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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

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1 The contractile activity of neurotensin and acetylcholine on rat isolated fundus strips was examined in preparations maintained in Tyrode buffer containing 2.5, 1.0 or 0 mM Ca^{2+}. While the neurotensin contractions depended markedly on the external Ca^{2+} concentration, the acetylcholine-induced muscular responses were not significantly affected by omission of calcium in the superfusion media.

2 Pre-incubation of rat fundus strips with nifedipine (0.03–3.8 μM), dialtiazem (0.5–3.5 μM) or methoxyverapamil (0.3–1.3 μM) antagonized in a non-surmountable fashion the contractile activity of neurotensin but not of acetylcholine.

3 Pretreatment with Bay K 8644 potentiated in a concentration-dependent fashion the contractile activity of rat fundus strips to neurotensin without modifying to any significant degree the acetylcholine-induced contractions.

4 Nifedipine blocked in a concentration-dependent manner the Bay K 8644-induced potentiation of the neurotensin contractile responses in the fundus.

5 Results demonstrate the dependence on external calcium of the contractile activity of neurotensin and the resistance of the muscarinic response to external calcium manipulations. The coupling of the neurotensin receptor to calcium channels is discussed.

Introduction

The gut-brain regulatory peptides have raised considerable interest among neurobiologists within the past few years. Neurotensin, a 13-amino acid neuropeptide is one of such substances; it was originally discovered and chemically characterized from bovine hypothalamus by Carraway & Leeman (1973). A few years later it was isolated from mammalian and human intestine (Kitabgi *et al.*, 1976; Hammer *et al.*, 1980). That neurotensin may play a role in the control of the gastro-intestinal activity is supported by the fact that picomoles of exogenously applied neurotensin to isolated gut preparations greatly modify the motility of the fundus and the intestines (for a review see Kitabgi, 1982). In addition, 85% of the total neurotensin body content is found in the intestines (Carraway & Leeman, 1976). Neurotensin could thus function in the gut as a neurotransmitter, a neuromodulator or as a hormone. Compatible with a hormonal role of neurotensin in the gastro-intestinal tract is the fact that the plasma concentrations of neurotensin are comparable to that of established gastro-intestinal hormones, such as cholecystokinin. Furthermore, several investigators demonstrated that food, particularly fats, cause a significant rise in the circulating level of neurotensin (Mashford *et al.*, 1978; Rosell & Rökaeus, 1979).

Our laboratory is interested in characterizing the physiology of neurotensin in the gastro-intestinal tract. In a recent publication on the rat fundus we demonstrated that neurotensin-induced contractile activity but not that of acetylcholine is antagonized in a non-competitive fashion by verapamil (Huidobro-Toro & Kullak, 1985). This result suggested that the activation of the neurotension receptor in the rat fundus is likely to be coupled with the influx of extracellular calcium ions, via the opening of Ca^{2+} channels. The objective of this investigation was to test further this working hypothesis. We have now

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examined the dependence of the contractile activity of neurotensin on external calcium and tested the effect of a variety of calcium channel blockers on the neurotensin-induced contractions. Results are contrasted with the contractile activity induced by muscarinic agents. In addition, we describe the effect of the novel 1,4 dihydropyridine, Bay K 8644 (Schramm *et al.*, 1983), on the muscular activity of neurotensin and acetylcholine.

Methods

Animals and tissue preparations

Adult Sprague Dawley rats (250–300 g) were killed by cervical dislocation; the abdomen was opened via an incision in the midline. The whole stomach was dissected, washed with the superfusion buffer solution and transferred to a Petri dish for tissue preparation and mounting in isolated organ baths according to the method described by Donoso & Huidobro-Toro (1985). The composition of the bathing buffer was as follows (mM): NaCl 118, KCl 5.4, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 23.8 and glucose 11.1. The buffer was gassed with 95% O_2:5% CO_2 to maintain a pH close to 7.4. Preparations were maintained under a tension of 1.5 g. Isometric muscular activity was measured with a Grass force displacement transducer (FT03C) connected to a Grass multichannel polygraph. Contractions from 3 separate strips could be recorded concurrently.

Quantification of results: concentration-response curves

Neurotensin and acetylcholine (ACh) concentration-response curves were performed non-cumulatively by adding increasing concentrations of the agonists to the tissue chambers, until maximum muscular responses occurred. Agonist applications were performed regularly at 10 min intervals. Results are expressed as the percentage of the contractile activity induced with 5 μM acetylcholine or as g of tension generated by either neurotensin or ACh. Each concentration-response curve was repeated at least 8 times in separate tissues.

