-adrenergic inhibitory neurons of the myenteric plexus. This peptide causes an excitation by stimulating cholinergic neurons of the myenteric plexus.

**KEYWORDS:** caerulein, gastrointestinal hormones, gastric motility, autonomic nerves, myenteric plexus

---

*PMID: 4091039 [PubMed - indexed for MEDLINE]

Acta Med. Okayama **39,** (6), 431-440 (1985)

# EFFECTS OF CAERULEIN ON THE GASTRIC MOTILITY OF RATS

Ze-Zhong JIN*, Toshiaki NEYA and Sosogu NAKAYAMA

*Department of Physiology, Okayama University Medical School, Okayama 700, Japan*

*Received July 8, 1985*

*Abstract.* The effects of caerulein on gastric motility in urethane-anesthetized rats were studied. Caerulein administered into the lateral cerebral ventricle (i.c.v.) and jugular vein (i.v.) caused predominantly an inhibitory effect on gastric motility but sometimes an excitatory or a biphasic effect. The inhibitory response was reduced after vagotomy and/or splanchnicotomy, or after guanethidine. The remaining inhibitory response was abolished by tetrodotoxin, but was resistant to atropine and guanethidine. The excitatory response was abolished by atropine. Discharges of the gastric branch of the vagus nerve were decreased by i.v. injection of caerulein but increased by i.c.v. injection, whereas those of the splanchnic nerve were increased by both i.v. and i.c.v. injection. These results suggest that caerulein causes an inhibition of gastric motility by centrally stimulating vagal non-adrenergic inhibitory nerves and splanchnic adrenergic nerves and inhibiting vagal cholinergic nerves, and by peripherally stimulating non-adrenergic inhibitory neurons of the myenteric plexus. This peptide causes an excitation by stimulating cholinergic neurons of the myenteric plexus.

*Key words :* caerulein, gastrointestinal hormones, gastric motility, autonomic nerves, myenteric plexus.

Caerulein presents a striking similarity to cholecystokinin. The action of caerulein is mainly exerted on the gastrointestinal tract and biliary system (1). In dogs, caerulein causes contractions of the stomach and small intestine by acting on myenteric neurons, and a tonic contraction of the gallbladder and a relaxation of the sphincter of Oddi by acting on their smooth muscles (2-4). As for the site of action of caerulein, Neya *et al.* (5) showed that this peptide administered intravenously stimulates not only myenteric neurons peripherally but also sympathetic nerves centrally.

The aims of the present experiments are to study whether caerulein administered into the lateral cerebral ventricle exerts different effects on gastric motility than when administered into the jugular vein, and whether caerulein acts on both the myenteric plexus and central nervous systems.

* Present address: Department of Physiology, Yenbian Medical College, Yenki, China

## MATERIAL AND METHODS

Experiments were carried out in 68 rats, weighing 220 - 470 g, fasted for 24 h and anesthetized with urethane (0.8 g/kg, i.p.). The abdominal cavity was opened by midline incision. The luminal contents of the stomach were washed out with warmed Tyrode solution through a hole made in the *pars proventricularis* and then a 2-cm-long balloon was introduced into the stomach through the hole. The gastric balloon, which was connected to a pressure transducer, was distended with 1 - 1.5 ml of water. Intragastric pressure waves produced by gastric contractions were recorded on a pen-oscillograph.

To study whether caerulein effects were mediated by extrinsic nerves, vagus nerves were sectioned at the neck or subdiaphragmatic level and the greater and lesser splanchnic nerves were sectioned at a site central to the celiac ganglia.

The multiunits efferent discharges were recorded from the gastric branch of the vagus nerve running to the cardia along the ventral surface of the esophagus and the gastric branch of the splanchnic nerve arising from the left celiac ganglion and running to the stomach along the left gastric artery. The discharges were monitored using an oscilloscope and the frequency histogram of discharges was manifested by counting every pulse of neural discharges through the discriminator.

