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Motility pattern of isolated rat proximal colon and excitatory action of neurotensin

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Abstract

The investigation concerned the effects of neurotensin on mechanical activity of isolated rat proximal colon. An isometric-isovolumic preparation was used. Colonic segments showed spontaneous contractile activity, consisting of regular changes in both endoluminal and isometric tension. Neurotensin (1 pM to 0.1 μM) induced a concentration-dependent tonic contraction of both circular and longitudinal muscle accompanied by high frequency oscillatory activity. Desensitization of the neurotensin receptors antagonized the contractile activity of neurotensin. The excitatory effects of neurotensin were partially blocked to the same degree by tetrodotoxin and atropine, indicating that a component of the neurotensin-mediated contraction involves the release of endogenous acetylcholine. The tetrodotoxin-resistant component of the neurotensin-induced effect seems to be due to a direct action on the smooth muscle cells.

Keywords: Neurotensin; Neurotensin receptor; Gastrointestinal motility; Colon, rat

1. Introduction

It is now well established that a variety of substances including neurotransmitters, hormones and neuromodulators are involved in the regulation of gastrointestinal motility. Neurotensin, first localized in the central nervous system (Emson et al., 1982), in the periphery is largely concentrated in the gastrointestinal tract. Immunohistochemical studies have shown that neurotensin is present in endocrine N-cells found throughout the small intestine and colonic mucosa (Daniel et al., 1985; Sundler et al., 1977). In addition, neurotensin-like immunoreactivity has been identified in nerve fibers of the enteric plexus of rat, guinea-pig and dog (Buchan and Barber, 1987; Schultzberg et al., 1980). Therefore, neurotensin may act as paracrine agent or hormone as well as neurotransmitter or neuromodulator, but its physiological role is still poorly understood.

Neurotensin has been found to induce inhibitory, excitatory or biphasic effects on gastrointestinal muscle (Christinck et al., 1992; Huidobro-Toro and Kullak, 1985; Huidobro-Toro and Way, 1982; Huidobro-Toro and Zhu, 1984; Kitabgi and Freychet, 1978, 1979; Sakai et al., 1984). The inhibitory effect of neurotensin seems to be due to a direct action on smooth muscle cells whereas the nature of the contractile response can be neuronal or involve a tetrodotoxin-resistant mechanism (Allesher et al., 1992; Hellstrom, 1985; Huidobro-Toro and Kullak, 1985; Huidobro-Toro and Yoshimura, 1983; Huidobro-Toro and Way, 1982; Huidobro-Toro and Zhu, 1984; Kitabgi and Freychet, 1978; 1979). Hence, neurotensin has been proposed as a candidate for the neurotransmitter of non-adrenergic, non-cholinergic (NANC) inhibitory nerves as well as of NANC excitatory nerves (Goedert et al., 1984; Komori et al., 1986, 1992).

Our laboratory is interested in characterizing the physiology of neurotensin in the gastrointestinal tract. Recently, we demonstrated that neurotensin exerts differential effects on longitudinal and circular muscle of rat duodenum and we suggested the presence of two

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types of neurotensin receptors or, alternatively, of two different transduction mechanisms (Mulè et al., 1992). The effects of neurotensin, however, have not been studied in the rat colon. The present study, therefore, was carried out to evaluate and compare the effects of neurotensin on the longitudinal and circular muscular layer of rat proximal colon and to determine the mechanism of action of neurotensin.

