Acta Medica Okayama

Volume 44, *Issue* 3 1990 *Article* 5

JUNE 1990

Capsaicin-sensitive and hexamethonium-insensitive circular muscle responses to mesenteric nerve stimulation in the isolated guinea pig ileum.

Miyako Takaki* Ji-Guang Jin†

Sosogu Nakayama‡

*Okayama University,

†Okayama University,

‡Okayama University,

Capsaicin-sensitive and hexamethonium-insensitive circular muscle responses to mesenteric nerve stimulation in the isolated guinea pig ileum.*

Miyako Takaki, Ji-Guang Jin, and Sosogu Nakayama

Abstract

Capsaicin-sensitive and hexamethonium-insensitive contractile and relaxatory motor responses of circular muscle induced by mesenteric nerve stimulation were studied. Desensitization to substance P or to neurokinin A, or the substance P antagonist, spantide largely reduced the initial contractile response. Desensitization to calcitonin gene-related peptide moderately reduced the late prolonged relaxation response. These results indicate that both responses of the circular muscle to mesenteric nerve stimulation may be attributed to a release of neuropeptides evoked by the 'efferent' stimulation of capsaicin-sensitive sensory nerves.

KEYWORDS: calcitonin gene-relatsd peptide, capsaicin, ileal motility, mesenteric nerve, substance P

---

*PMID: 1696423 [PubMed - indexed for MEDLINE]

Acta Med Okayama 44 (3) 135-139 (1990)

Capsaicin-Sensitive and Hexamethonium-Insensitive Circular Muscle Responses to Mesenteric Nerve Stimulation in the Isolated Guinea Pig Ileum

Miyako Takaki*, Ji-Guang Jin and Sosogu Nakayama

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

Capsaicin-sensitive and hexamethonium-insensitive contractile and relaxatory motor responses of circular muscle induced by mesenteric nerve stimulation were studied. Desensitization to substance P or to neurokinin A, or the substance P antagonist, spantide largely reduced the initial contractile response. Desensitization to calcitonin gene-related peptide moderately reduced the late prolonged relaxation response. These results indicate that both responses of the circular muscle to mesenteric nerve stimulation may be attributed to a release of neuropeptides evoked by the 'efferent' stimulation of capsaicin-sensitive sensory nerves.

Key words : **calcitonin gene-related peptide, capsaicin, ileal motility, mesenteric nerve, substance P**

Recently, it has been shown that substance P, neurokinin A, other tachykinins, and calcitonin gene-related peptide (CGRP) coexist in the sensory nerves of various species. In most instances, these nerves have been shown to be capsaicin-sensitive (1-4). We have reported that the longitudinal muscle responses to electrical mesenteric nerve (MN) stimulation in the presence of guanethidine and hexamethonium in the guinea pig ileum are attributed to antidromic activation of capsaicin-sensitive (neuropeptide-containing) sensory afferent nerve fibers (5, 6) as suggested by other investigators (7-10). The circular muscle and myenteric plexus of the guinea pig small intestine also contain many neuropeptide-containing nerve fibers that appear to be of extrinsic origin (1, 11, 12). In fact, MN stimulation produced a slow depolarizing response similar to that produced by substance P in myenteric neurons of the guinea pig ileum (15). The contribution of circular muscles to whole-gut motility is not less important than that of longitudinal muscles. However, it is not clear how neuropeptide-containing nerve fibers influence the circular muscle motility of the small intestine. The aim of the present experiments was, therefore, to study the motor responses of the circular muscle induced by MN stimulation in the isolated guinea pig ileum.

Materials and Methods

Adult guinea pigs of both sexes were stunned and exsanguinated. The 20-cm-long terminal portion of the ileum was not used. A 0.8- to 1.0-cm-long segment of the ileum, with the mesenteric vessel and nerve attached, was excised and placed in an organ bath containing 15 ml of

* To whom correspondence should be addressed.

Krebs solution, which was maintained at 36 and 37°C and aerated with 5% CO_2 in O_2. The composition of modified Krebs solution was (mM): NaCl, 120.9; KCl, 5.9; $NaHCO_3$, 14.4; $NaH_2PO_4 \cdot 2H_2O$, 1.1; $CaCl_2 \cdot 2H_2O$, 1.9; $MgCl_2 \cdot 6H_2O$, 1.2, and glucose, 11.5. Preparations were left to settle in the organ bath for 1.5–2h. The tissues were treated with guanethidine (2μM) before and during the experiments. The mesenteric vessel and nerve were placed between a pair of annular platinum electrodes. Stimulation was given for 30s at 5.5 (rarely 10.5) min intervals. The mechanical activity of the circular muscle was recorded at the middle of the whole gut segment, which was suspended transversely through an isotonic transducer (14). Tension of 0.25g was applied to record the activity of circular muscle. In some experiments, the mechanical activity of the longitudinal muscle was recorded according to the method described in previous reports (5, 6).