To determine the latency of a response, the time lag between the starting point of the drug injection and the onset of a mechanical or neural response was measured from the recordings. Statistical analysis was made by Student's *t*-test.

Caerulein dissolved in 0.9 % saline solution was administered into the lateral cerebral ventricle (i.c.v.) and external jugular vein (i.v.). To inject caerulein into the ventricle, the animal was fixed on a stereotaxic head holder. According to the stereotaxic atlas of the rat brain (6), the tip of the needle of a 10 $\mu$l microsyringe was inserted into the lateral ventricle 0.6 mm caudal to the bregma. Then, 5 $\mu$l of caerulein solution was injected over a 30-second period. In the intravenous administration of caerulein, 0.3 ml of solution was infused over a 1-min period.

Atropine sulphate (Merck), guanethidine sulphate (Tokyo-Kasei) and tetrodotoxin (Sigma) were injected into the external jugular vein. Caerulein was kindly given by Kyowa-Hakko, Tokyo.

## RESULTS

*Caerulein effects on the innervated stomach.* Caerulein administered through the i.c.v. or i.v. route produced an excitatory, an inhibitory or a biphasic effect on gastric motility, while 0.9 % saline solution had no effect. In individual rats, the response to caerulein was reproducible when the drug was repeatedly administered at intervals of 30 min. If the drug was administered at intervals shorter than 30 min, tachyphylaxis was observed. Therefore, caerulein was administered at intervals longer than 30 min.

The threshold dose of caerulein to produce an effect varied among individual animals, but a difference in the threshold dose between the routes of drug administration was not observed. In 8 rats studied to determine the threshold dose, it was in the range of 1 to 5 ng/kg.

Typical responses produced by caerulein at a dose of 20 ng/kg are shown in Fig. 1. Both i.c.v. and i.v. injection of caerulein produced a rapid fall in the basal

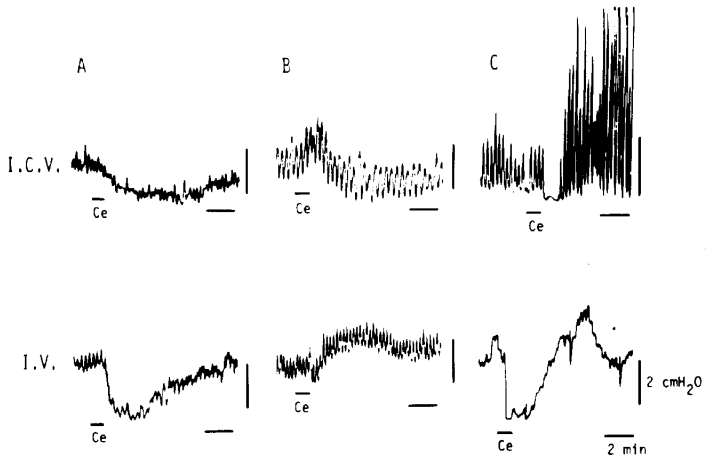


Fig. 1. Patterns of gastric responses induced by caerulein (Ce, 20 ng/kg) administered into the lateral cerebral ventricle (i.c.v.) and jugular vein (i.v.). A, B and C show excitatory, inhibitory and biphasic responses, respectively. The responses were obtained from 6 different rats.

