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Neurotensin Enhances Gastric Motility in Antral Circular Muscle Strip of Guinea-pig

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Many reports suggest that neurotensin (NT) in the gastrointestinal tract may play a possible role as a neurotransmitter, a circulating hormone, or a modulator of motor activity. NT exerts various actions in the intestine; it produces contractile and relaxant responses in intestinal smooth muscle. This study was designed to investigate the effect of NT on motility of antral circular muscle strips in guinea-pig stomach. To assess the role of Ca^{2+} influx in underlying mechanism, slow waves were simultaneously recorded with spontaneous contractions using conventional intracellular microelectrode technique. At the concentration of 10^{-7} M, where NT showed maximum response, NT enhanced the magnitude (863±198%, mean±SEM, n=13) and the frequency (154±10.3%, n=11) of spontaneous contractions. NT evoked a slight hyperpolarization of membrane potential, tall and steep slow waves with abortive spikes (278±50%, n=4). These effects were not affected by atropine (2 μM), guanethidine (2 μM) and tetrodotoxin (0.2 μM). NT-induced contractile responses were abolished in Ca^{2+}-free solution and reduced greatly to near abolition by 10 μM of verapamil or 0.2 mM of $CdCl_2$. Verapamil attenuated the effects of NT on frequency and amplitude of the slow waves. Taken together, these results indicate that NT enhances contractility in guinea-pig gastric antral circular muscle and Ca^{2+} influx through the voltage-operated Ca^{2+} channel appears to play an important role in the NT-induced contractile mechanism.

Key Words: Neurotensin, Spontaneous contractions, Slow waves, Verapamil, Ca^{2+} influx

INTRODUCTION

Neurotensin (NT), a linear tridecapeptide which was isolated from bovine hypothalamus (Carryway & Leeman, 1973), has been shown to exert a variety of biological effects in the periphery and in the central nervous system. In addition to brain, NT has also been found in nerve tissue of gastrointestinal tract in rats, guinea-pigs and dogs. In fact, most NT (over 85%) has been found to be distributed in gastrointestinal tract, especially in distal ileum (Orchi et al, 1976; Sundler et al, 1977), where it is stored in a population of specific endocrine-type cells of mucosal layer called the N-cells (Doyle et al, 1985). All these facts strongly suggest a possible role of NT in gastrointestinal tract as a neurotransmitter or neuromodulator involved in the regulation of intestinal motility and secretion (Schultzberg et al, 1980; Buchan & Barber, 1987; Allescher et al, 1991).

There is a regional and species difference in the mode of action of NT to contract and relax the smooth muscle of gastrointestinal tract; NT reduces the pressure of lower esophageal sphincter (Rosell et al, 1980), inhibits gastric motility (Hellstrom et al, 1982; Keinke et al, 1986), blocks migrating motor activity (Al-Saffar & Rosell, 1981), and enhances motility of large intestine (Thor & Rosell, 1986). In

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guinea-pig ileum, NT potentiated contractions of longitudinal muscles, whereas it attenuated those of circular muscles (Yamanaka et al, 1987). Ohashi et al (1994) found that NT exerts a direct action to contract longitudinal and circular smooth muscles of guinea-pig small intestine, in addition to its well-documented indirect action brought about by the release of acetylcholine from cholinergic nerves (Kitabgi & Freychet, 1978, 1979; Kitabgi, 1982), and enhances the voltage-dependent inward Ca^{2+} current in ileal smooth muscle cells of the guinea-pig (Ohashi et al, 1994). NT also exerted an apamin-sensitive inhibitory action to relax longitudinal and circular smooth muscles or inhibited muscarinic receptor-mediated contraction in circular smooth muscle of the guinea-pig intestine (Ohashi et al, 1994).

There have been a few experiments about the effects of NT on gastric muscle of guinea-pig. The longitudinal muscle strips showed biphasic response, an initial trasient relaxation followed by lasting tonic contraction (Katsoulis & Conlon, 1988). On the contrary, Mandrek & Milenov (1991) showed different response of longitudinal muscle strip which did not respond to NT, in contrast to circular muscle strip showing strong tonic contraction. Single cells isolated from circular muscle showed relaxation to NT (Chijiiwa et al, 1993).

All these results demonstrate diverse nature of gastrointestinal smooth muscle in the response to NT, showing further investigations are still needed. Therefore, this experiment was designed to characterize the profile of contractile response to NT in antral circular muscle strips of guinea-pig stomach and to assess the role of Ca^{2+} influx in the mechanism.

