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Short communication

Mode and mechanism of neurotensin action in rat proximal colon

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Abstract

This study examined the mechanism of action of neurotensin on intraluminal pressure in rat proximal colon. The direct and indirect contractile response to neurotensin (100 nM) was abolished in Ca^{2+}-free solution, and was antagonized by nifedipine (1–5–10 nM) and potentiated by Bay K 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate) (10–100–1000 nM). Neurotensin, in the presence of nifedipine (10 nM) and atropine (1 μM), induced a tetrodotoxin-insensitive inhibitory effect, which was antagonized by SR 48692 (2[(1-(7-chloro-4-quinolinyl)-5-(2,6-dimethoxy-phenyl)pyrazol-3-yl) carbonyl amino]tricyclo (3.3.1.1.^{3,7}) decan-2-carboxylic acid) (300 nM) or apamin (0.1 μM). The results demonstrate that the neurotensin response is dependent on the influx of Ca^{2+} via L-type channels and results from summation of excitatory and inhibitory effects.

Keywords: Neurotensin; Colon; Ca^{2+} channel, L-type; Mechanical activity

1. Introduction

The mechanisms coupling the activation of neurotensin receptors to the biochemical and electrophysiological changes in the intestinal smooth muscle cells are not fully clear. Studies with small intestine of different species suggest that the direct inhibitory effects of neurotensin are mediated by the opening of apamin-sensitive Ca^{2+}-dependent K^+ channels (Allescher et al., 1992; Christinck et al., 1992; Huidobro-Toro, 1983; Mulè et al., 1992; Ohashi et al., 1994). The direct contractile activity of neurotensin depends on the increase in the intracellular Ca^{2+} concentration produced either by influx of extracellular Ca^{2+} through voltage-dependent channels (Christinck et al., 1992; Donoso et al., 1986; Mulè et al., 1992; Snape et al., 1987) or by release of Ca^{2+} from internal stores (Komori et al., 1992). Little is known about the transduction mechanism underlying the neuronal release of intestinal neurotransmitters such as acetylcholine and substance P, that is induced by neurotensin and responsible for its indirect excitatory effects.

Our previous studies with rat proximal colon have shown that neurotensin exerts contractile effects, acting both directly on smooth muscle cells and indirectly, through acetylcholine release (Mulè et al., 1995). The present work was undertaken to further characterize the mode and the mechanism of action of neurotensin in rat proximal colon.

2. Materials and methods

A 2-cm-long segment of proximal colon removed from Wistar rats (400–500 g) killed by cervical dislocation was mounted in a 5-ml horizontal organ bath continuously perfused with Krebs solution at 37°C, bubbled with 95% O_2 and 5% CO_2. The composition of the Krebs solution was the following (mM): NaCl 119; KCl 4.5; $MgSO_4$ 2.5; $NaHCO_3$ 25; KH_2PO_4 1.2; $CaCl_2$ 2.5; glucose 11.1. An isometric-isovolumic preparation was used as previously described (Mulè et al., 1995). The mechanical changes in intraluminal pressure were displayed on an ink-writing polygraph recorder (Grass model 7D). The preparation was filled with Krebs solution to obtain an initial pressure of 10 cmH_2O and was allowed to equilibrate for at least 30 min before the start of the experiment. Agonists were added into the bath after the perfusion was switched off. The tissue was incubated with neurotensin for 5 min or with bethanechol for 2 min.

To study the influence of Ca^{2+} on the contractile activity of neurotensin and bethanechol, the maximal response to neurotensin and to bethanechol was tested: (i)

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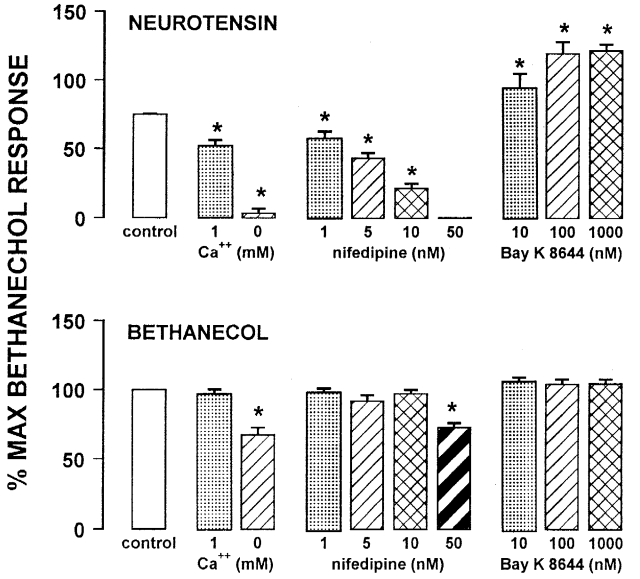

Fig. 1. Influence of (i) manipulations of external Ca^{2+} concentration, (ii) nifedipine and (iii) Bay K 8644 on contractile response induced by neurotensin (100 nM) and by bethanechol (10 μM). Data are expressed as percentages of the contractile response induced by 10 μM bethanechol and are mean values ± S.E. obtained from five preparations. $^{*}P < 0.05$.

under control conditions (regular buffer: 2.5 mM Ca^{2+}), (ii) after 45-min incubation of the preparation with buffer solution containing 1 mM Ca^{2+}, or no Ca^{2+} (this buffer was additionally supplemented with 100 μM EGTA), (iii) after 30-min pretreatment with nifedipine (1 nM to 50 nM), L-type Ca^{2+} channel blocker, or BAY K 8644 (10 nM to 1 μM), L-type Ca^{2+} channel activator.