Manipulations of the external calcium concentration

To study the influence of calcium on the contractile activity of neurotensin and ACh, the superfusion buffer solutions were prepared without calcium (this buffer was supplemented additionally with 100 μM ethylene glycol *bis* (β-aminoethyl ether) N, N'-tetra acetic acid (EGTA), 1.0 or 2.5 mM calcium (regular buffer). The protocol called initially for a complete neurotensin and ACh concentration-response curve in a 2.5 mM Ca^{2+} buffer. After completion of the two response curves, the preparations were incubated for 45 min in buffer containing 1.0 mM Ca^{2+} or no Ca^{2+} (buffered with 100 μM EGTA), and the contractile activity of neurotensin and ACh was re-evaluated. Results are expressed as a percentage of the muscular tension generated by 5 μM ACh in the buffer solution containing 2.5 mM Ca^{2+}. In these protocols, each preparation served as its own control.

Studies with drugs that block calcium channels

Neurotensin and ACh concentration-response curves were performed in the absence and in the presence of calcium channel blockers. The same fundus preparation was used to test the activity of neurotensin and ACh and one concentration of the various calcium channel blockers was studied. Three concentrations of nifedipine were tested: 0.03, 0.5 and 3.8 μM. Diltiazem was examined at 0.5 and 3.5 μM; methoxyverapamil (D600, gallopamil) was tested at 0.3 and 1.3 μM. The channel blocking drugs were added to the tissue chambers for 2 min before the addition of the concentrations of neurotensin or ACh. Each concentration of agonist was added regularly at 10 min intervals. Results are expressed as a percentage of the muscular tension generated by 5 μM ACh in the absence of calcium channel blockers. Experiments were replicated in at least 6 different preparations.

In the particular case of nifedipine, the contractile activity of angiotensin II and 5-hydroxytryptamine (5-HT) was also examined.

Studies with Bay K 9644 and its interaction with nifedipine

To evaluate the effect of the alleged calcium channel agonist, Bay K 8644, fundus strips were preincubated for 2 min before the addition of 2 nM neurotensin or 50 nM ACh, with various concentrations of Bay K 8644 which remained in the solution when neurotensin or ACh was added to the chambers. In other experiments, neurotensin and ACh response curves were constructed in the absence and, in the presence, of Bay K 8644 92 nM. Tissues were always preincubated with Bay K 8644 for 2 min before the addition of the agonists.

In a separate series of experiments, to test whether the potentiating action of Bay K 8644 is due to the activation of a calcium channel, we investigated the displacement by nifedipine of the activity induced by Bay K 8644. For this purpose, tissues were preincubated with 92 nM Bay K 8644 followed a minute later by the application of various concentrations of nifedipine in the continued presence of Bay K 8644. A minute after the addition of nifedipine, all preparations were challenged with 2 nM neurotensin or 50 nM ACh. Results express the activity as the muscular

tension (g) developed in response to neurotensin or acetylcholine.

In a parallel series of experiments, neurotensin or ACh concentration-response curves were obtained in the absence and in the presence of both 92 nM Bay K 8644 plus 1.9 μM nifedipine.

Statistical analysis

The paired or non paired Student's *t* test was used. Significance was set at a *P* value of less than 0.05.

Drugs sources

Neurotensin, angiotensin II, 5-hydroxytryptamine creatine sulphate, acetylcholine were obtained from Sigma Chemical Co. (St. Louis, MO). Nifedipine and Bay K 8644 were kindly provided by Dr F. Hoffmeister (Bayer A.G., Wüppertal, Germany). The dihydropyridines were dissolved, immediately before each testing, in a mixture of propyleneglycol, ethyl alcohol and water (1.5:1.5:7). To avoid photo-decomposition, the drugs were maintained in dark containers; the experiments were carried out during daytime with no external lighting. The solvent proved to be without effect on the contractile activity of the fundus. Diltiazem HCl was provided by Marion Labs (Kansas, U.S.A.) and methoxyverapamil HCl (D600, gallopamil) was a gift from Prof. A. Pinto Corrado (Ribeirao Preto, Brasil).

