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Teruhiro Yamasato* Sosogu Nakayama†

*Okayama University,
†Okayama University,

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Teruhiro Yamasato and Sosogu Nakayama

Abstract

Effects of the mesenteric nerve stimulation (MNS) on the twitch contraction induced by field stimulation were investigated regarding the relationship between myenteric neurons and extrinsic cholinergic nerves in the guinea-pig mesenteric nerve-ileal preparation. The twitch contraction was inhibited after MNS. The inhibition of the twitch contraction after MNS was induced twice, just after MNS (1st inhibition) and 2-3 min later (2nd inhibition) (type I), or once, just after MNS (1st inhibition) (type II), in recovery course of twitch contraction for 6-8 min. The 1st inhibition was slightly decreased by guanethidine and hexamethonium. The inhibitory response (1st inhibition) in both types I and II was recovered to the control level by pretreatment with naloxone (recovered twitch contraction), but the late inhibitory response (2nd inhibition) was markedly observed after 2-3 min in types I and II. Either the 1st or the 2nd inhibition was not altered by capsaicin, desensitization to calcitonin gene-related polypeptide (CGRP), vasoactive intestinal polypeptide (VIP), somatostatin, or galanin. The recovered twitch contraction in types I and II was decreased by CGRP-desensitization, or capsaicin. These results suggest that the first inhibitory response was induced by enteric opioid neurons connected with extrinsic cholinergic nerves, but the 2nd inhibition was induced by unknown substances other than CGRP, VIP, somatostatin, and galanin. The twitch contraction may partly be induced by endogenous neurokinin-like substances. And, some CGRP containing neurons, which connect with extrinsic cholinergic nerves, probably activate the intrinsic excitatory neurons.

KEYWORDS: mesenteric nerve, myenteric neuron, twitch contraction, 1st inhibition, 2nd inhibition

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Interaction of Myenteric Neurons and Extrinsic Nerves in the Intestinal Inhibitory Response Induced by Mesenteric Nerve Stimulation

Teruhiro Yamasato* and Sosogu Nakayama

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

Effects of the mesenteric nerve stimulation (MNS) on the twitch contraction induced by field stimulation were investigated regarding the relationship between myenteric neurons and extrinsic cholinergic nerves in the guinea-pig mesenteric nerve-ileal preparation. The twitch contraction was inhibited after MNS. The inhibition of the twitch contraction after MNS was induced twice, just after MNS (1st inhibition) and 2-3min later (2nd inhibition) (type I), or once, just after MNS (1st inhibition) (type II), in recovery course of twitch contraction for 6-8min. The 1st inhibition was slightly decreased by guanethidine and hexamethonium. The inhibitory response (1st inhibition) in both types I and II was recovered to the control level by pretreatment with naloxone (recovered twitch contraction), but the late inhibitory response (2nd inhibition) was markedly observed after 2-3min in types I and II. Either the 1st or the 2nd inhibition was not altered by capsaicin, desensitization to calcitonin gene-ralated polypeptide (CGRP), vasoactive intestinal polypeptide (VIP), somatostation, or galanin. The recovered twitch contraction in types I and II was decreased by CGRP-desensitization, or capsaicin. These results suggest that the first inhibitory response was induced by enteric opioid neurons connected with extrinsic cholinergic nerves, but the 2nd inhibition was induced by unknown substances other than CGRP, VIP, somatostatin, and galanin. The twitch contraction may partly be induced by endogenous neurokinin-like substances. And, some CGRP containing neurons, which connect with extrinsic cholinergic nerves, probably activate the intrinsic excitatory neurons.

Key words: mesenteric nerve, myenteric neuron, twitch contraction, 1st inhibition, 2nd inhibition

It has been known that various neuropeptides exist in the myenteric plexus. They induce an excitatory or inhibitory response of the motility of the small intestine in cats, dogs, rats and guinea pigs. Some of them have been known to be transmitters or neuromodulators in the myenteric plexus.