Drugs used were: acetylcholine chloride (Daiichi Seiyaku Co., Ltd., Tokyo, Japan), atropine sulfate (Dainippon Seiyaku Pharmaceutical Co., Ltd., Osaka, Japan), capsaicin (Sigma Chemical Co., St. Louis, MO, USA), guanethidine sulfate (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan), hexamethonium bromide (Sigma Chemical Co.), human calcitonin generelated peptide (hCGRP, CGRP), neurokinin A, substance P and [D-Arg[1], D-Trp[7,9], Leu[11]] SP(1–11) (Spantide) (Peptide Institute Minoh, Osaka, Japan) and tetrodotoxin (Sankyo Co., Ltd., Tokyo, Japan). Capsaicin (10 mM) was dissolved in 100% dimethylsulfoxide (DMSO). When used, the stock solution was diluted one-hundredfold with distilled water. DMSO at 0.1% had no effect of its own. Substance P, spantide and CGRP were dissolved in and diluted with 0.02M acetic acid. Desensitization to substance P and neurokinin A was carried out by a single application of 0.2μM of substance P and neurokinin A, which abolished the contractile response induced by 10nM of substance P and neurokinin A, respectively. Desensitization to CGRP was carried out by applying CGRP (25nM) twice for 10–14min, which reduced the response induced by 2.5nM of CGRP by about 70%. All values are presented as the mean ± SE. Statistical significance of differences between means was estimated by Student's *t*-test.

Results

Supramaximal MN stimulation (20Hz, 0.5ms) constantly induced a maximal circular muscle contraction of the guinea pig ileal loop after an adrenergic neuron blockade by guanethidine (2 μM). The pattern of contractile response to MN stimulation showed a prompt and transient phasic contraction (Fig. 1) or a burst of phasic contractions (Fig. 2, A). The duration of the circular muscle contraction was 50.0 ± 4.3s (n = 7), which was not significantly different from that of the longitudinal response (36.6 ± 5.1s, n = 17, see Ref. (6)). These contractions were composed of local contractions at the recording site and/or peristalses that spread over a whole or half loop. The frequency of these contractions fluctuated

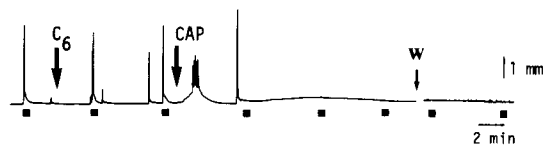

Fig. 1 Effects of hexamethonium (C_6, 50μM) and capsaicin (CAP, 1μM) on the circular muscle contractile response to mesenteric nerve (MN) stimulation (20Hz, 0.5ms, for 30s, at ■). C_6 (at the first arrow) did not affect the response, CAP (at the second arrow) abolished it. The response did not recover after washout (W) for 2h.

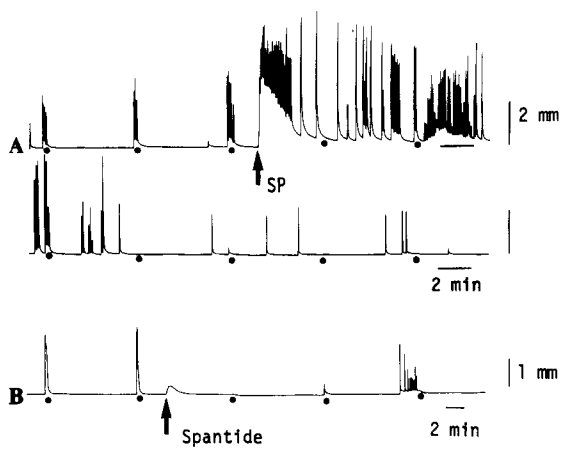

Fig. 2 Effects of substance P-desensitization attained by a single application of 0.2μM of substance P (SP) (A) and spantide (10μM) (B) on the circular contractile response to MN stimulation (●). A and B, data from different preparations of different animals. In A, SP-desensitization abolished the response. In B, spantide abolished or largely attenuated the response.

between 1 and 7 waves (3.6 ± 0.3, n = 34) during each MN stimulation for 30 sec.