2. Matherials and methods

2.1. General

Adult male Wistar rats (200–300 g) were killed by cervical dislocation and the abdomen was opened via an incision in the midline. A 2-cm long segment of proximal colon was rapidly removed and placed in Krebs solution of the following composition (mM): NaCl 119; KCl 4.5; $MgSO_4$ 2.5; $NaHCO_3$ 25; KH_2PO_4 1.2, $CaCl_2$ 2.5, glucose 11. The content of the excised segment was gently flushed out with Krebs solution. Colonic segments were mounted in a 5-ml organ bath continuously perfused with oxygenated (95% O_2 and 5% CO_2) and heated (37°C) Krebs solution. An isometric-isovolumic preparation was used. The distal end of the colonic segment was tied around the mouth of a J-tube and this was connected via a T-catheter to a syringe and to a pressure transducer (Statham mod. P23XL). The ligated proximal end was secured with a silk thread to an isometric force displacement transducer (Grass FT03). The colonic segment and the connections were filled with Krebs solution and the total volume was set by means of the syringe. The mechanical signals were detected as changes in both endoluminal pressure, which measures mainly circular muscle activity, and isometric tension, which is an index of longitudinal muscle responses. Changes in endoluminal pressure and isometric tension were recorded on a multichannel Grass model 7D polygraph recorder. Preparations, distended with 0.5–0.8 ml Krebs solution, were subjected to an initial tension of 1 g and were allowed to equilibrate for at least 30 min. Since the circular muscular layer exhibited periodicity which was temporarily similar to the periodicity of the longitudinal muscle activity, we needed to establish whether the mechanical recordings did not result from technical artefacts. So, in some experiments, local distension was carried out with a rubber balloon connected to a syringe by thin polyethylene tubing and introduced into the lumen from the anal end of the colonic segment. The balloon was inflated with 0.25 ml of warm water for 15 s. In experiments in which tetrodotoxin was used to eliminate neuronal responses, this was checked by blockade of the response to electrical field stimulation. Electrical field stimulation was applied, by Grass S88 stimulator, in 5-s trains (0.5 ms, 30 Hz, supramaximal voltage), via platinum ring electrodes and it produced in both muscle layers a sudden cessation of spontaneous contractions followed at the end of the stimulus by a rebound contraction. At the beginning of each experiment, the preparation was challenged with 1 μM carbachol until a constant response was achieved. Agonists were added to the bath after switching off the perfusion for the testing time. Antagonists were added to the perfusing solution at least 20 min before the agonist. The tissue was incubated with neurotensin for 5 min. Neurotensin concentration-response curves were obtained non-cumulatively by adding increasing concentrations of the neuropeptide at intervals of 30 min. The results are expressed as the percentage of the maximal neurotensin response achieved.

2.2. Desensitization protocol

Desensitization was used to study receptor specifity. The preparation was pretreated with a concentration of neurotensin (1, 5 or 10 nM) and subsequently, after 20 min, the effect of the added neurotensin was tested. To test the specificity of the neurotensin-induced desensitization the response to carbachol (1 μM) was evaluated before and after desensitization. Desensitization was reversible, with recovery of the amplitude of the response to neurotensin after extensive washout.

2.3. Data analysis

An increase in baseline pressure or tension of more than 1 min in duration with and without superimposed phasic contractions is called a tonic contraction. The amplitude of this tonic contraction was calculated. All data are given as means ± S.E.; n indicates the number of different animal preparations. S.E. is only presented in the figures if it exceeds the dimension of the symbol. The concentration of neurotensin required to cause a half-maximal contractile effect (EC_{50}) was calculated by interpolation from the respective concentration-response curves. Statistical significance was estimated by using paired and impaired Student's t-test and one-way analysis of variance. A P value less than 0.05 was considered to be significant.

2.4. Drugs

The following drugs were used: neurotensin acetate salt, carbamylcholine chloride (carbachol), atropine sulfate, tetrodotoxin, substance P, nifedipine. All drugs were purchased from Sigma. Stock solutions were prepared using distilled water, except nifedipine which was dissolved in absolute ethanol, and kept frozen. The stock solution was then diluted with Krebs on the day of the experiment.