METHODS

Guinea-pigs of either sex weighing 300~350 g were exsanguinated after stunning. The stomach was isolated and cut in the longitudinal direction along the lesser curvature in phosphate-buffered Tyrode solution. The antral part of stomach was cut and the mucosal layer was separated from the muscle layers.

Measurement of isometric contractions and intracellular recording of the electrical activity

Muscle strips (2~3 mm wide, 10~12 mm long) from the proximal part of antrum were cut parallel to the circular fibers, and mounted in a 100 ml vertical chamber. One end was fixed and the other was connected to a force transducer (Isometric Transducer, Harvard Bioscience, USA) to measure isometric contractions. Another strip was mounted in a 2 ml horizontal chamber. The strip was pinned out at one end with tiny pins and the other end was connected to a force transducer to record the isometric contractions. The strip was constantly perfused at a rate of 2~3 ml/min with CO_2/bicarbonate-buffered Tyrode solution. Electrical activities were recorded using conventional intracellular microelectrode recording technique in which glass microelectrodes were filled with 3 M KCl and only the ones with tip resistance of 40~80 MΩ were used. Mechanical and electrical responses of smooth muscle cells were simultaneously recorded by a chart recorder (MX-6, Device Ltd, Britain).

Solutions and drugs

Phosphate-buffered Tyrode solution contained (in mM) NaCl 147, KCl 4, $MgCl_2 \cdot 6H_2O$ 1.05, $CaCl_2 \cdot 2H_2O$ 2, $NaH_2PO_4 \cdot 2H_2O$ 0.42, $Na_2HPO_4 \cdot 12H_2O$ 1.81, glucose 5.5, and pH was adjusted to 7.35. CO_2/bicarbonate buffered-Tyrode solution contained (in mM) NaCl 135, KCl 5.4, $MgSO_4 \cdot 7H_2O$ 1, $CaCl_2 \cdot 2H_2O$ 1.8, $NaHCO_3$ 15.3, $NaH_2PO_4 \cdot 2H_2O$ 1, glucose 5.6, and pH was adjusted to 7.35 and bubbled with 5% CO_2~95% O_2. All drugs used in this experiments were purchased from Sigma Chemicals (St. Louis, MO, USA).

Statistics

All values are expressed as means ± SEM. Statistical analysis was performed using the Student's *t* test. Differences were considered to be significant when P value was less than 0.05.

RESULTS

Effects of neurotensin on the spontaneous contractions

Smooth muscle cells of guinea-pig stomach generally generate spontaneous contractions without external stimuli as shown in Fig. 1. These con-

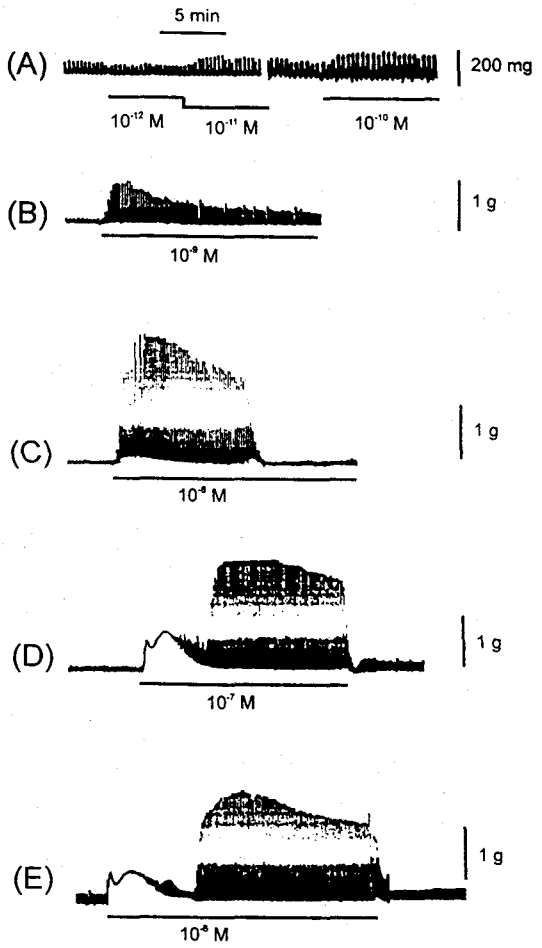

Fig. 1. Effect of neurotensin on the spontaneous contractions of antral circular muscle in guinea-pig stomach. Neurotensin (NT) was applied to the bath solution from low concentration (10^{-12} M) as shown in the figure. NT increased the amplitude of spontaneous contractions in a dose-dependent manner and transiently increased the basal tone at high concentration range ($>10^{-9}$ M). The frequency of spontaneous contractions was also markedly increased. Maximal potentiating effect of NT appeared around 10^{-7} M.