Results

Contractile activity of neurotensin and acetylcholine: calcium dependence

Picomolar concentrations of neurotensin caused a concentration-dependent contraction of the rat fundus. The maximal tension developed by neurotensin reached generally between 50 to 60% of that attained by ACh. The median effective concentration of the peptide was 1.5 nM, whereas the potency of ACh was 250 nM, a value approximately 150 times larger than that of the peptide (Figure 1).

The contractile activity induced by neurotensin, was markedly dependent on the external concentration of calcium in the Tyrode buffer. Reduction of the external calcium to 1.0 mM, significantly decreased the maximum neurotensin-induced tension and flattened the peptide response curve. Omission of calcium from the incubation media, abolished the neurotensin-induced contractions except at the highest concentrations of the neuropeptide where modest responses were still recorded (Figure 1). In marked contrast to the neurotensin-induced contractile activity, the muscarinic responses caused by ACh were relatively unaffected by the same changes in external calcium. As shown in Figure 1b, reduction of the external calcium concentration to 1.0 mM caused a minor but significant reduction in the ACh contractile effect. Omission of calcium from the incubation buffer (zero Ca^{2+} plus EGTA) caused a modest effect on the ACh-induced contractility of the fundus.

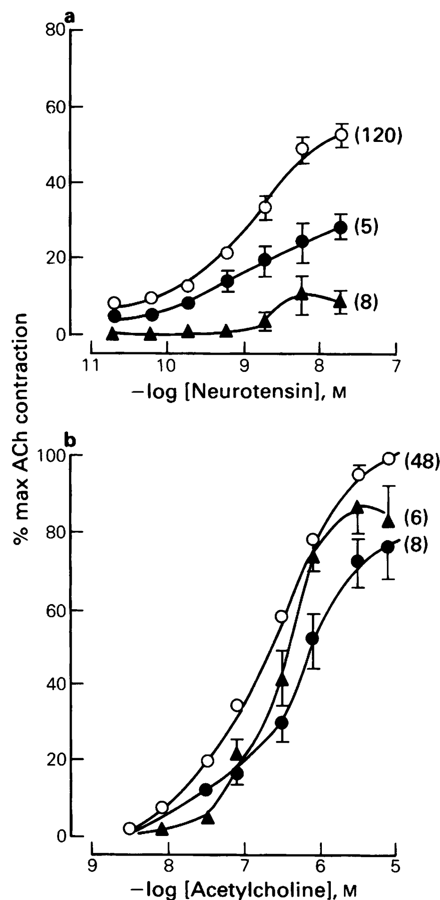

Figure 1 Determination of neurotensin and acetylcholine concentration-response curves in buffer solutions containing various concentrations of external calcium. Agonist response curves were obtained in rat fundus strips maintained in 2.5 mM Ca^{2+} (○), 1.0 mM Ca^{2+} (●) or no calcium plus 0.1 mM EGTA (▲). All tissues were challenged with 5 μM acetylcholine in Tyrode buffer containing 2.5 mM Ca^{2+} before changing the calcium concentration in the tissue superfusion media. Symbols indicate the mean values expressed as a percentage of the contraction induced with 5 μM acetylcholine; vertical lines denote s.e.mean. Numbers in parentheses denote the times the experiment was repeated in separate tissues.

Effect of calcium channel blocking drugs

(a) *Nifedipine.* Tissue pretreatment with 0.03 or 0.5 μM nifedipine substantially decreased the neurotensin-induced contractile activity of the fundus. In contrast, these concentrations of nifedipine did not modify the muscular effects induced by ACh (Figure 2). Further increases in the concentration of nifedipine to 3.8 μM abolished almost entirely the contractions induced by neurotensin while producing a modest five fold displacement to the right of the ACh-induced response curve. Pretreatment with 3.8 μM nifedipine reduced by no more than 25% the maximum tension generated by acetylcholine.