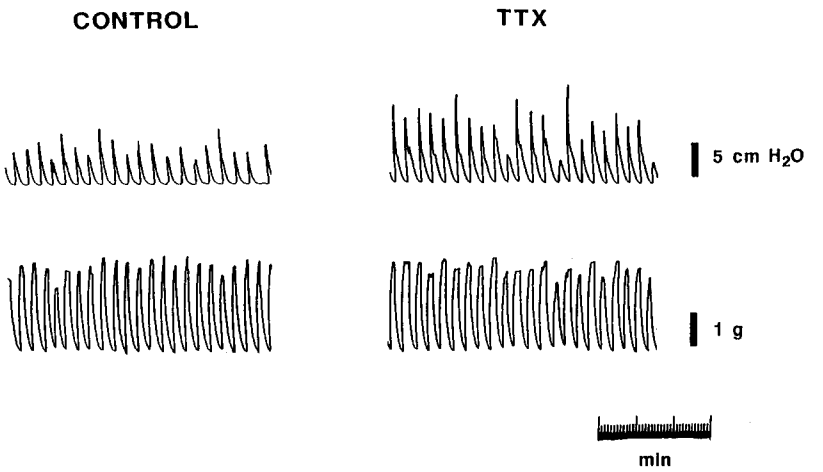

Fig. 1. Motility pattern of isolated rat proximal colon before (control) and after tetrodotoxin (TTX) pretreatment (1 μM). Changes in endoluminal pressure (upper trace) reflect circular muscle activity, changes in isometric tension (lower trace) are an index of longitudinal muscle motor activity. Tetrodotoxin increases the amplitude of the pressure waves, without affecting the force waves.

3. Results

3.1. General

Rat colonic segments exibited spontaneous contractile activity, consisting of phasic changes in both endoluminal pressure (from 5 to 15 cm H_2O) and isometric tension (from 1 to 2.5 g) (Fig. 1). In a single preparation, the amplitude and duration of the pressure and force waves were very regular, varying little with time. The contractions of the two recordings were almost simultaneous, beginning to appear with a delay of about 6 s in the pressure recordings, and the tension peaks were usually earlier than the pressure peaks. The frequency of the spontaneous contractions was not significantly different in the two muscle layers, being 2.52 ± 0.4 c/min for circular muscle and 2.54 ± 0.3 c/min for longitudinal muscle. Atropine (1 μM, $n = 5$) had no effect on the motor activity of the isolated rat proximal colon. Tetrodotoxin (1 μM) significantly increased the amplitude of the pressure waves from 13 ± 0.8 cm H_2O to 23.9 ± 2.4 cm H_2O ($n = 8$; $P < 0.01$) without affecting the force wave amplitude (1.9 ± 0.1 g in control and 1.8 ± 0.2 g after drug, $n = 8$; $P > 0.05$) (Fig. 1). The frequency of the contractions was unaffected by tetrodotoxin ($P > 0.05$). On local distension, colonic segments showed changes in mechanical activity. In fact, after deflation of the balloon, a relaxation followed by contraction of the circular muscle was observed, while longitudinal muscle only contracted.

3.2. Effects of neurotensin on the colonic segment

Neurotensin (1 pM to 0.1 μM) changed the regular pattern of contractions of uniform duration and constant frequency into a pattern characterized by the occurrence of a contraction, persisting throughtout the

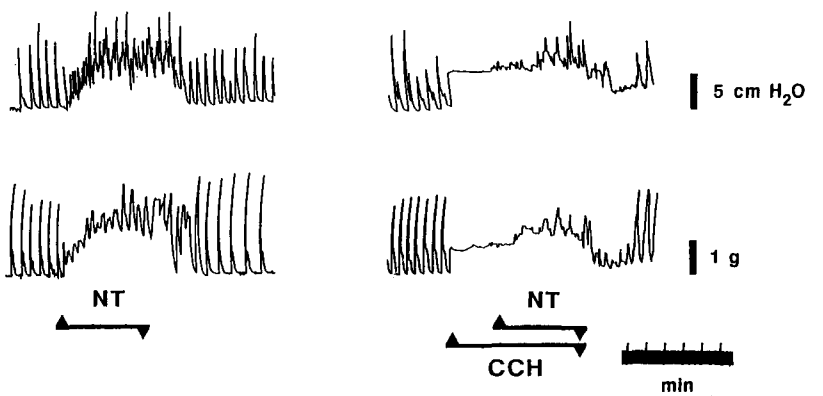

Fig. 2. Effects induced by neurotensin (NT) (0.1 μM) on endoluminal pressure (upper trace) and on isometric tension (lower trace) of isolated rat proximal colon before (control) (left panel) and after carbachol (CCH) (10 nM) (right panel). Note that neurotensin changes the spontaneous motility pattern, inducing a tonic contraction with superimposed phasic oscillations and, in the presence of carbachol, keeps on inducing excitatory effects.