tractions were potentiated by the application of neurotensin (NT) at the concentration of 10^{-11} M and higher. At the concentration range of 10^{-11} M to 10^{-8} M, the magnitudes of both spontaneous phasic contractions and tonic contraction were increased (Fig. 1A, B, C). At the concentration range above 10^{-8} M, however, the response was biphasic, showing an initial development of tonic contraction followed by late reappearance of spontaneous contractions which were bigger than control magnitude and attenuation of tonic contraction (Fig. 1D, E).

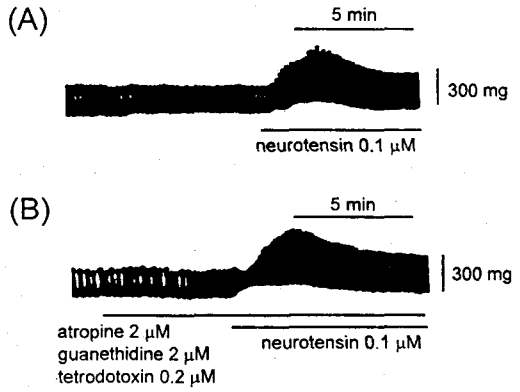

Fig. 2. Neurotensin (NT) enhanced the spontaneous contractions even in the presence of autonomic nerve blockers. A: NT (0.1 μM) increased the amplitude of spontaneous phasic contractions. However, tonic contraction was induced transiently and was not maintained throughout. B: These effects were not affected by autonomic nerve blockers (atropine 2 μM, guanethidine 2 μM, and tetrodotoxin 0.2 μM).

At the concentration of 10^{-7} M, where NT produced maximum response, NT enhanced both magnitude (863±198%, n=13) and frequency (154±10.3%, n=11) of spontaneous contractions.

Effect of NT in the presence of autonomic nerve blockers on gastric contractility

It is well known that gastrointestinal tract is profusely innervated with both intrinsic and extrinsic nervous plexus. To rule out the influence of auto nomic nervous system the muscle strips were treated with typical autonomic nerve blockers of atropine, guanethidine and tetrodotoxin (Fig. 2). Pretreatment with these blockers did not make a significant difference to the overall result (Fig. 2B). This finding indicated that NT exerts a direct action to contract the antral circular muscle strips of guinea-pig stomach. To elucidate the underlying mechanism for neurotensin-induced contractile responses the concentration around 10^{-7} M NT showing maximum contractile responses was chosen in this experiment.

Role of extracelllular Ca^{2+} influx

Increase in either intracellular Ca^{2+} concentration or in calcium sensitivity of contractile apparatus is essential for enhancing smooth muscle contraction. The former could be achieved by either Ca^{2+} release

from intracellular stores such as sarcoplasmic reticulum or Ca^{2+} influx through ionic channels. To assess the degree to which Ca^{2+} influx pathways contribute the NT effect of potentiating contractions, calcium ions were removed from normal Tyrode solution (Fig. 3A). Calcium removal prevented any contractile acitivity of the muscle strip from the excitatory effect of NT including activation of large phasic contractions and tonic contraction. However, carbachol (CCh) known as an agent releasing Ca^{2+} from intracellular calcium stores evoked tonic contraction even in the absence of external Ca^{2+} (Fig. 3A). Treatment with cadmium chloride ($CdCl_2$), an inorganic Ca^{2+} channel blocker, abolished spontaneous contractions, leaving tiny contraction which was suppressed by NT. In the presence of $CdCl_2$ and NT CCh brought about a similar tonic contraction (Fig. 3B). Verapamil, an organic Ca^{2+} channel blocker, also suppressed all the contractions of the strip of which tonic contraction was able to be activated transiently by NT. In this condition CCh produced lasting tonic contraction (Fig. 3C). All the results point to the fact that blocking Ca^{2+} entry into the cell has led to complete or nearly complete abolition of contractile responses, but tonic contraction could still be produced if Ca^{2+} is released from intracellular stores.