Hexamethonium (C_6, 50 μM) had no effect on these circular muscle contractions (109.4 ± 6.5 % of the control amplitude remained, n = 9, Figs. 1 and 3,A), but atropine (1 μM) abolished them. Capsaicin (1 μM) irreversibly (at least for 2 h) abolished or reduced the amplitude of the contractile response by MN stimulation to 13.3 ± 6.8 % (n = 22) (Figs. 1 and 3, B). In 4 other preparations, capsaicin (1 μM) had no effect on the contractile response, which was reduced to 45.8 ± 20.6 % (n = 4) by the following application of C_6 (50 μM). Tetrodotoxin (0.31 μM) completely abolished the remaining circular response to MN stimulation. Substance P at any dose exclusively caused contractions. The desensitization to substance P reversibly abolished the contractile response to MN stimulation in 7 out of 10 preparations (Fig. 2, A). However, in the remaining 3 preparations, it did not affect the amplitude of the contractile response, the frequency of which sometimes was increased. Altogether, the desensitization to substance P reduced the amplitude of the contractile response to 31.3 ± 15.4 % of the control (n = 10) (Fig. 3-D). Neurokinin A (15–17), the substance P-related peptide which acts on neurokinin-2 receptors, caused a circular muscle contractile response similar to that caused by substance P at the same range of dose. The desensitization to neurokinin-2 receptor by neurokinin A reversibly abolished or reduced the amplitude of the circular muscle contractile response by MN stimulation to 26.5 ± 8.9 % of the control in all 10 preparations (Fig. 3-E). It also reduced the amplitude of the longitudinal muscle contraction induced by MN stimulation to 32.2 ± 10.5 % of the control (n = 6). Spantide (10 μM), a substance P antagonist (18), reduced the amplitude of the contractile response to 41.3 ± 16.5 % (n = 6) without any effect on the contraction induced by acetylcholine (0.1 μM) (Fig. 3-F).

The late prolonged relaxation of the circular muscle by MN stimulation could be detected after

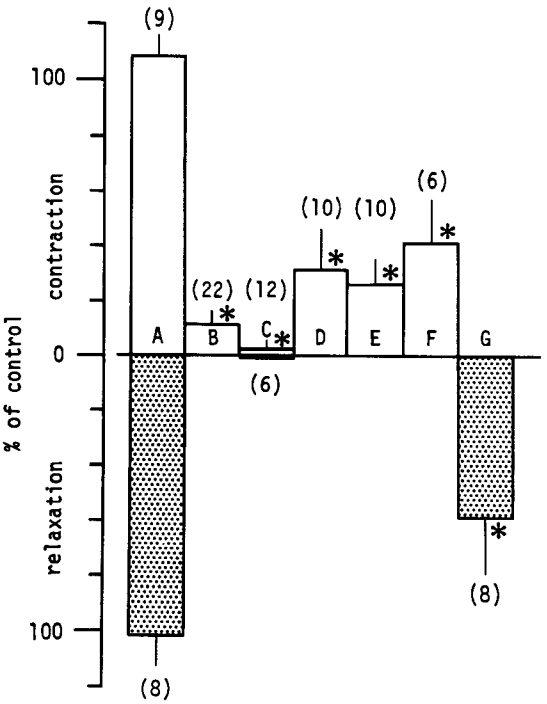

Fig. 3 Effects of hexamethenium (C_6), capsaicin (CAP), substance P (SP), neurokinin A (NKA)-desensitization and spantide and calcitonin gene-related peptide (CGRP)-desensitization on the circular responses to mesenteric nervre (MN) stimulation. Open column, the contractile response to MN stimulation; dotted column, the relaxation response to MN stimulation. A, C_6 (50 μM); B, CAP (1 μM); C, C_6 (50 μM) plus CAP (1 μM) D, SP-desensitization; E, NKA-desensitization; F, Spantide (10 μM); G, CGRP-desensitization attained by 2 applications of 25 nM of CGRP. Numbers in parentheses denote the number of preparations. * significantly different ($p < 0.005$) vs. the control.