application time, in both muscle layers. Superimposed on this prolonged contraction were much briefer contractions of high frequency (Fig. 2). Regardless of the concentration used, the onset of the effect occurred after about one minute. When neurotensin was tested in preparations precontracted with carbachol (10 nM), neurotensin kept on inducing its contractile effect on both muscle layers (Fig. 2). No relaxation was observed in such conditions.

Neurotensin's excitatory effect was dependent on the concentration used. A maximal contractile effect of 14 ± 0.4 cm H_2O and 2.5 ± 0.3 g, which was about 70% of the 1-μM carbachol contracture, was obtained. Fig. 3 illustrates the concentration-response curves for the increase in tone induced by neurotensin on circular and longitudinal muscle in the control and after various neurotensin concentrations had been applied to desensitize the neurotensin receptors. No difference was found in the potency of neurotensin for inducing the excitatory effects on endoluminal pressure or isometric tension, the EC_{50} values being not significantly different (Table 1). Moreover, the neurotensin concentration-response curves gradually shifted to the right and downwards after desensitization of the neurotensin receptors (Fig. 3). Preincubation of colonic segments with a low concentration of neurotensin (1 nM) did not significantly modify the concentration-response curve for the peptide. Increasing the concentration of neurotensin to 5 nM, led to a significant reduction of the maximal effect of neurotensin (Table 1). With 10 nM neurotensin there was an almost complete loss of the tissue response to the peptide. Desensitization was a reversible process. After extensive washout the sensitivity of the tissue to neurotensin recovered in a time-dependent fashion. The 10 nM carbachol-induced contractile effects were not modified in the presence of neurotensin receptor desensitization ($P > 0.05$, $n = 5$).

Table 1
Excitatory effects of neurotensin (NT) on endoluminal pressure and isometric tension of rat proximal colon before (control) and after various desensitizing concentrations of the peptide

	Endoluminal pressure		Isometric tension	
	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)
NT	0.7 ± 0.09	100 ± 0	0.5 ± 0.1	100 ± 0
NT after NT 1 nM	0.8 ± 0.09	95.0 ± 2	0.6 ± 0.2	96.5 ± 2.5
NT after NT 5 nM	2.8 ± 0.5 [a]	45.8 ± 4.2 [a]	2.6 ± 0.3 [a]	50.6 ± 8.8 [a]
NT after NT 10 nM	10.1 ± 0.8 [a]	18.0 ± 4.0 [a]	9.8 ± 0.7 [a]	20.8 ± 3.2 [a]

The values are given as means ± S.E. for five preparations. [a] $P < 0.01$ as compared to the control value. EC_{50} = neurotensin concentration producing half-maximum effect. E_{max} = maximum effect expressed as percentage reduction of the maximum response.

3.3. Effect of tetrodotoxin and atropine on the neurotensin contractile response

Pretreatment of the colon with 1 μM tetrodotoxin, sufficient to block the response induced by electrical field stimulation, caused a marked reduction of the contractile response to a maximal concentration of neurotensin in both muscle layers. In fact, in the presence of tetrodotoxin the excitatory responses to neurotensin (0.1 μM) were significantly different from the control, being 10.8 ± 0.2 cm H_2O and 2.5 ± 0.2 g before and 5.4 ± 0.1 cm H_2O and 1.4 ± 0.2 g after neurotoxin ($P < 0.001$). However, the response to the peptide exibited a tetrodotoxin-resistant component. The effect of the toxin was reversible. Following extensive