The twitch contraction was inhibited by endogenous enkephalin released after repeptitive field stimulation (1-5). The peristaltic motility of the small intestine terminated by enkephalin (6, 7) is antagonized by naloxone (3, 4, 8). Calcitonin gene-ralated polypeptide (CGRP) (9 - 11), somatostatin (12, 13), galanin (14, 15) and

* To whom correspondence should be addressed.

vasoactive intestinal polypeptide (VIP) (16) have been known as the substances which induce the inhibitory response in the motility of the small intestine. These inhibitory effects were found to be caused by inhibiting acetylcholine release from the terminals of myenteric nerves. On the other hand, galanin and VIP induced the excitatory response in the rat ileum (17) and guinea-pig small intestine (18, 19). However, it has not been known what actions these polypeptides in the myenteric neurons exert on the motility of small intestine when the extrinsic nerve was stimulated electrically. The aim of this study is to examine the interaction between intrinsic peptidergic neurons and extrinsic nerves in the isolated guinea-pig small intestine.

Materials and Methods

Sixteen guinea pigs of both sexes, weighing between 250–400 g, were used. They were killed by a blow upon the head and bled from carotid arteries. The mesenteric nerve-ileal preparation of 3-cm long was removed, except 10 cm oral to the ileocecal junction, and placed in aerated Tyrode solution (mM; 145 NaCl, 2.7 KCl, 1.5 $CaCl_2$, 0.7 $MgCl_2$, 4.8 $NaHCO_3$, 0.3 NaH_2PO_4, 11.1 gulcose). After the intraluminal contents were rinsed, the specimens were fixed to a holder with two pairs of electrodes, which was set up in a 15-ml organ bath containing the Tyrode solution at 35.0 ± 0.5°C and aerated with 95 % O_2 + 5 % CO_2; one pair of electrodes was used to induce the twitch contraction by field stimulation, and the other pair of electrodes was used for stimulation of the mesenteric nerves (Fig. 1A). The longitudinal contraction of the ileal specimen was recorded with an isotonic transducer (TD-112S, Nihon Kohden, Tokyo, Japan) under the resting load of 0.5 g, and a pen oscillograph (RJG-3026, Nihon Kohden, Tokyo, Japan).

The twitch contraction was induced by field stimulation (0.1 Hz, 0.5 msec, max. current) and the mesenteric nerve was stimulated for 20 sec (20 Hz, 0.5 msec, max. current) at 7–10 min intervals (Fig. 1B).

The specimens were equilibrated for 60–90 min and washed out every 30 min. The specimens were washed out 5 times after the response was recorded. 5 min later an additional rinsing was carried out 4 times.

In order to examine effects of antagonists and blocking agents on the twitch contraction after mesenteric nerve stimulation, these agents were added 10–15 min before mesenteric nerve stimulation. Desensitization to polypeptides was performed 3–5 min before mesenteric

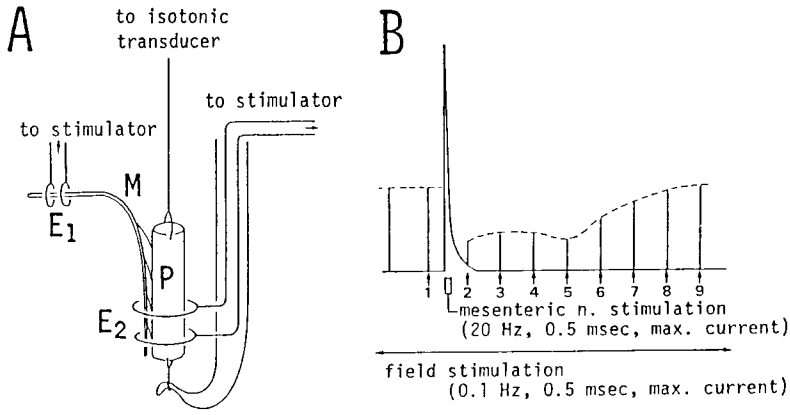

Fig. 1 Schematic diagram for the experimental procedure and data analysis. In figure A: E_1, electrodes for mesenteric nerve stimulation; E_2 electrodes for field stimulation which was used to induce the twitch contraction; M, mesenteric nerve; P, ileum-mesenteric nerve preparation. In figure B: the height of the twitch contraction of point 2 was measured at 30 sec after mesenteric nerve stimulation (MNS). Other points were measured at every 1 min interval. The height of the twitch contraction was measured between baseline of the tone of the preparation and maximal point of the twitch contraction on every point.