In a parallel series of experiments, we examined further the effect of nifedipine on the contractile activity induced by angiotensin II and 5-hydroxytryptamine (5-HT) on the rat fundus. Figure 2 shows that

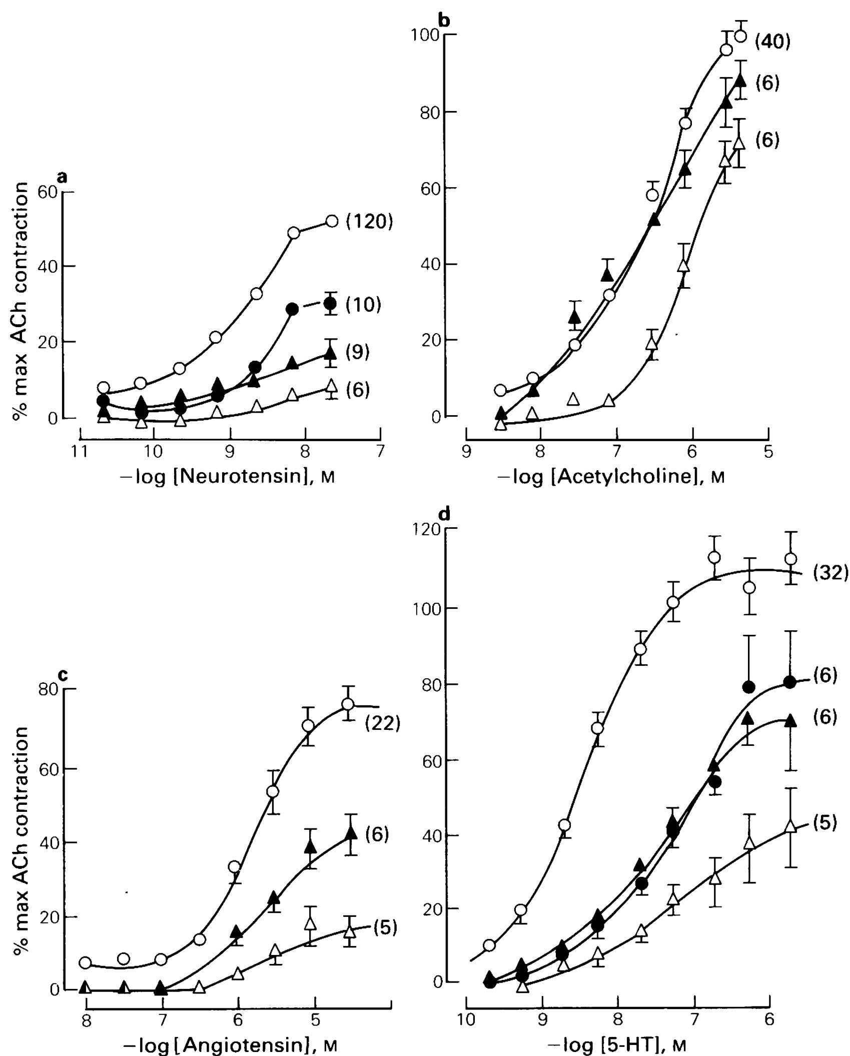

Figure 2 Effect of nifedipine on the contractile activity of neurotensin and other agonists. Neurotensin (a), acetylcholine (b), angiotensin II (c) and 5-hydroxytryptamine (5-HT) (d) concentration-response curves were obtained in the absence and in the presence of 0.03 (●), 0.5 (▲) or 3.8 (△) μM nifedipine; (○) control. Each tissue was used to evaluate the effect of only one concentration of the calcium entry blocker. Points indicate the mean contractile activity expressed as a percentage of that induced by 5 μM acetylcholine; vertical lines denote s.e.mean. Numbers in parentheses indicate the times each experiment was repeated in a separate preparation.

in contrast to the ACh-induced contractions, nifedipine caused a non-surmountable blockade of the contractile activity of these agents. When comparing this family of curves, it can be observed that neurotensin and angiotensin II were the most sensitive to nifedipine; 5-HT exhibited an intermediate sensitivity and ACh was the least affected by the blocking action of nifedipine.

(b) *Diltiazem.* Preincubation of fundus strips with 0.5 or 3.5 μM diltiazem substantially antagonized the contractile activity of neurotensin without significantly affecting the muscarinic potency of ACh. As shown in Figure 3, diltiazem 3.5 μM flattened the neurotensin-response curve. The muscular tension generated in response to 30 mM neurotensin was reduced by 40 and 80% with 0.5 and 3.5 μM diltiazem respectively; the activity of acetylcholine remained little altered by the same treatment.