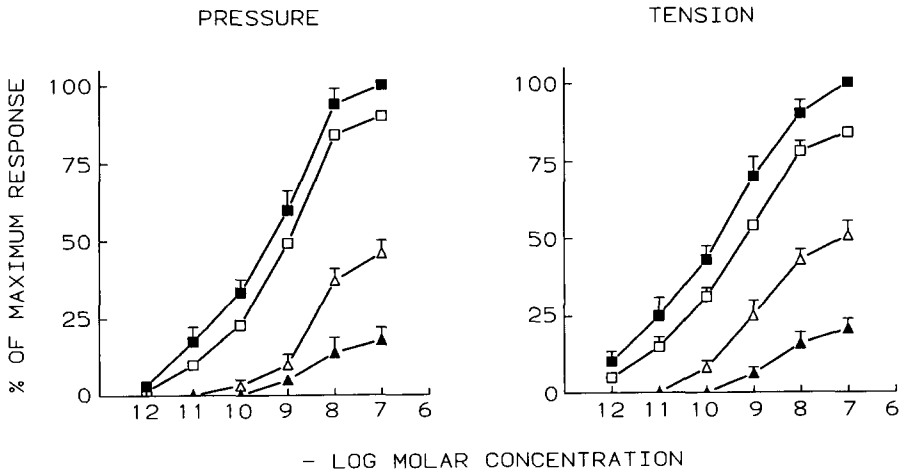

Fig. 3. Concentration-response curves for the contractile effects induced by neurotensin on endoluminal pressure and isometric tension, before (control) and after different desensitizing concentrations of the peptide. The effects are expressed as a percentage of the neurotensin maximal response. Symbols represent the mean of the values obtained from five preparations and bars show the S.E. Responses to peptide were determined prior to (filled square) and following the perfusion of different desensitizing concentrations of neurotensin (1 nM: unfilled square; 5 nM: unfilled triangle; 10 nM: filled triangle). No difference was found in the potency of neurotensin for inducing effects on endoluminal pressure and isometric tension.

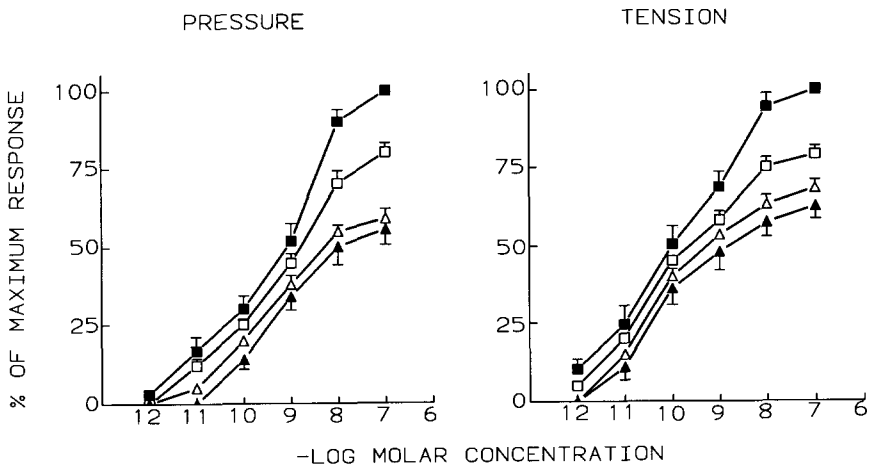

Fig. 4. Concentration-response curves for the contractile effects induced by neurotensin on endoluminal pressure and isometric tension in the absence (filled square) and in the presence of different concentration of atropine (0.1 μM: unfilled square; 0.5 μM: unfilled triangle; 1 μM: filled triangle). The effects are expressed as a percentage of the neurotensin maximal response. Symbols represent the mean of the values obtained from five preparations and bars show the S.E. Atropine partially and non-competitively antagonized the contractile effects of neurotensin.

washout the neurotensin-induced tonic contraction recovered almost completely.