an initial contraction by measurement at a higher gain. The amplitude of the circular muscle relaxation induced by MN stimulation tended to be smaller than the longitudinal one. The duration of the circular response was 76.2 ± 5.0 s (n = 7), almost equal to longitudinal one (75.5 ± 4.1 s, n = 17, see Ref. (6)). C_6 (50 μM) did not affect the amplitude of the relaxation response (101.8 ± 10.7 % of the control remained, n = 8) (Fig. 3-A). However, an additional application of capsaicin (1 μM) irreversibly (at least for 2 h) abolished it in all 6 preparations (Fig. 3-C). CGRP-desensitization was carried out according

to the method previously described (6, 19). The first application of CGRP (25 nM) caused a marked, prolonged relaxation, *i. e.*, CGRP decreased the tone of the ileal circular muscle in 6 out of 8 preparations and induced a phasic contraction superimposed on a tonic contraction in the remaining 2 preparations. Both inhibitory and excitatory effects induced by a second application of CGRP (25 nM) were greatly reduced, indicating that the tissue was desensitized to this peptide. In the presence of a desensitizing concentration of CGRP (25 nM×2), the tone gradually recovered to the control level within 5–15 min. After complete recovery, the relaxation response induced by MN stimulation was still suppressed. This suppression by CGRP-desensitization usually disappeared within 20 to 30 min without washout. CGRP-desensitization maximally reduced the amplitude of the late relaxation to 58.5 ± 10.8 % of the control ($n = 8$) (Fig. 3-G) without significant influence on the initial contraction (96.1 ± 10.7 % of the control amplitude remained, $n = 7$).

Discussion

The capsaicin-sensitive and C_6-insensitive circular muscle contraction induced by MN stimulation was reversibly reduced by substance P- or neurokinin A-desensitization, or spantide. These results indicate that the capsaicin-sensitive and C_6-insensitive contractile response is due to the release of sensory neuropeptides, such as substance P and neurokinin A, in accordance with the response of the longitudinal muscle to MN stimulation (5, 7, 8, 10). Infrequent capsaicin-insensitive and C_6-sensitive contractile responses seem to be due in part to activation of a small proportion of vagal parasympathetic fibers as suggested in the longitudinal muscle response of the rabbit (20) and guinea-pig small intestine (5).

The capsaicin-sensitive and C_6-insensitive late circular muscle relaxation induced by MN stimulation, similar to the longitudinal muscle relaxation (6), was moderately suppressed by CGRP-desensitization. This result indicates that, at least in part, the circular relaxation response is due to the release of CGRP from sensory nerve fibers. On the other hand, in the present study, CGRP (25 nM) by itself predominantly induced a relaxation of the circular muscle, although it also induced a phasic contraction in a few cases. Recently, it has been found that CGRP(0.3–30 nM) has not only excitatory actions on the circular muscle, but also inhibitory actions on the ascending enteric reflex contraction (21), while the predominant action of CGRP on the intestinal longitudinal muscle has previously been found to be a relaxation (6, 19, 22). These findings indicate a heterogeneity and topical selectivity in the motor actions of CGRP on the gut differently from substance P and neurokinin A. Such heterogeneity and selectivity might be reasons why CGRP-desensitization slightly inhibited the circular muscle relaxation induced by MN stimulation.

In conclusion, in the isolated guinea pig ileum, MN stimulation induced a circular muscle contraction due to the release of sensory neuropeptides, such as substance P and neurokinin A, and a circular muscle relaxation partly due to the release of a sensory neuropeptide, CGRP. The time course of the circular muscle response mimicked that of the longitudinal one. It is therefore likely that the 'efferent' stimulation of the capsaicin-sensitive sensory nerves synchronously affects the circular and longitudinal muscle motilities (5, 6). This event is triggered by chemical stimulation of the sensory nerves in the inflammatory responses, facilitating the formation of spasm of the intestine.

References

1. Gibbins IL, Furness JB, Costa M, Macintyre I, Hillyard CJ and Girgis S: Co-localization of calcitonin gene related peptide-like immunoreactivity with substance P in cutaneous, vascular and visceral sensory neurons of guinea pigs. Neurosci Lett (1985) **57**, 125–130.
2 Lee Y, Takami K, Kawai Y, Girgis S, Hillyard CJ,

Macintyre I, Emson PC and Tohyama M: Distribution of calcitonin gene-related peptide in the rat peripheral nervous system with reference to its coexistence with substance P. Neuroscience (1985) **15**, 1227-1237.

3 Skofitsch G and Jacobowitz DM: Calcitonin gene-related peptide coexists with substance P in capsaicin-sensitive neurons and sensory ganglia of the rat. Peptides (1985) **6**, 747-754.