TABLE 1. FREQUENCIES OF THE RESPONSES INDUCED BY CAERULEIN IN THE INNERVATED AND DENERVATED STOMACH

| Caerulein (ng/kg) | | Type of responses | | | | Total |
|---|---|---|---|---|---|---|
| | | Excitatory | Inhibitory | Biphasic | None | |
| Innervated stomach | | | | | | |
| ICV | 20 | 4 (15.4) | 16 (61.6) | 5 (19.2) | 1 ( 3.8) | 26 (100) |
| ICV | 40 | 1 ( 7.7) | 8 (61.5) | 4 (30.8) | 0 ( 0 ) | 13 (100) |
| IV | 20 | 7 (16.3) | 30 (69.8) | 5 (11.6) | 1 ( 2.3) | 43 (100) |
| Denervated stomach | | | | | | |
| ICV | 20 | 0 ( 0 ) | 9 (81.8) | 0 ( 0 ) | 2 (18.2) | 11 (100) |
| ICV | 40 | 4 (30.8) | 2 (15.3) | 4 (30.8) | 3 (23.1) | 13 (100) |
| IV | 5 | 0 ( 0 ) | 5 (83.3) | 0 ( 0 ) | 1 (16.7) | 6 (100) |
| IV | 20 | 8 (32.0) | 10 (40.0) | 2 ( 8.0) | 5 (20.0) | 25 (100) |

ICV: intra cerebral ventricular, IV: intravenously.
Percentages are given in parentheses.
The results were obtained from 47 aminals.

tone and a decrease in amplitude of rhythmic contractions (inhibitory response). However, the same dose of the drug caused an increase in the basal tone with or without an increase in amplitude of contractions (excitatory response) and an

TABLE 2. THE LATENCIES AND DURATIONS OF THE RESPONSES PRODUCED BY CAERULEIN IN THE INNERVATED AND DENERVATED STOMACH

| Caerulein (ng/kg) | | Response | Latency* (sec) | Duration* (sec) | n |
|---|---|---|---|---|---|
| Innervated stomach | | | | | |
| ICV | 20 | Inhibitory | 58.4 ± 4.5 | 720 ± 105 | 15 |
| IV | 20 | Inhibitory | 44.0 ± 2.0 | 257 ± 19 | 29 |
| Denervated stomach | | | | | |
| ICV | 20 | Inhibitory | 97.7 ± 11.4 | 618 ± 64 | 7 |
| IV | 20 | Inhibitory and Excitatory | 86.1 ± 10.4 | 437 ± 76 | 14 |

* Mean ± SE. ICV, IV ; See Table 1.

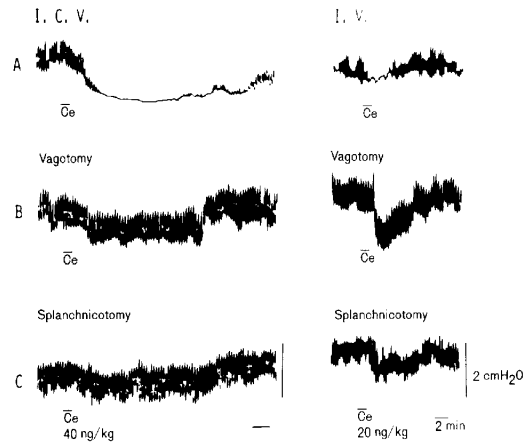


Fig. 2. Effects of vagotomy (B) and subsequent splanchnicotomy (C) on gastric inhibition induced by caerulein (Ce) administered through i.c.v. (40 ng/kg) and i.v. (20 ng/kg) routes. A, Control response. The results were obtained from 2 different rats.

inhibition followed by an excitation (biphasic response). The predominant response was inhibitory in both i.c.v. and i.v. administrations (Table 1), and the mean latency and duration of the response to i.c.v. injection were longer than those to i.v. injection (Table 2). The differences were significant in latency ($p<0.001$) and in duration ($p<0.001$).

The inhibitory effect of caerulein (20 ng/kg) injected either i.c.v. or i.v. on the gastric motility was reduced after bilateral section of the greater and lesser splanchnic nerves (8 rats in i.v. and 4 in i.c.v.). After subsequent bilateral section of vagus nerves, the response was inhibited largely. In some animals, the response was reversed to an excitatory response or changed to a biphasic response after