nerve stimulation and effects of polypeptides on the twitch response were tested again 10 min after mesenteric nerve stimulation. In the present study, 16 out 23 specimens were equilibrated with Tyrode's solution containing guanethidine (1 μM) and hexamethonium bromide (10 μM).

The procedure to measure the amplitude of twitch response is shown in Fig. 1B. The amplitude (%) after mesenteric nerve stimulation was calculated in comparison with the control amplitude. Statistical analysis was performed according to Wilcoxon's test for pair differences. Values of $p < 0.05$ were regarded as significant.

The following drugs were used: capsaicin (Sigma, St. Louis, USA), atropine sulfate (Sigma), hexamethonium bromide (C_6) (Sigma), guanethidine hydrochloride (Sigma), tetrodotoxin (TTX) (Sankyo, Tokyo, Japan), somatostatin (Peptide Inst., Osaka, Japan), vasoactive intestinal polypeptide (VIP) (Peptide Inst.), galanin (Peptide Inst.), calcitonin gene-related polypeptide (CGRP) (Peptide Inst.).

Results

The twitch contraction induced by field stimulation was not changed by guanethidine (1 μM) or C_6 (10 μM), but it was terminated by atropine (1 μM) or TTX (0.31 μM) (Fig. 2A-E).

Mesenteric nerve stimulation (MNS) for 20 sec elicited an intense intestinal contraction followed by an inhibition of the twitch contraction induced by field stimulation during 3 to 4 min after MNS. The MNS-induced contraction was enhanced by guanethidine (1 μM) and reduced by C_6 (10 μ M) (Fig. 2B and C).

The MNS-induced intense contraction was reversed to an inhibitory response by atropinization (1 μM) in 5 out of 10 preparations (Fig. 2D), but the small contraction was induced by MNS in the other 5 preparations. The relaxation and remaining small contraction were abolished by application of TTX (0.31 μM) (Fig. 2E) or cutting the mesenteric nerves.

Two types of inhibition after MNS were observed during the 6–8 min recovery course of the ileal twitch contraction after termination of MNS (control respone). In Type I, the amplitude of the twitch contraction was markedly decreased to about 15 % of the control level just after MNS (1st inhibition). After the 1st inhibition recovered to 50–60 % of the control level, it was inhibited again 2–3 min after MNS (to about 25 % of the control level) (2nd inhibition), and gradually recovered to the control level (Fig. 3). In Type II, the twitch contraction was markedly decreased to about 5 % of the control level after MNS, and then gradually recovered to the control level 6–8

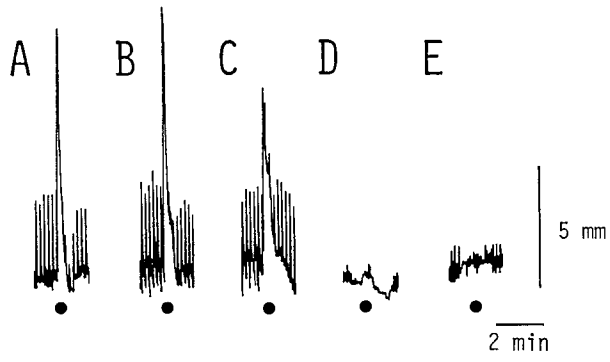

Fig. 2 Effects of guanethidine, hexamethonium, atropine and tetrodotoxin on the MNS-induced ileal contraction and the twitch contraction. A, control; B, after guanethidine (1 μM); C, after hexamethonium (10 μM); D, after atropine (1 μM); E, after tetrodotoxin (0.31 μM); ●, mesenteric nerve stimulation (20 Hz, 0.5 msec, max. current).