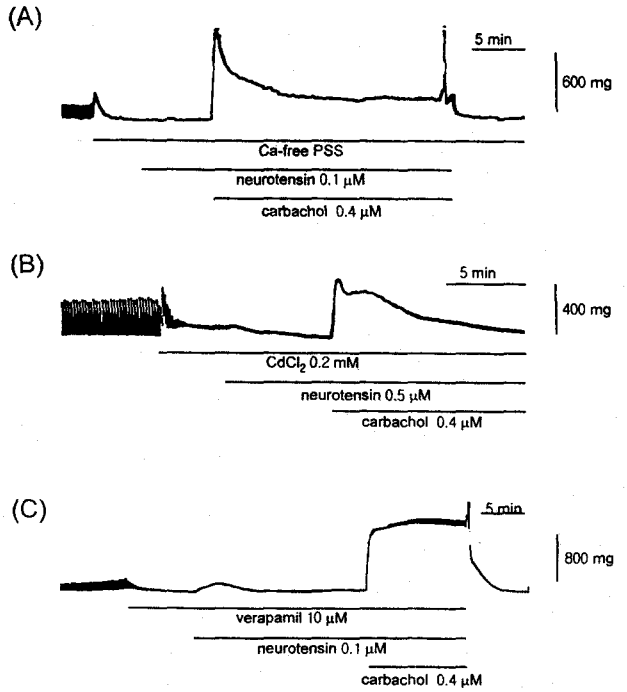

Fig. 3. Effects of external Ca^{2+} on the contractile responses induced by neurotensin (NT). A: External calcium ions were eliminated by replacing normal Tyrode solution with Ca^{2+}-free normal Tyrode solution. Spontaneous contractions were abolished and NT had no effect at all in this condition. However, carbachol (CCh) still produced prominent tonic contraction without phasic contractions. B: Treatment with $CdCl_2$ also abolished spontaneous contractions. NT had no effect and CCh produced the similar contractile response as above. C: With verapamil the basic results were the same. However, NT could produce slight tonic contraction transiently and CCh could produce small phasic contractions on large tonic contraction.

Role of verapamil-sensitive Ca^{2+} channels

Antral circular muscle of guinea-pig stomach always

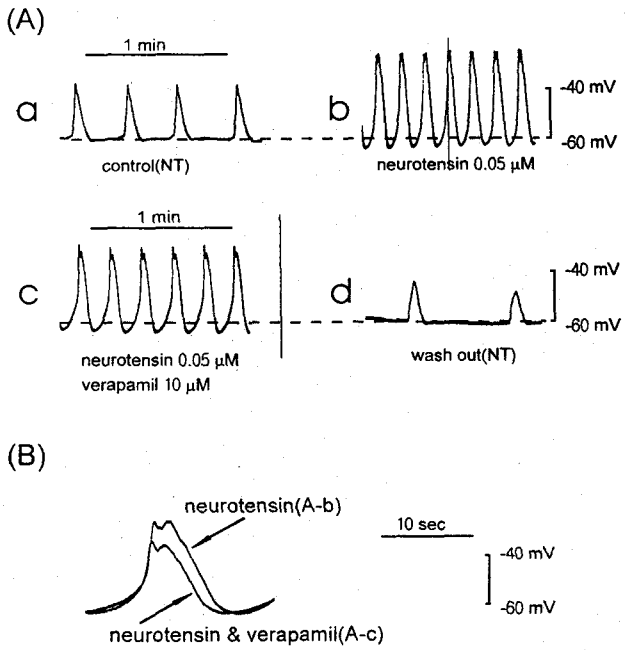

Fig. 4. Effects of neurotensin (NT) on slow waves. A: Slow waves were recorded continuously in the same smooth muscle cell (a to d). Both the amplitude and the frequency of slow waves were markedly increased by NT and hyperpolarization of membrane potential was induced with tall and steep slow waves (b). In this condition verapamil attenuated these responses that were reversible (c). B: The effect of NT (A-b) and that of added verapamil (A-c) were compared in expanded time scale. Verapamil resulted in reduction of slow waves frequency and attenuated their amplitude no by reducing the hyperpolarization of resting membrane potential but by reducing the depolarization of peak potential.