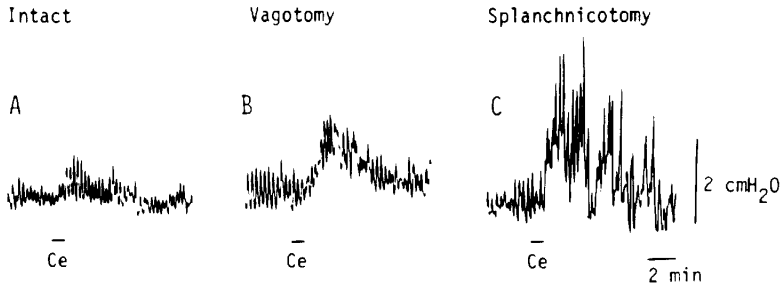


Fig. 3. Effects of vagotomy (B) and subsequent splanchnicotomy (C) on the gastric excitation induced by intravenous injection of caerulein (Ce, 20 ng/kg). A, Control response.

the denervation. When vagotomy was carried out first, the inhibitory effect of caerulein was reduced, and the effect was reduced further after subsequent splanchnicotomy (5 animals). Examples are shown in Fig. 2. After vagotomy the fall in the basal tone by i.c.v. injection was reduced, but the decrease in amplitude of rhythmic contractions did not occur. A slight decrease in the basal tone was induced even after splanchnicotomy. In i.v. injection, the inhibition of the amplitude of contractions induced by caerulein was largely reduced after vagotomy although some potentiation of the fall in basal tone was observed. The decrease in basal tone by caerulein was reduced after splanchnicotomy.

When caerulein (20 ng/kg, i.v.) had an excitatory effect on the stomach (3 rats), the effect was augmented after vagotomy and further potentiated after subsequent splanchnicotomy (Fig. 3). Therefore, it is suggested that caerulein stimulates the excitatory neurons in the myenteric plexus of the stomach, and that vagal and splanchnic efferents exert an inhibitory action on the stomach.

*Caerulein effects on the denervated stomach.* In the stomach denervated by bilateral section of both vagus and splanchnic nerves, caerulein produced somewhat different actions by i.c.v. and i.v. injection, as shown in Table 1. Lower doses of caerulein (20 ng/kg in i.c.v. and 1-5 ng/kg in i.v.) produced only an inhibition. In contrast, higher doses of caerulein (40 ng/kg in i.c.v. and 20 ng/kg in i.v.) caused excitatory, inhibitory and biphasic responses (Table 1).

The latency and the duration of the response to i.c.v. injection of caerulein (20 ng/kg) were longer than those to i.v. (Table 2). The differences between i.c.v. and i.v. injection were not significant in either the latency or duration ($p>0.05$). However, the latencies in the denervated stomach were significantly longer than those in the innervated stomach ($p<0.01$). The prolongation in latency after denervation may be due to the fall in systemic blood pressure and blood flow rate to the peripheral organs produced after bilateral splanchnicotomy.

*Effects of autonomic drugs on caerulein actions.* In the innervated stomach, inhibitory effects of caerulein (20 or 40 ng/kg) injected i.c.v. or i.v. on gastric motility were resistant to atropine (1 mg/kg, i.v.) and guanethidine (5 mg/kg, i.v.) but

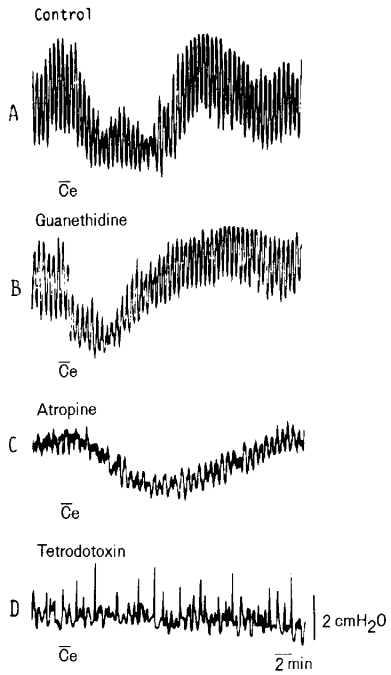


Fig. 4. Effects of guanethidine (5 mg/kg, i.v.) (B), atropine (1 mg/kg, i.v.) (C) and tetrodotoxin (10 $\mu$g/kg, i.v.) (D) on caerulein-induced inhibition of motility in the denervated stomach. Caerulein (Ce) was administered into the lateral cerebral ventricle (20 ng/kg). A, Control response.