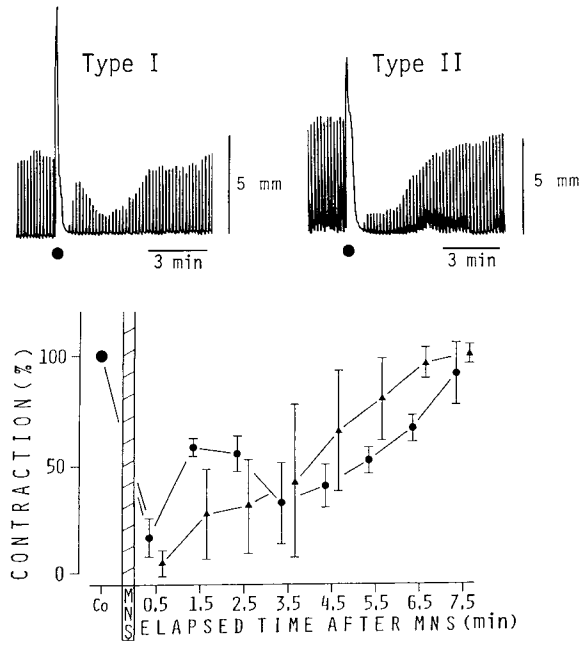

Fig. 3 Two types inhibitory responses induced by mesenteric nerve stimulation on the ileal twitch contraction. ●–●, Type I (n = 7); ▲–▲, type II (n = 5); ● and MNS, mesenteric nerve stimulation (20 Hz, 0.5 msec, max. current).

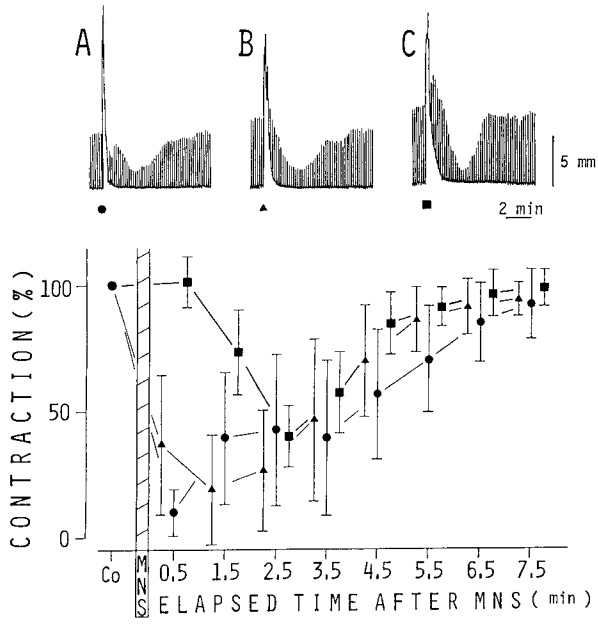

Fig. 4 Effects of guanethidine, C_6 and naloxone on the MNS-induced inhibition to the twitch contraction. Co, control; ●–● (A), control response; ▲–▲ (B), after guanethidine (1 μM) and C_6 (10 μM); ■–■ (C), after additional application of naloxone (0.5 μM); MNS, mesenteric nerve stimulation (20 Hz, 0.5 msec, max. current). n = 7

min after MNS (Fig. 3).

In the 1st inhibition of types I and II, the twitch contraction was decreased to 30 % of the control levl after treatment with guanethidine (1 μM) and C_6 (10 μM) in combination (Fig. 4B) ($p < 0.05$), but not by guanethidine or C_6 alone ($p > 0.05$ as compared with control response).

The amplitude of the twitch contraction in the 1st inhibition of types I and II was recovered to the control level by application of naloxone (0.5 μM) (recovered twitch contraction) ($p < 0.05$ as compared with control response), and the 2nd inhibition was markedly reduced after additional application of naloxone followed by guanethidine and C_6 in types I and II (Fig. 4C) (about 30 % of the control level).