4. Sundler F, Brondin E, Ekblad E, Håkanson R and Uddmann R: Sensory nerve fibers: Distribution of substance P, neurokinin A and calcitonin gene-related peptide; in Tachykinin Antagonists, Håkanson and Sundler eds, Elsevier, Amsterdam (1985) p 3.

5. Takaki M, Jin J-G and Nakayama S: Involvement of hexamethonium-sensitive and capsaicin-sensitive components in cholinergic contractions induced by mesenteric nerve stimulation in guinea-pig ileum. Biomed Res (1987) **8**, 195-203.

6. Takaki M, Jin J-G and Nakayama S: Possible involvement of calcitonin gene-related peptide (CGRP) in non-cholinergic non-adrenergic relaxation induced by mesenteric nerve stimulation in guinea pig ileum. Brain Res (1989) **478**, 199-203.

7. Barthó L, Holzer P, Lembeck F and Szolcsányi J: Evidence that the contractile response of the guinea-pig ileum to capsaicin is due to release of substance P. J Physiol (1982) 332, 157-167.

8. Grbović L and Radmanović BZ: The influence of substance P on the response of guinea-pig isolated ileum to periarterial nerve stimulation. Br J Pharmacol (1983) **78**, 681-685.

9. Szolcsányi J and Barthó L: New type of nerve-mediated cholinergic contractions of the guinea-pig small intestine and its selective blockade by capsaicin. Naunyn-Schmied Arch Pharmacol (1978) **305**, 83-90.

10. Wali FA: Possible involvement of substance P in the contraction produced by periarterial nerve stimulation in the rat ileum. J Autonom Pharmacol (1985) **5**, 143-148.

11. Costa M, Furness JB and Gibbins IL: Chemical coding of enteric neurons. Prog Brain Res (1986) **68**, 217-239.

12. Fehér E, Burnstock G, Varndell IM and Polak JM: Calcitonin gene-related peptide immunoreactive nerve fibres in the small intestine of the guinea-pig: Electronmicroscopic immunocytochemistry. Cell Tiss Res (1986) **245**, 353-358.

13. Takaki M and Nakayama S: Effects of mesenteric nerve stimulation on the electrical activity of myenteric neurons in the guinea pig ileum. Brain Res (1988) **442**, 351-353.

14. Jin J-G, Neya T and Nakayama S: Myenteric 5-HT-containing neurones activate the descending cholinergic excitatory pathway to the circular muscle of guinea-pig ileum. Br J Pharmacol (1989) **98**, 982-988.

15. Regoli D, D'Orleans-Juste P, Drapeau G, Dion S and Escher E: Pharmacological characterization of substance P antagonists; in Tachykinin Antagonists, Håkanson and Sundler eds, Elsevier, Amsterdam (1985) p 277.

16. Regoli D, Drapeau G, Dion S and D'Orleans-Juste P: Receptors for neurokinins in peripheral organs; in Substance P and Neurokinins, Henry, Couture, Cuello, Pelletier, Quirion and Regoli eds, Montreal, July 10-23, Springer-Verlag, New York (1986) p 99.

17. Regoli D, Drapeau G, Dion S and D'Orleans-Juste P: Pharmacological receptors for substance P and neurokinins. Life Sci (1987) **40**, 109-117.

18. Folkers K, Håkanson R, Hönig J, Jie-Cheng X and Leander S: Biological evaluation of substance P antagonists. Br J Pharmacol (1984) **83**, 449-456.

19. Barthó L, Lembeck F and Holzer P: Calcitonin gene-related peptide is a potent relaxant of intestinal muscle. Eur J Pharmacol (1987) **135**, 449-451.

20. Barthó L and Szolcsányi J: The mechanism of the motor response to periarterial nerve stimulation in the small intestine of the rabbit. Br J Pharmacol (1980) **70**, 193-195.

21. Holzer P, Barthó L, Matusak O and Bauer V: Calcitonin gene-related peptide action on intestinal circular muscle. Am J Physiol (1989) **256**, G546-G552.

22. Maggi CA, Manzini S, Giuliani S,Santicioli P and Meli A: Calcitonin gene-related peptide activates non-adrenergic, non-cholinergic relaxations of the rat isolated duodenum. J Pharm Pharmacol (1987) **39**, 327-328.

Received April 2, 1990; accepted April 27, 1990.