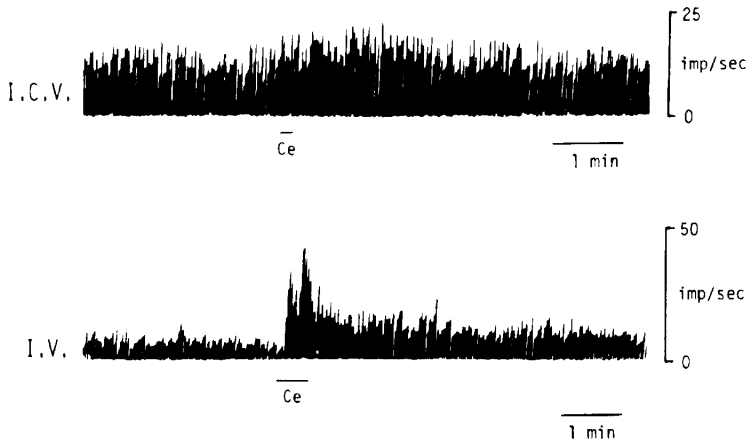


Fig. 5. Effects of caerulein on the discharge rate of the gastric branch of the splanchnic nerve. The discharge rate was enhanced after injection of caerulein (Ce, 20 ng/kg) through i.c.v. and i.v. routes.

TABLE 3. THE LATENCIES AND DURATIONS OF THE RESPONSE IN THE DISCHARGE RATE IN THE GASTRIC BRANCH OF THE VAGUS AND SPLANCHNIC NERVES

| Caerulein (ng/kg) | Response | Latency* (sec) | Duration* (sec) | n |
|---|---|---|---|---|
| Vagus branch | | | | |
| ICV 20 | Excitatory | 55.4 ± 11.6 | 868 ± 133 | 11 |
| IV 20 | Inhibitory | 15.4 ± 1.1 | 200 ± 27 | 12 |
| Splanchnic branch | | | | |
| ICV 20 | Excitatory | 70.7 ± 17.0 | 405 ± 168 | 4 |
| IV 20 | Excitatory | 24.2 ± 8.9 | <1000 | 3 |

* Mean ± SE. ICV, IV; See Table 1.

were abolished by tetrodotoxin (10 μg/kg, i.v.), while excitatory responses were converted into inhibitory responses after atropine.

The inhibitory component of the biphasic response produced by caerulein in the innervated stomach was reduced, and the excitatory response was potentiated after guanethidine.

In the denervated stomach, the inhibitory response (7 rats) to caerulein did not change after application of both guanethidine and atropine, but it was terminated by tetrodotoxin (Fig. 4). An excitatory response was reversed to an inhibitory response after atropine. This inhibitory response was also resistant to guanethidine.

*Caerulein effects on sympathetic and parasympathetic nerve discharges.* The rate of discharges of the gastric branch of splanchnic nerves was enhanced by i.c.v. injection of caerulein (20 ng/kg) in 4 animals and by i.v. in 3 (Fig. 5). As compared with the increase in the discharge rate induced by i.v. injection, however, the response to i.c.v. injection was not as clear in all rats. The mean latency of the response to i.v. injection was shorter than that to i.c.v. injection ($p<0.05$), and the duration