always exhibits regular fluctuations of membrane potential called slow waves ranging from −60 mV to −40 mV, constituting amplitude of around 20 mV. The more depolarization slow waves reach, the larger contractions are developed. Abortive spikes on the top of slow waves which trigger even bigger contractions are evoked when slow waves reach the depolarization potential above a certain threshold value. The effects of NT on slow waves are shown in Fig. 4. The amplitude of slow waves was 20 mV and the frequency was 3/min (Fig. 4A-a). NT increased the amplitude of slow waves by slight hyperpolarization of resting membrane potential and considerable depolarization of peak plateau potential (278±50%, n=4). The frequnecy was also increased to 6/min (Fig. 4A-b). Verapamil resulted in reduction of the frequency of slow waves from 6/min to 4.8/min and attenuated their amplitude not by reducing the hyperpolarization of resting membrane potential but by reducing the depolarization of peak potential (Fig. 4A-c). The effects of verapamil on slow waves showed again more clearly in Fig. 4B, where the slow wave treated with NT and that treated with both NT and verapamil were superimposed for comparison in faster time scale. Removal of NT and verapamil from the solution eventually reversed the frequency and amplitude to control value, though Fig. 4A-d shows rather reduction of them than control values because of remaining verapamil that requires longer time to wash out. Effect of verapamil can be seen more clearly in Fig. 5, in which contractile and electrical responses were recorded simultaneously. Initially slow waves showed very small amplitude of 8~9 mV. However, upon administration of NT slow waves became larger in amplitude and accompanied with abortive spikes which transformed small amplitude of contractions into much larger ones (Fig. 5A). This effect of NT was conspicuously weakened by pretreatment with verapamil as evidenced by membrane depolarization and tall, steep slow waves without Ca^{2+}-spikes (Fig. 5B).

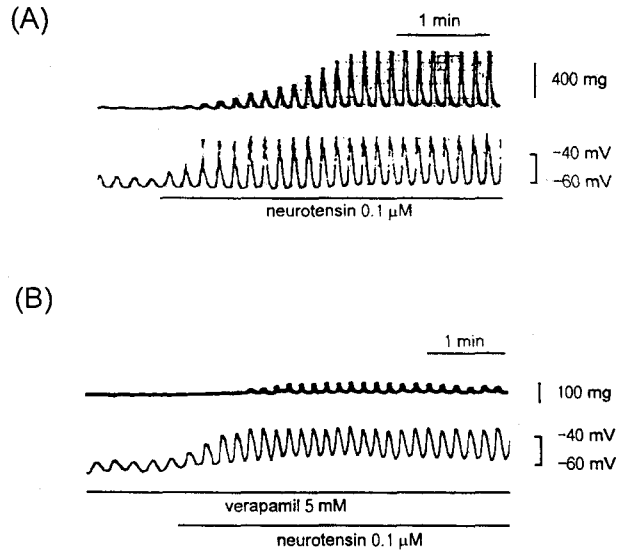

Fig. 5. Comparison of the effects of neurotensin (NT) between control and verapamil- pretreated conditions on the electrical and mechanical responses. The membrane potential and mechanical contraction were simultaneously recorded. A: NT increased enormously the amplitude of both spontaneous contractions and slow waves and induced prominent Ca^{2+}-spikes. B: In the same muscle strip the pretreatment with verapamil greatly attenuated the effect of NT on the electrical and mechanical activities. However, even in the presence of verapamil NT potentiated phasic contractions coupled with changes in slow waves; membrane depolarization and tall slow waves without abortive spikes.

DISCUSSION

The present results show that the contractile response of guinea-pig antral circular muscle strip to NT is induced by increase in Ca^{2+} influx through L-type voltage-dependent Ca^{2+} channels.

Studies with gastrointestinal smooth muscle of different species suggest that NT has a variety of effects on contractility: the direct inhibitory and excitatory effects on smooth muscle as well as the indirect excitatory effects via neural release of neurotransmitters. The direct contractile activity of NT depended on the increase in the intracellular Ca^{2+} concentration produced either by influx of extracellular Ca^{2+} through voltage-dependent channels (Donoso et al, 1986; Snape et al, 1987; Christinck et al, 1992; Mulé et al, 1992; Mulé & Serio, 1997), or Ca^{2+} influx through nonselective cation channels (Komori et al, 1992), or release of Ca^{2+} from internal stores (Komori et al, 1992). The direct inhibitory effects of NT were mediated by the opening of apamin-sensitive Ca^{2+}-dependent K^+ channels (Huidobro-Toro, 1983; Allecher et al, 1992; Christinck et al, 1992; Mulé et al, 1992; Ohashi et al, 1994) or by the increase in intracellular cGMP level (Chijiiwa et al, 1993). The indirect excitatory effects of NT were

induced by the neuronal release of acetylcholine and substance P (Mulé & Serio, 1997).