(c) *Methoxyverapamil (D600).* Like other calcium channel blockers, D600 antagonized in an unsurmountable fashion the contractile activity of

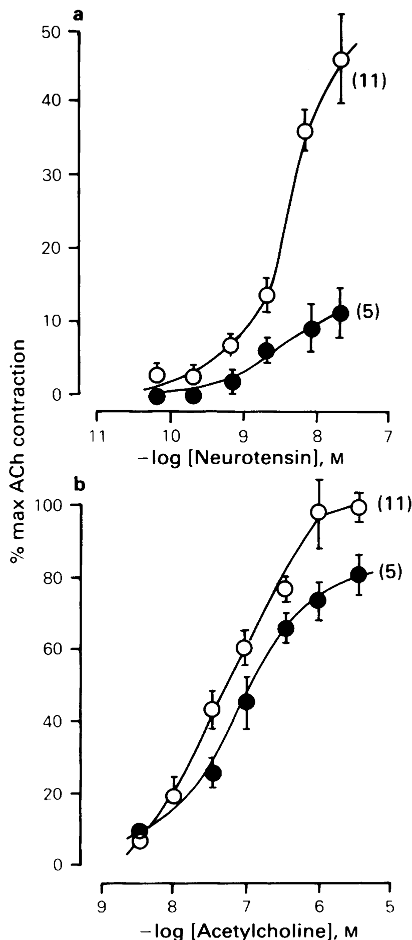

Figure 3 Effect of diltiazem (3.5 μM, ●) on neurotensin (a) and acetylcholine (b) concentration-response curves; (○) control. The calcium channel blocker was added to the tissue superfusion media 2 min before agonist application. Symbols express the mean percentage of the contractile response to 5 μM acetylcholine; vertical lines denote s.e.mean.

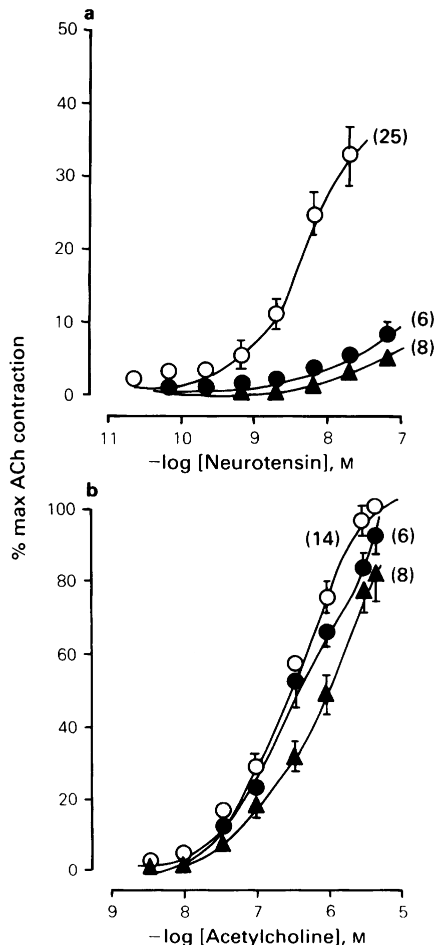

Figure 4 Effects of preincubation with methoxyverapamil on the contractile activity induced by neurotensin and acetylcholine. Concentration-response curves to neurotensin (a) and ACh (b) were obtained in the absence and in the presence of either 0.3 or 1.3 μM methoxyverapamil (D600). Full points denote the mean percentage of the contractile activity induced with 5 μM acetylcholine; vertical lines indicate s.e.mean. Figures in parentheses indicate the number of times the experiment was repeated in separate preparations.

neurotensin but to a much lesser extent that induced by ACh. Figure 4 shows that D600 1.3 μM substantially reduced the neurotensin activity without modifying to nearly the same extent the potency of ACh.

Potentiation of the neurotensin activity by Bay K 8644

Preincubation of rat fundus strips with as little as 9.2 nM Bay K 8644 produced a significant potentiation of the contractile response induced with 2 nM neurotensin. The effect of Bay K 8644 was concentration-dependent (Figure 5); preincubation of the tissues with 920 nM Bay K 8644 tripled the tension generated by 2 nM neurotensin ($P < 0.01$). In contrast, the muscular activity induced by 50 nM ACh was not significantly affected by applications of Bay K 8644; although there was a minor potentiation it did not reach statistical significance (Figure 5).