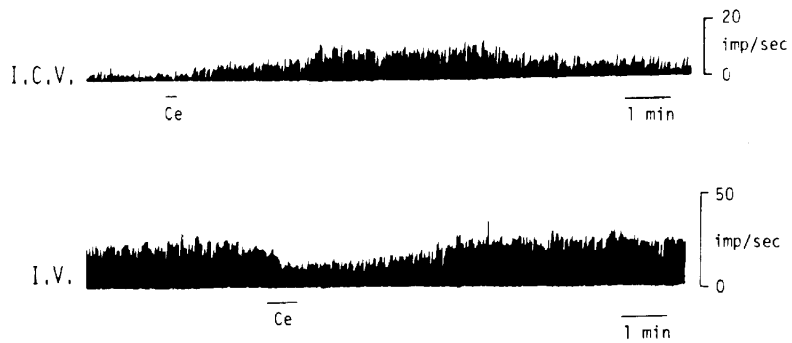


Fig. 6. Effects of caerulein on the discharge rate of the gastric branch of the vagus nerve. The discharge rate was enhanced after i.c.v. injection of caerulein (Ce, 20 ng/kg) but diminished after i.v. injection (Ce, 20 ng/kg).

of the response to i.v. injection was longer than that to i.c.v. injection (Table 3).

The discharge rate of the gastric branch of the vagus nerve was decreased in 12 animals and increased in two animals by i.v. injection of caerulein (20 ng/kg), while it was enhanced by i.c.v. injection of the drug in all 7 animals examined (Fig. 6). The mean latency and duration of the inhibitory response to i.v. injection were shorter than those of the excitatory response to i.c.v. injection. The differences were significant in latency ($p<0.01$) and duration ($p<0.001$).

## DISCUSSION

In the present experiments, caerulein administered through the i.c.v. and i.v. routes predominantly caused an inhibition of the motility of the innervated stomach in rats. The inhibitory response was reduced after vagotomy and/or splanchnicotomy, while the excitatory response was potentiated after vagotomy and splanchnicotomy. These facts indicate that the inhibitory response is, in a part, mediated by sympathetic and parasympathetic nerves. The gastric branches of both the vagus and splanchnic nerves responded to i.c.v. and i.v. injection of caerulein, and the mean latencies of the neural responses were shorter than those of the gastric responses. It is, therefore, suggested that caerulein has central actions and causes gastric inhibition by acting on vagal and splanchnic preganglionic neurons. Such an action of a caerulein-like peptide on the vagal preganglionic neurons was demonstrated by Ewart and Wingate (7), who showed that most neurons in the rat dorsal vagal nucleus responding to the gastric distension were excited by cholecystokinin octapeptide applied iontophoretically to the neurons, although some neurons were inhibited. The stimulatory action of caerulein on sympathetic nerves observed in the present study is in agreement with the result demonstrated in the dog by Neya *et al.* (5). They showed that an excitatory effect of caerulein administered intravenously on intestinal motility was enhanced after splanchnicotomy. However, Nakamura *et al.* (4) concluded that caerulein had no central action in the dog since the drug (1 - 10 ng/kg) injected into the cerebral ventricle failed to induce any effects on gastric and ileal motility although i.v. injection caused an excitatory effect. This discrepancy in the central action of caerulein can not be explained from the present study.

As described above, both vagus and splanchnic nerve efferents may exert an inhibitory action on the gastric motility. Since the discharges of the vagal branch were increased by i.c.v. injection of caerulein and decreased predominantly by i.v. injection, caerulein may stimulate vagal preganglionic neurons, which connect with postganglionic non-adrenergic inhibitory neurons and inhibit vagal preganglionic neurons, which connect with postganglionic cholinergic excitatory neurons. It is, however, difficult to explain the reason why the differences in the actions of caerulein on vagus nerves and in the latencies of the responses of the vagus nerves were obtained by i.c.v. and i.v. routes of the drug administration.

Perhaps these differences reflect the different mechanisms of the action of caerulein on vagal inhibitory and excitatory neurons. An inhibitory response to caerulein in the innervated stomach was reduced by guanethidine, whereas the discharges of the gastric branch of the splanchnic nerve were enhanced by caerulein. These facts indicate that the postganglionic neurons of the splanchnic nerve inducing gastric inhibition are adrenergic.