At the lower concentration range of NT (10^{-11} M~10^{-8} M), both the magnitude of spontaneous phasic contraction and that of tonic contraction were increased. However, at the concentration range above 10^{-8} M the response was biphasic, showing initial transient disappearance of spontaneous phasic contractions and development of tonic contraction followed by late reappearance of much bigger spontaneous contractions (Fig. 1). Even in the presence of autonomic nerve blockers, atropine, guanethidine and tetrodotoxin, such contractile responses to NT were not affected (Fig. 2). This result indicated that the contractile reponses result from direct action of NT on gastric smooth muscle cells. Therefore, this experiment was focussed on the source and the role of Ca^{2+} in the direct contractile effects evoked by NT in gastric smooth muscle.

The observation that the excitatory mechanical action of NT is sensitive to both extracellular Ca^{2+} and drugs (verapamil, $CdCl_2$) which block L-type voltage-dependent Ca^{2+} channels, while the contractile response to carbachol is not affected (Fig. 3), suggest that activation of NT receptors may lead to the opening of L-type voltage-dependent Ca^{2+} channels, whereas that of muscarinic receptors induces tonic contraction via release of Ca^{2+} from internal Ca^{2+} stores. Recently, Gully et al (1993) reported the pharmacological profile of a nonpeptide NT antagonist, SR 48692. Using this antagonist, the existence of NT receptor subtypes has been suggested (Dubuc et al, 1994; Labbé-Jullié et al, 1994; Mulé et al, 1996; Nguyen-Le et al, 1997; Croci et al, 1999; Unno et al, 1999). The cloning of rodent (Tanaka et al, 1990) and human NT-receptors from the HT 29 cell line (Vita et al, 1993) resulted in sites with high-affinity in vitro specific binding (NT_1); a low-affinity site (NT_2) was also cloned from rat brain (Chalon et al, 1996). Recently the human NT_2 receptor was also cloned from a brain cortex cDNA library and stably expressed in CHO cells (Vita et al, 1998). Since NT is a 13-amino acid peptide, its excitatory action seems to be mediated not by activation of intracellular receptor but by that of membrane NT receptor.

NT evoked characteristic changes in slow waves which were recorded simultaneously with spontaneous contractions: membrane hyperpoarization and frequent, tall, steep slow waves with abortive spikes (Fig. 4, 5). These results suggest that NT may activate a K^+ channels and L-type voltage-dependent Ca^{2+} channels. Our previous studies with guinea-pig antral circular muscle strips and cells have shown that guinea-pig gastric myocytes have L-type Ca^{2+} channels current which is closely linked with changes in intracellular Ca^{2+} concentration (Kim et al, 1997) and the increased Ca^{2+} current can induce tall, steep slow waves with abortive spikes, and also have apamin-sensitive, Ca^{2+}- dependent K^+ channels which are activated by norepinephrine to induce membrane hyperpolarization (Lee et al, 1991). NT produced complex species- and region-dependent actions on gastrointestinal motor activity. In the guinea-pig stomach, the presence of inhibitory (Kastsoulis & Conlon, 1988) or excitatory (Mandrek & Milenov, 1991) NT receptors on smooth muscle cells has been suggested. The results of slow waves changes recorded from this study using conventional intracellular microelectrode technique suggested that the direct excitatory effects of NT via Ca^{2+} influx through L-type channels prevails over the inhibitory effects induced by activation of apamin-sensitive Ca^{2+}-dependent K^+ channels.

In conclusion, present study provides evidence for the coexistence of inhibitory effects with contractile excitatory effects induced by NT in guinea-pig gastric antral circular muscle. The influx of Ca^{2+} through L-type voltage-dependent channels induces the contractile excitatory effects of NT.

REFERENCES

Al-Saffar A, Rosell S. Effects of neurotensin and neurotensin analogues on the migrating myoelectrical complexes in the small intestine of rats. *Acta physiol Scand* 112(2): 203−208, 1981

Allescher HD, Ahmad S, Classen M, Daniel EE. Interaction of trimebutine and Jo-1196 (fedotozine) with opoid receptors in the canine ileum. *J Pharmacol Exp Ther* 257(2): 836−842, 1991

Allescher HD, Fick H, Schusdziarra V, Classen M. Mechanisms of neurotensin-induced inhibition in rat ileal smooth muscle. *Am J Physiol* 263: G767−G774, 1992

Buchan AMJ, Barber DL. Neurotensin containing neurons in the canine enteric innervation. *Neurosci Lett* 76: 13−17, 1987

Carryway R, Leeman SE. The isolation of a new hypotensive peptide, neurotensin, from bovine hypothalami.