The statistical significance of differences was determined with the paired Student's *t*-test and *P* values less than 0.05 were considered significant.

The drugs used were: neurotensin acetate salt, carbamyl β-methyl-choline chloride (bethanechol), atropine sulphate, apamin, tetrodotoxin, nifedipine, ethylene glycol bis (β-aminoethyl ether *N,N′*-tetraacetic acid (EGTA) (all from Sigma, St. Louis, MO, USA), SR 48692 (2[(1-(7-chloro-4-quinolinyl)-5-(2,6-dimethoxy-phenyl)pyrazol-3-yl) carbonyl amino]tricylo (3.3.1.1.^{3,7}) decan-2-carboxylic acid), a gift from Sanofi Recherche (Montpellier, France), BAY K 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate), a gift from Bayer (Milan, Italy). Nifedipine was dissolved in absolute ethanol. Bay K 8644 and SR 48692 were dissolved in dimethyl sulfoxide and successive dilutions were made in distilled water.

3. Results

The application of neurotensin (1 pM to 100 nM) to proximal colon produced a concentration-dependent contractile effect, as previously described (Mulè et al., 1995).

The response to the peptide was strongly dependent on the external Ca^{2+} concentration. Reduction of the external Ca^{2+} to 1 mM, which reduced the spontaneous phasic contractions, significantly decreased the response of the preparation to the peptide (100 nM). Omission of Ca^{2+} from the Krebs solution abolished the spontaneous phasic contractions and the response to the peptide. In contrast, the muscarinic contractile response caused by bethanechol (10 μM) was unaffected by the lowering of external Ca^{2+} and was only slightly, although significantly, reduced in Ca^{2+}-free solution (Fig. 1). Nifedipine pretreatment (1 nM) failed to modify the spontaneous mechanical activity; the increase of the concentration up to 10 nM slightly reduced the resting tone and the amplitude of the spontaneous phasic contractions. Nifedipine (1–5–10 nM) dose dependently decreased the contractile activity induced by 100 nM neurotensin without affecting the bethanechol muscle response. Nifedipine, 50 nM, abolished the mechanical spontaneous activity and the effects of neu-

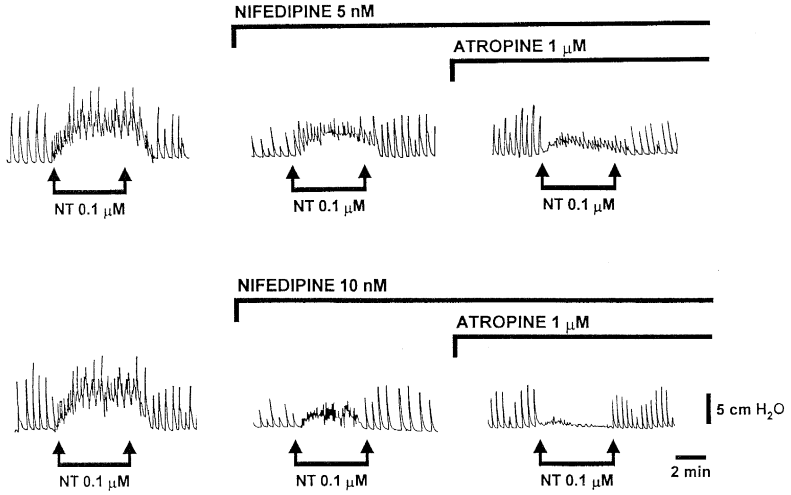

Fig. 2. Representative tracings of the effects induced by neurotensin (100 nM) in the control, after nifedipine and after a combination of nifedipine and atropine.

rotensin and slightly reduced the response to bethanecol (Fig. 1). Bay K 8644 (10–100–1000 nM) increased resting tone and spontaneous activity in a concentration-dependent manner. It produced a significant potentiation of the contractile response induced by 100 nM neurotensin, while it failed to affect the contractile response to 10 μM bethanechol (Fig. 1).