The MNS-induced contraction and the twitch contraction were reduced to about 70 and 80 % of the control level after treatment with capsaicin (0.5 μM), respectively. The 1st inhibition was not altered after application of capsaicin (0.5 μM). The 2nd inhibition after naloxone (0.5 μM) was not altered by additional application of capsaicin (0.5 μM), but the recovered twitch contraction was decreased to about 80 % of the control level (Fig. 5) ($p < 0.05$ as compared with that before application of capsaicin) and the recovery time course after application of capsaicin was shorter than that of the control response.

CGRP (0.5, 1 μM) caused an inhibitory effect on the twitch contraction. Desensitization to CGRP (1 μM) occurred after 2 or 3 times application of it (1 μM). The 1st inhibition and the 2nd inhibition after naloxone were unchanged by CGRP-desensitization ($p > 0.05$) (Fig. 6).

VIP (0.5 μM, 1 μM) inhibited the twitch contraction. The desensitization to VIP was induced by 2 or 3 times VIP (1 μM) applications. After previous application of naloxone, the recovered twitch contraction was increased to 105 % of control level by VIP-desensitization, but the 2nd inhibition was not altered by it (Fig. 7) ($p > 0.05$ as compared with control response).

The desensitization to somatostatin and galanin did not affect the 1st inhibition, the 2nd inhibition after naloxone or the recovered twitch contraction.

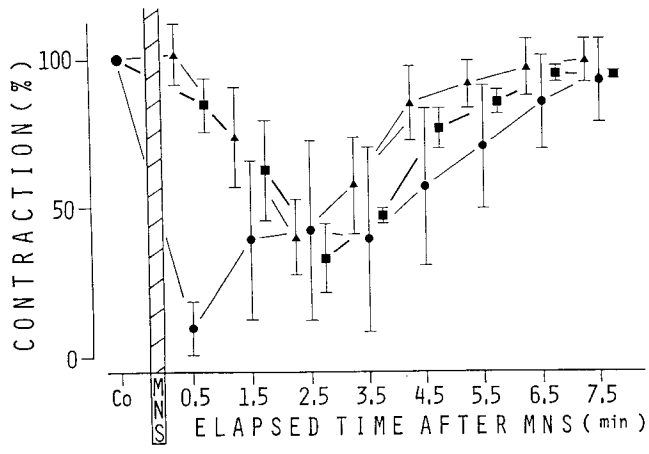

Fig. 5 Effects of capsaisin on the 2nd inhibition and the recovered twitch contraction after application of naloxone. Co, control; ●–●, control response; ▲–▲, after pretreatment of naloxone (0.5 μM); ■–■, after additional preapplication of capsaicin (0.5 μM) followed by naloxone; MNS, mesenteric nerve stimulation (20 Hz, 0.5 msec, max. current). n = 5

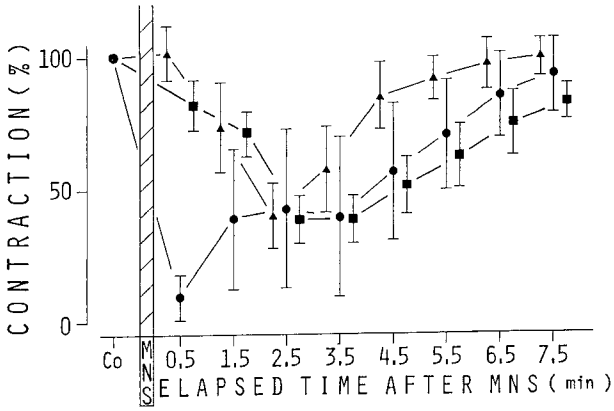

Fig. 6 Effects of desensitization to CGRP on the 2nd inhibition and the recovered twitch contraction after application of naloxone. Co, control; ●–●, cotrol response; ▲–▲, after pretreatment of naloxone (0.5 μM); ■–■, after additional CGRP-desensitization (1 μM × 3 times) followed naloxone; MNS, mesenteric nerve stimulation (20 Hz, 0.5 msec, max. current). n = 5