J Biol Chem 248: 6854−6861, 1973

Chijiiwa H, Kawakami O, Misawa T, Kabemura T, Nawata H. Direct inhibitory effect of neurotensin on isolated gastric smooth muscle cells of guinea pig via the cyclic GMP system. *Digestion* 54(3): 135−138, 1993

Christinck F, Daniel EE, Fox-Threlkeld JET. Inhibitory and excitatory mechanisms of neurotensin action in canine intestinal circular muscle in vitro. *Can J Physiol Pharmacol* 70: 1423−1431, 1992

Chalon P, Vita N, Kaghad M, Guillemot N, Bonnin J, Delpech B, Le Fur G, Ferrara P, Caput D. Molecular cloning of a levocabastine-sensitive neurotensin binding site. *FEBS Lett* 386: 91−94, 1996

Croci T, Aureggi G, Guagnini F, Manara L, Gully D, Le Fur G, Maffrand JP, Mukenge S, Ferla G, Ferrara P, Chalon P, Vita N. In vitro functional evidence of different neurotensin-receptors modulating the motor response of human colonic muscle strips. *Br J Pharmacol* 127: 1922−1928, 1999

Donoso MV, Huidobro-Toro JP, Kullak A. Involvement of calcium channels in the contractile activity of neurotensin but not acetylcholine: studies with calcium channel blockers and Bay K 8644 on the rat fundus. *Br J Pharmacol* 88: 837−846, 1986

Doyle H, Greeley GH, Mate L, Sakamoto T, Townsend CM, Thompson JC. Distribution of neurotensin on the canine gastrointestinal tract. *Surgery* 97: 337−341, 1985

Dubuc I, Costentin J, Terranova JP, Barnouin MC, Soubrie P, Le Fur G, Rostene W, Kitabgi P. The nonpeptide neurotensin antagonist, SR 48692, used as a tool to reveal putative neurotensin receptor subtypes. *Br J Pharmacol* 112: 352−354, 1994

Gully D, Canton M, Boigegrain R, Jeanjean F, Molimard R, Brouard A, Pelaprat D, Labbe-Jullie C, Mazella J, Soubrie P, Maffrand JP, Rostene W, Kitabgi P, Le Fur G. Biochemical and pharmacological profile of a potent and selective nonpeptide antagonist of the neurotensin receptor. *Proc Natl Acad Sci USA* 90: 65−69, 1993

Hellstrom PM, Nylander G, Rosell S. Effects of neurotensin on the transit of gastrointestinal contents in the rat. *Acta Physiol Scand* 115: 239−243, 1982

Huidobro-Toro JP. Non-neuronal excitatory neurotensin receptors on the taenia-coli of the guinea-pig: lack of influence of tetrodotoxin and dynorphin. *Neurosci Lett* 38: 309−314, 1983

Katsoulis S, Conlon JM. Neurotensin and prostaglandin interactions in smooth muscle of the guinea pig stomach. *Eur J Pharmacol* 158: 251−256, 1988

Keinke O, Wulschke S, Ehrlein HJ. Neurotensin slows gastric emptying by a transient inhibition of gastric and a prolonged inhibition of duodenal motility. *Digestion* 34: 281−288, 1986

Kim SJ, Ahn SC, Kim JK, Kim YC, So I, Kim KW. Changes in intracellular Ca^{2+} concentration induced by L-type Ca^{2+} channel current in ghinea pig gastric myocytes. *Am J Physiol* 273: C1947−C1956, 1997

Kitabgi P, Freychet P. Effects of neurotensin on isolated intestinal smooth muscles. *Eur J Pharmacol* 50: 349−357, 1978

Kitabgi P, Freychet P. Neurotensin contracts the guinea-pig longitudinal ileal smooth muscle by inducing acetylcholine release. *Eur J Pharmacol* 56: 403−406, 1979

Kitabgi P. Effects of neurotensin on intestinal smooth muscle: application to the study of structure-activity relationship. *Ann NY Acad Sci* 400: 37−55, 1982

Komori S, Matsuoka T, Kwon SC, Takewaki T, Ohashi H. Membrane potential and current responses to neurotensin in the longitudinal muscle of the rectum of the fowl. *Br J Pharmacol* 107: 790−796, 1992

Labbè-Julliè C, Deschaintres S, Gully D, Le Fur G, Kitabgi P. Effect of the nonpeptide neurotensin antagonist, SR 48692, and two enantiomeric analogs, SR 48527 and SR 49711, on neurotensin binding and contractile responses in guinea-pig ileum and colon. *J Pharmacol Exp Ther* 271: 267−276, 1994