After combined pretreatment with 5 nM nifedipine and 1 μM atropine, neurotensin produced a response which was lower than that evoked in the control or in the presence of nifedipine alone. After combined pretreatment with 10 nM nifedipine and 1 μM atropine, neurotensin produced an inhibitory effect (Fig. 2). Such an inhibitory effect was evoked at neurotensin concentrations ranging from 0.1 nM to 100 nM and it always appeared as complete suppression of the phasic contractions. Exposure to SR 48692, the non-peptide antagonist of the neurotensin receptors (Gully et al., 1993), at a concentration (300 nM) previously shown to antagonize effects of neurotensin (Mulè et al., 1996), resulted in complete antagonism of the inhibitory response to the peptide. In addition, the inhibitory response, unmasked by atropine and nifedipine, was unaffected by pretreatment with tetrodotoxin (1 μM) and was abolished in the presence of apamin (0.1 μM).

Lastly, 30-min apamin (0.1 μM) pretreatment significantly increased the contractile response to neurotensin (0.1 μM) from 15 ± 0.6 cmH_2O to 20 ± 0.5 cmH_2O ($n = 4$; $P < 0.05$), while it failed to affect the contractile response to bethanechol (10 μM) (20.5 ± 0.5 cm H_2O; $n = 4$; $P > 0.05$).

4. Discussion

The present results show that the contractile response of rat proximal colon to neurotensin is dependent on external Ca^{2+} and results from summation of excitatory and inhibitory effects.

The source and the role of Ca^{2+} in the contractile effects evoked by neurotensin in gastrointestinal muscle are controversial (Regoli et al., 1994) and they have been investigated regarding only the direct myotropic effect (Donoso et al., 1986; Huidobro-Toro and Kullak, 1985; Snape et al., 1987). Our experimental model was chosen to investigate the role of Ca^{2+} in neurally mediated effects of neurotensin also, since we have previously shown that the peptide acts via a double mechanism (Mulè et al., 1995).

In our study, in agreement with the results obtained with other gastrointestinal preparations (Donoso et al., 1986; Huidobro-Toro and Kullak, 1985; Snape et al., 1987) the direct component of the response to neurotensin is dependent on extracellular Ca^{2+}. Since manipulations of external Ca^{2+} concentration also block the indirect component of the contractile response to the peptide, the entire excitatory mechanical response to neurotensin appears to be dependent on extracellular Ca^{2+}. The observation that the response to bethanechol is quite resistant to omission of Ca^{2+} suggests that Ca^{2+} is involved in neurotransmitter release but not in the muscarinic transduction mechanism.

The observation that the excitatory mechanical action of neurotensin is sensitive to drugs that block (nifedipine) or that activate (Bay K 8644) L-type voltage-dependent Ca^{2+} channels, while the contractile response to bethanechol is not attenuated or potentiated by the same drugs, suggests that activation of neurotensin receptors may lead to the opening of L-type voltage-dependent Ca^{2+} channels, whereas the muscarinic receptors are not essentially linked to the same channels.

We wondered why nifedipine antagonized the entire response to neurotensin since the muscarinic effects are not dependent on Ca^{2+} influx through L-type voltage-dependent channels. We, therefore, tested atropine effects on the residual response to neurotensin in the presence of nifedipine, in order to verify if, under these conditions, acetylcholine release was impaired. Atropine antagonized the response to the peptide after nifedipine, suggesting that neurotensin was still able to induce acetylcholine release. Therefore, L-type Ca^{2+} channels are not involved in the acetylcholine mobilization by neurotensin. On the other hand, in peripheral preparations, L-type Ca^{2+} channel effects appear to be restricted to post-junctional elements (Wessler et al., 1990).

It is worth noting that neurotensin in the presence of atropine and 10 nM nifedipine, which respectively block indirect and direct contractile effects, induces the complete suppression of spontaneous phasic mechanical activity. Therefore, in rat proximal colon, the peptide is able to induce an inhibitory response, apart from contractile effects, suggesting that the entire response to neurotensin results from summation of excitatory and inhibitory responses. Thus, the progressive reduction of the entire neurotensin response, observed in the presence of nifedipine, could be due to a gradual prevalence of the inhibitory effect over the excitatory one. Additional evidence in support of our hypothesis is provided by the observation that apamin, which antagonized the unmasked inhibitory effect, increases the contractile effects of the peptide, without affecting the bethanechol-induced response.

The inhibitory effect of neurotensin is antagonized by SR 48692, indicating that it is mediated by specific and selective receptor sites. The resistance of this inhibitory effect to tetrodotoxin suggests that it does not involve neuroaxonal transmission and could be due to a direct action on the smooth muscle cells or to release of certain inhibitory transmitters from prejunctional sites. In our preparation, the inhibitory effect of neurotensin was blocked by apamin, suggesting an involvement of Ca^{2+}-dependent potassium channels in the mechanism of action of neurotensin. This is in agreement with the findings with small intestine of various species (Allescher et al., 1992; Christinck et al., 1992; Kullak et al., 1987; Mulè et al., 1992).

In conclusion, our study provides evidence for the coexistence of inhibitory effects with contractile effects induced by neurotensin in rat proximal colon. The involvement of an influx of Ca^{2+} through L-type voltage-dependent channels in the direct contractile action of neurotensin seems obvious, whereas acetylcholine mobilization by neurotensin does not appear to be dependent on the opening of L-type calcium channels.

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