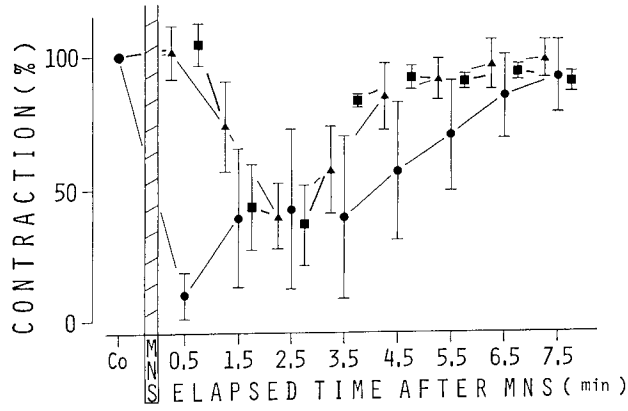

Fig. 7 Effects of vasoactive intestinal polypeptide (VIP)-desensitization on the 2nd inhibition and the recovered twitch contraction after application of naloxne. Co, control; ●–●, control response; ▲–▲, after application of naloxone (0.5 μM); ■–■, after additional VIP-desensitization (0.5 μM × 3 times) followed naloxone treatment; MNS, mesenteric nerve stimulation (20 Hz, 0.5 msec, max. current). n = 5

Discussion

In the present experiment, effects of the mesenteric nerve stimulation (MNS) on the twitch responses of the guinea-pig ileum were studied in relation to myenteric neurons.

Stimulation of the MNS induced a contraction in the isolated ileal preparation. Since the MNS-induced contraction was terminated by cutting the mesenteric nerve, the response was not due to direct electrical stimulation of the preparation. MNS-induced contractile response which was reduced by C_6 and enhanced by guanethidine was reversed to an inhibitory response by atropine. These results suggest that the mesenteric nerve contains the presynaptic fibers to excite the excitatory cholinergic neurons via nicotinic receptors and the adrenergic nerves

which inhibit the cholinergic nerves (20, 21).

In our study, the twitch contraction was inhibited just after MNS in the isolated guinea-pig ileum. The inhibition of control response seems to consist of the 1st and the 2nd inhibitions. The 1st inhibition was slightly decreased by C_6 with guanethidine, but not the 2nd inhibition. It is generally accepted that adrenergic nerves terminate the cholinergic neurons and inhibit the acetylcholine release from them (20, 21). Nicotinic receptors were blocked by C_6. The present results suggest that a part of the 1st inhibition is induced by adrenergic fibers which terminate at cholinergic neurons and presynaptic cholinergic neurons which make synaptic connection with the inhibitory neurons.

The inhibitory effect on the twitch contraction produced after repetitive field electrical stimulation of the guinea-pig small intestine was antagonized by naloxone (3), and electrically stimulated myenteric neurons of the guinea-pig ileum inhibited its motility by opioid-like material released from them (2, 4). Enkephalin reduced the ACh release from the myenteric neurons in the guinea-pig ileum (1, 5) and the peristalsis terminated by enkephalin was recovered by naloxone in the isolated guinea-pig ileum (6, 7, 18, 22).

The 1st inhibition of control response was terminated by naloxone after guanethidine with C_6. These results suggest that the twitch contraction caused by ACh release is reduced by endogenous opioid (2, 8) released by the MNS, resulting in the 1st inhibition. Such a response has not been found in the recent physiological and anatomical studies.