Caerulein also acts on intrinsic nerves as reported by Bertaccini *et al.* (2), Nakayama *et al.* (3) and Nakamura *et al.* (4), since this peptide exerts inhibitory, excitatory or biphasic actions on the denervated stomach which are terminated by tetrodotoxin. The facts that an inhibitory effect could be observed even after administration of both atropine and guanethidine, but an excitatory effect was abolished after atropine indicate that caerulein stimulates non-adrenergic inhibitory neurons and excitatory cholinergic neurons in the myenteric plexus, and that the excitatory, inhibitory and biphasic responses in gastric motility may be attributed to the predominancy of net activity between the two groups of neurons stimulated by caerulein. In addition, in the denervated stomach, inhibitory responses were observed more frequently at lower doses of caerulein, and excitatory responses at higher doses. Thus, the lower concentration of caerulein in circulating blood may stimulate the non-adrenergic inhibitory neurons rather than the excitatory neurons, and the higher concentration of the drug may stimulate both neuron groups.

It has been reported that caerulein slowed gastric emptying of liquid and solid diets in rats (8, 9). This slowing may be due to a spasmogenic effect of caerulein on the pyloric sphincter (10), but it may also be due to the marked fall in the basal tone of the gastric body accompanied with the decrease in amplitude of the contractions as shown in the present study.

It is concluded that caerulein causes predominantly an inhibition of rat gastric motility by acting centrally on both vagal and splanchnic preganglionic neurons and peripherally on postganglionic myenteric neurons in the stomach.

*Acknowledgement.* The authors wish to thank Mr. M. Watanabe for his excellent technical assistance.

## REFERENCES

1. Bertaccini, G.: Peptide of the amphibian skin active on the gut. I. Tachykinins (substance P-like peptide) and caeruleins. Isolation, structure and basic functions, in *Comprehensive Endocrinology Series, Gastrointestinal Hormones*, ed. J. Glass, Raven Press, New York. pp. 315-342, 1980.
2. Bertaccini, G., De Care, G., Endean, R., Erspamer, V. and Impicciatore, M.: The actions of caerulein on the smooth muscle of the gastrointestinal tract and the gall bladder. *Br. J. Pharmacol.* **34**, 291-310, 1968.
3. Nakayama, S., Neya, T., Tsuchiya, K., Takeda, M. Yamasato, T. and Watanabe, K.: Effects of caerulein on the movements of the gastrointestinal and the biliary system. *Ohyo-Yakuri (Tokyo)* **6**, 1163-1173, 1972 (in Japanese).
4. Nakamura, N., Shiozaki, S., Koyama, Y., Kojima, T. and Marumo, H.: Pharmacological studies

of caerulein, II. The possibility of mediation through the central nervous system. *Jpn. J. Pharmacol.* **25**, 241-250, 1975.

5. Neya, T., Tsuchiya, K., Watanabe, K., Takeda, M. and Yamasato, T.: Effect of caerulein on the intestinal movements, especially its effect through the central nervous system. *Jpn. J. Smooth Muscle Res.* **9**, 63-68, 1973 (in Japanese).
6. Pellegrino, L.J., Pellegrino, A.S. and Cushman, A.T. *A Stereotaxic Atlas of the Rat Brain*, Plenum Press, New York, 1979.
7. Ewart, W.R. and Wingate, D.L.: Cholecystokinin octapeptide and gastric mechanoreceptor activity in rat brain. *Am. J. Physiol.* **224**, G613-G617, 1983.
8. Scarpignato, C., Capovilla, T. and Bertaccini, G.: Action of caerulein on gastric emptying of the conscious rat. *Arch. Int. Pharmacodyn.* **246**, 286-294, 1980.
9. Anika, M.S.: Effects of cholecystokinin and caerulein on gastric emptying of the conscious rat. *Arch. Int. Pharmocodyn.* **246**, 286-294, 1982.
10. Bertaccini, G., Impicciatore, M. and De Caro, G.: Action of caerulein and related substances on the pyloric sphincter of anesthetized rat. *Eur. J. Pharmacol.* **22**, 320-324, 1973.