Lee TJ, Kim JH, Kim KW. Excitatory influences of noradrenaline on the spontaneous contractions and electrical activity of antral circular muscle of the guinea-pig stomach. *Kor J Physiol* 25(2): 147−158, 1991

Mandrek K, Milenov K. Effects of neurotensin on gastric smooth muscle of dog and guinea pig. *J Gastrointest Motil* 3: 5−11, 1991

Mulè F, Postorino A, Geraci A, Serio R. Neurotensin: dual effect on the motor activity of rat duodenum. *Eur J Pharmacol* 212: 215−224, 1992

Mulè F, Serio R, Postorino A, Vetri T, Bonvissuto F. Antagonism by SR 48692 of mechanical responses to neurotensin in rat intestine. *Br J Pharmacol* 117: 488−492, 1996

Mulè F, Serio R. Mode and mechanism of neurotensin action in rat proximal colon. *Eur J Pharmacol* 319: 269−272, 1997

Nguyen-Le XK, Neugebauer W, Gobeil F, Pheng LH, Nsa Allogho S, Regoli D. Pharmacological heterogeneity of neurotensin receptors: an in vitro study. *Can J Physiol Pharmacol* 75: 547−551, 1997

Ohashi H, Takewaki T, Unno T, Komori S. Mechanical and current responses to neurotensin in the smooth muscle of guinea-pig intestine. *J Auton Pharmacol* 14: 239−251, 1994

Orchi L, Baetens O, Rufener C, Brown M, Vale W, Guilemin R. Evidence for immunoreactive neurotensin in dog intestinal mucosa. *Life Sci* 19: 559−562, 1976

Rosell S, Thor K, Rokaeus A, Nyquist O, Lewenhaupt A, Kager L, Folkers K. Plasma concentration of neurotensin-like immunoreactivity (NTLI) and lower esophageal sphincter (LES) pressure in man following infusion of (Gln^4)-neurotensin. *Acta Physiol Scand* 109(4): 369−375, 1980

Schultzberg M, Hokfelt M, Nilsson G, Terenus L, Rehfeld JF, Brown M, Elde R, Goldstein M, Said S. Distribution of peptide and catecholamine-containing neurons in the gastrointestinal tract of rat and guinea pig: immunohistochemical studies with antisera to substance P, vasoactive intestinal peptide, enkephalin, somatostatin, gastrin, cholecyctokinin, neurotensin and dopamine β-hydroxylase. *Neuroscience* 5: 689−744, 1980

Snape Jr WJ, Hyman PE, Mayer EA, Sevy N, Kao HW, Root D. Calcium dependence of neurotensin stimulation of circular colonic muscle of the rabbit. *Gastroenterology* 93: 823−828, 1987

Sundler F, Hakanson R, Hammer RA, Alumets J, Carraway R, Leeman SE, Zimmerman EA. Immunohistochemical localization of neurotensin in endocrine cells of the gut. *Cell Tiss Res* 178: 313−321, 1977

Tanaka K, Massu M, Nakanishi S. Structure and functional expression of the cloned rat neurotensin receptors. *Neuron* 4: 847−854, 1990

Thor K, Rosell S. Neurotensin increases colonic motility. *Gastroenterology* 90(1): 27−31, 1986

Unno T, Komori S, Ohashi H. Characterization of neurotensin receptors in intestinal smooth muscle using a nonpeptide antagonist. *Eur J Pharmacol* 369: 73−80, 1999

Vita N, Laurent P, Lefort S, Chalon P, Dunont X, Kaghad M, Gully D, Le Fur G, Ferrara P, Caput D. Cloning and expression of a complementary DNA encoding a high affinity human neurotensin receptor. *FEBS Lett* 317: 139−142, 1993

Vita N, Oury-Donat F, Chalon P, Guillemot M, Kaghad M, Bachy A, Thurneyssen O, Garcia S, Poinot-Chazel C, Casellas P, Keane P, Le Fur G, Maffrand JP, Soubrie P, Caput D, Ferrara P. Neurotensin is an antagonist of the human neurotensin NT2 receptor expressed in chinese hamster ovary cells. *Eur J Pharmacol* 360: 265−272, 1998

Yamanaka K, Kitamura K, Kuriyama H. Effects of neurotensin on electrical and mechanical properties of smooth muscles in longitudinal and circular layers of the guinea-pig ileum. *Pflügers Arch* 408: 10−17, 1987