Atropine also antagonized non-competitively and in a concentration-dependent fashion the contractile effect of neurotensin on both muscle layers (Fig. 4). The maximum antagonism was reached at 0.5 μM. Atropine partially and significantly reduced the maximal excitatory action of neurotensin on endoluminal pressure and isometric tension to the same degree as that produced by 1 μM tetrodotoxin. The joint application of atropine (1 μM) plus tetrodotoxin (1 μM), however did not have an additive effect (Fig. 5).

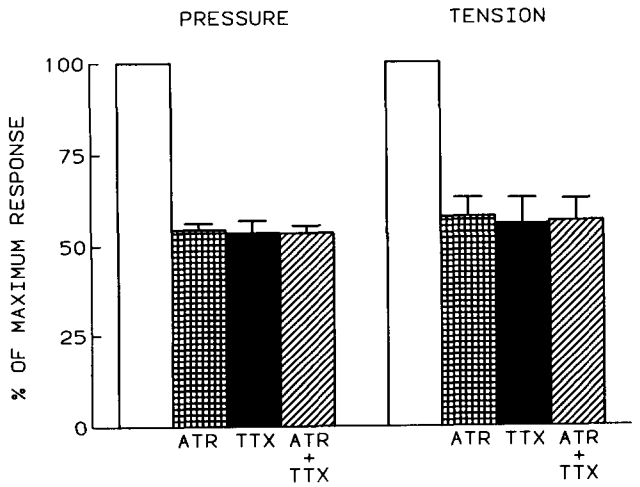

Fig. 5. Histogram showing the effects of atropine (ATR) (1 μM), tetrodotoxin (TTX) (1 μM) or a combination of both on the contractile effect induced by neurotensin (0.1 μM) on endoluminal pressure and isometric tension. Data obtained from five preparations are expressed as means ± S.E. and are reported as a percentage of the maximal response obtained in the control. Atropine and tetrodotoxin antagonized the response to neurotensin to the same degree and tetrodotoxin failed to further reduce the atropine-resistant contractile component.

Furthermore, the contractions induced by neurotensin were also elicited after desensitization of the preparation to substance P. Successive additions (six additions, one every minute) of substance P (50 nM) enhanced the baseline of the two recordings, which gradually returned to its initial level after 10 min. Under such conditions, neurotensin kept on exhibiting its excitatory effect. Lastly, the effect of neurotensin disappeared in the presence of nifedipine (1 nM) (data not shown).

4. Discussion

The results from the present investigation demonstrate that rat colon in vitro shows spontaneous motility which may be modified by neurotensin.

In rat proximal colon, the spontaneous mechanical activity recorded from circular and longitudinal muscle layers showed a regular pattern of contractions of uniform duration, regular force and constant frequency. It is well known that in colon muscle contractions depend on electrical signals referred to as pacemaker activity (Christensen, 1989). Dominance by a single common pacemaker seemed to occur in our preparation since the observed frequency of the spontaneous phasic contractions was not significantly different between circular and longitudinal muscle. Therefore, although data about electrical activity are lacking in this study, it seems that the two muscle layers interact in such a way that periods of contractions in them tend to coincide. Data from tetrodotoxin experiments suggest that the genesis and the resting rate of the contractions were not modulated by neuronal in-

puts. Indeed, tetrodotoxin increased the amplitude of the pressure waves without affecting that of the force waves, indicating that the contractile activity of the circular muscle was tonically suppressed. Tetrodotoxin could act by removing a nervous inhibitory influence which represses circular muscle only, according to the previous reports for rat intestine (Hata et al., 1990; Postorino et al., 1990). In addition, the observed differential effects of tetrodotoxin on circular and longitudinal muscle provide evidence that, in our preparation, the two mechanical recordings correspond to the activities of the two muscle layers. Independence of the two sets of recordings is also indicated by the different response of the two muscle layers observed after local distension of the segment.