The myenteric neurons having immunoreactivity to substance P were observed in the guinea-pig small intestine (23, 24). Capsaicin acts on the primary sensory nerve terminals, resulting in release and reduction of the neurokinin in the nerve terminals (25, 26). MNS-induced contraction after atropine is induced by nourokinin (27). In the present experiment, the control twitch contraction, the recovered twitch contraction and MNS-induced contraction were reduced by application of capsaicin. These results suggest that neurokinin released from the intrinsic capsaicin-sensitive nerves by MNS enhance the twitch contraction and that MNS-induced contraction is caused not only by the nenrokinin containing nerve, but also by postganglionic cholinergic neurons to which the mesenteric nerves make synaptic connection. However, it has been reported that decrease of the twitch contraction after capsaicin is caused by reduction of the neurokinin in the nerve terminals. Therefore, a part of the twitch contraction was probably caused by endogenous neurokinin (25, 26), and most of it was probably induced by endogenous acetylcholine which was released from the myenteric neurons.

CGRP induced the relaxant response on the guinea-pig ileum (10), stomach (28) and opossum oesophageal smooth muscle (29). Maggi *et al.* (11) reported that an inhibition of the twitch contraction following contractions after application of capsaicin was induced by endogenous CGRP. On the other hand, desensitization to CGRP did not affect the inhibitory response to capsaicin in the rat stomach (30). In the present exeperiment, CGRP-desensitization remained unaffected in the 1st and the 2nd inhibition after naloxone treatment. These results suggest that CGRP has no relation to these inhibitory responses induced after stimulation of mesenteric nerves.

Galanin induced the relaxation in the guinea-pig small intestine (14) and terminated the nicotinic excitatory presynaptic potentials in the myenteric plexus of guinea-pig small intestine (15), while it induced the contractile response in the rat ileum (17). In the present experiment, galanin induced the inhibitory effect on the twitch contraction, but desensitization to galanin did not affect the 1st inhibition, the 2nd inhibition, or the recovered twitch contraction. These results suggest that mesenteric nerves probably have no direct interaction with galanin-containg myenteric neurons. They also suggest that galanin containing neurons may act as the interneurons in the myenteric plexus, because only the peristaltic

reflex occurred.

Somatostatin induced the relaxant response in the guinea-pig small intestine (18, 31) and inhibited the release of acetylcholine from myenteric neurons of guinea-pig intestine (13, 32) and canine stomach (33). Somatostatin containing neurons act as the facilitatory interneurons of the descending pathway of the enteric neuron in the guinea-pig colon (12). In the present experiment, somatostatin inhibited the twitch contraction, but desensitization to somatostatin did not affect the 1st inhibition, the 2nd inhibition after naloxone, or the recovered twitch contmaction. These results suggest that the extrinsic cholinergic nerves do not make synaptic connection to somatostatin containing myenteric neurons, to which sympathetic nerves probably make synaptic connection. But, our study could not demonstrate that sympathetic nerves make synaptic connection to somatostatin containing neurons in the myenteric plexus.

VIP containing neurons were found in the guinea-pig small intestine (23), and VIP induced the contractile response in the guinea-pig small intestine (19). On the other hand, it induced the relaxant response in the feline (34) and lamb (35) stomach. Distension of the small intestine induced the release of VIP which caused the descending inhibition of the peristaltic reflex in the feline small intestine (16). In the present experiment, VIP inhibited the ileal twitch contraction and VIP-desensitization had no effect on the 1st inhibition, and the 2nd inhibition after naloxone, and slightly enhanced the recovered twitch contraction. Grider (36) reported that VIP- and somatostatin-containing neurons have an interrelated action on regulation of each neuron in the myenteric plexus, but the present results could not indicate the evidence that extrinsic nerves make synaptic connection to the VIP-containing neuron. But VIP-containing neurons probably act as the interneurons in the myenteric plexus on the regulation of intestinal motility as reported by previous investigators (16, 19, 34, 35).

Finally, the present study indicates that the extrinsic preganglionic cholinergic nerves in the mesenteric nerve may make synaptic connections to the intrinsic cholinergic, opioidergic, and neurokinin containing neurons, and CGRP-containing neurons which excite the intrinsic excitatory neurons. The present experiment could not demonstrate that mesenteric nerves make synaptic connection to the somatostatin-, galanin- and VIP-containing neurons in the myenteric plexus.

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