In rat colon, neurotensin exerted marked excitatory effects, changing the resting motility pattern in both muscle layers into a long-lasting contraction accompanied by high-frequency oscillatory activity. Indeed, neurotensin has been shown to have excitatory, inhibitory or biphasic effects on gastrointestinal motility, depending on the species, the segment or the muscular layer examined. Previously, we showed that neurotensin has a dual effect on the motor activity of rat duodenum (Mulè et al., 1992). Nevertheless, we may conclude that in rat proximal colon the peptide exclusively induces excitatory effects, since it failed to induce inhibitory responses even when our preparation was pre-contracted by carbachol.

Based on the desensitization experiments, it is possible to conclude that neurotensin-induced excitatory effects are due to occupation of specific receptors for the peptide. Desensitization is used as useful pharmacological tool in receptor studies (Huidobro-Toro and Kullak, 1985; Huidobro-Toro and Yoshimura, 1983; Huidobro-Toro and Zhu, 1984). In our preparation, tissue desensitization to neurotensin is a selective process, failing to affect the contraction in response to carbachol. In the presence of desensitizing concentrations of neurotensin the concentration-response curve for neurotensin was shifted to the right and downwards, indicating a concentration-dependent loss of potency and of the maximal effect.

With regard the mechanism of action of neurotensin, studies in canine, guinea-pig and mouse intestine suggest that the excitatory effect of neurotensin can be mediated by intramural neurons as well as directly on smooth muscle cells (Fontaine and Lebrun, 1985; Fox et al., 1987; Huidobro-Toro, 1983; Kitabgi and Freychet, 1978; 1979). In our preparation, tetrodotoxin partially and significantly reduced the excitatory effects of neurotensin, indicating that the action of neurotensin on rat proximal colon involves neural as well as non-neural factors. As tetrodotoxin blocks neuronal conduction, the tetrodotoxin-sensitive component of the neurotensin contractile effect appears to be due to stimulation of excitatory neurons and the tetrodotoxin-resistant component may be due to stimulation of smooth muscle cells and/or stimulation of the nerve terminals of excitatory neurons. Moreover, the observation that the response to neurotensin was partially antagonized by atropine suggests that the excitatory effect of neurotensin on the rat colon is partially mediated by the mobilization of endogenous acetylcholine. However, the antagonism by tetrodotoxin of the neurotensin-induced excitatory effect was of the same degree as that due to atropine. In addition, no additive effect was observed after the joint application of tetrodotoxin and atropine. These findings allow us to conclude that the tetrodotoxin-sensitive component of the response to neurotensin is due to activation of cholinergic neurons. The lack of effects of desensitization to substance P on the response induced by neurotensin excludes that, in our preparation, the release of substance P is involved in the mechanism of action of neurotensin, as reported for mouse colon (Fontaine and Lebrun, 1985). Consistent with other reports (Huidobro-Toro and Way, 1982; Huidobro Toro et al., 1984), we speculate that a set of excitatory neurotensin receptors is localized on cholinergic nerve cells whereas another subset could be distributed on the smooth muscle membrane. Data obtained with calcium channel inhibitors support this hypothesis. In fact, nifedipine blocked the contractile effect of neurotensin, suggesting that the action of the peptide depends on calcium influx through muscular L-type voltage-dependent calcium channels.

The question arises about the physiological role of neurotensin in the control of colonic motility. In the present study the concentrations needed for a half-maximal contractile effect were in good agreement with those used in other functional in vitro studies (Allescher et al., 1992; Kitabgi and Freychet, 1978; 1979), but were higher than the plasma neurotensin levels. However, it has to be taken into account that the half-life of neurotensin in the rat circulation is only 30 s (Leeman and Carraway, 1982) and that the concentration in the local intestinal circulation might be much higher. Our interpretation is that neurotensin is a local modulator of colonic motility, since the concentrations needed for the motor effects could be reached by local release from its storage cells and/or nerve endings.

Taken together, our data indicate that neurotensin induces marked excitatory effects on both muscle layers of the rat colon. It appears that at least two mechanisms are involved in tha action of neurotensin. There is a cholinergic, tetrodotoxin-sensitive component and a component resistant to the action of both atropine and tetrodotoxin. This latter mechanism seem to be related to a direct action of neurotensin on the muscle cells.

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