Bay K 8644 itself produced a slow rising contractile response when applied to the fundus in concentrations greater than 92 nM (Figure 6). Pretreatment with 92 nM Bay K 8644 caused a shift in the neurotensin concentration-response curve to the left, significantly increasing the potency and the maximum tension generated by neurotensin (Figure 6). In contrast to the effects of neurotensin, Bay K 8644 92 nM did not significantly modify the potency of ACh. Pretreatment of the same tissue with 480 nM nifedipine, a structural analogue of Bay K 8644, caused a non-parallel displacement of the neurotensin contractile response curve to the right without significantly altering the potency of ACh (Figure 6).

Blockade of the potentiating action of Bay K 8644 by nifedipine

Nifedipine inhibited the Bay K 8644-induced potentiation of neurotensin contractile response. The simultaneous application of Bay K 8644 (92 nM) (plus nifedipine (1 μM) completely blocked the potentiating

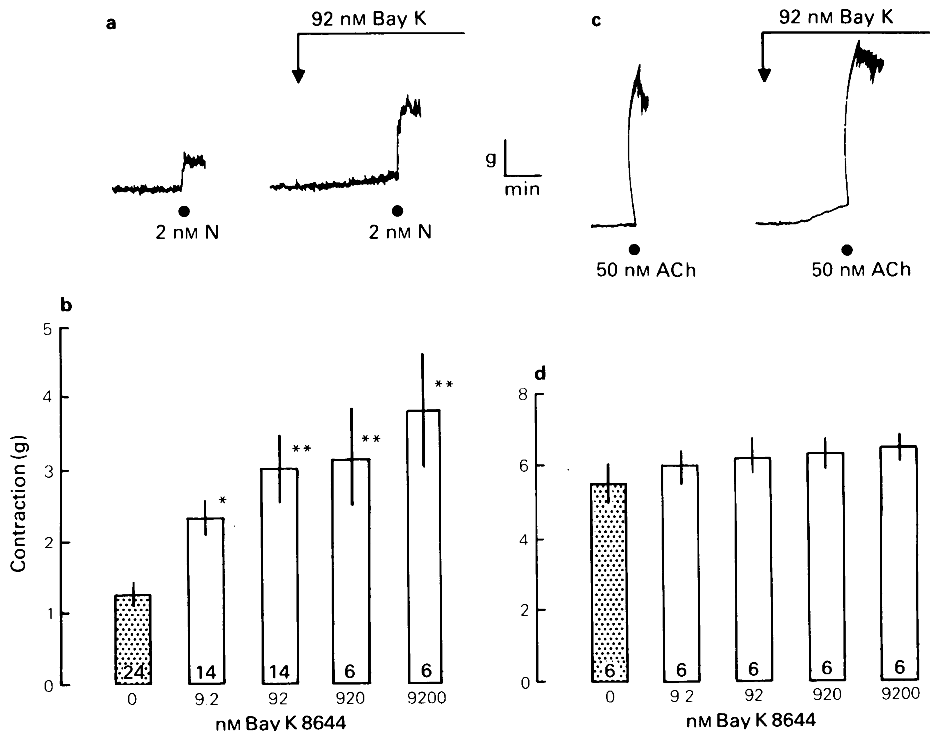

Figure 5 Bay K 8644-induced potentiation of the neurotensin but not muscarinic contractile responses. The contractile effect caused by 2 nM neurotensin (N) or 50 nM acetylcholine (ACh) was measured in the absence and in the presence of various concentrations of Bay K 8644. (a) and (c) show representative recordings of the agonists' effects obtained before and after 92 nM Bay K 8644. Columns in (b) and d) show the effect of increasing concentrations of Bay K 8644 on the agonist-induced contractions. $*P < 0.05$; $**P < 0.01$.

effect of Bay K 8644 compound; higher concentrations of nifedipine substantially reduced the contractions generated by 2 nM neurotensin in the presence of Bay K 8644 (92 nM) (Figure 7a). Neurotensin concentration-response curves elicited in the presence of both Bay K 8644 (92 nM) plus nifedipine(1.9 μM) were shifted to the right in a parallel fashion consistent with a competitive blockade of the Bay K 8644-induced potentiation by nifedipine (Figure 7a). Neither Bay K 8644 nor nifedipine modified the contractile activity of 50 nM ACh (Figure 7b).

Discussion

It is clear from the present results that, although the neurotensin-induced contractile response was sensitive to manipulations of the external calcium concentration and to the effect of agents that perturb calcium fluxes via blockade of the calcium channel, the muscarinic responses of ACh were markedly resistant to these manoeuvres. The flattening of the neurotensin-response curves either by changes in the external calcium concentration or by action of the channel

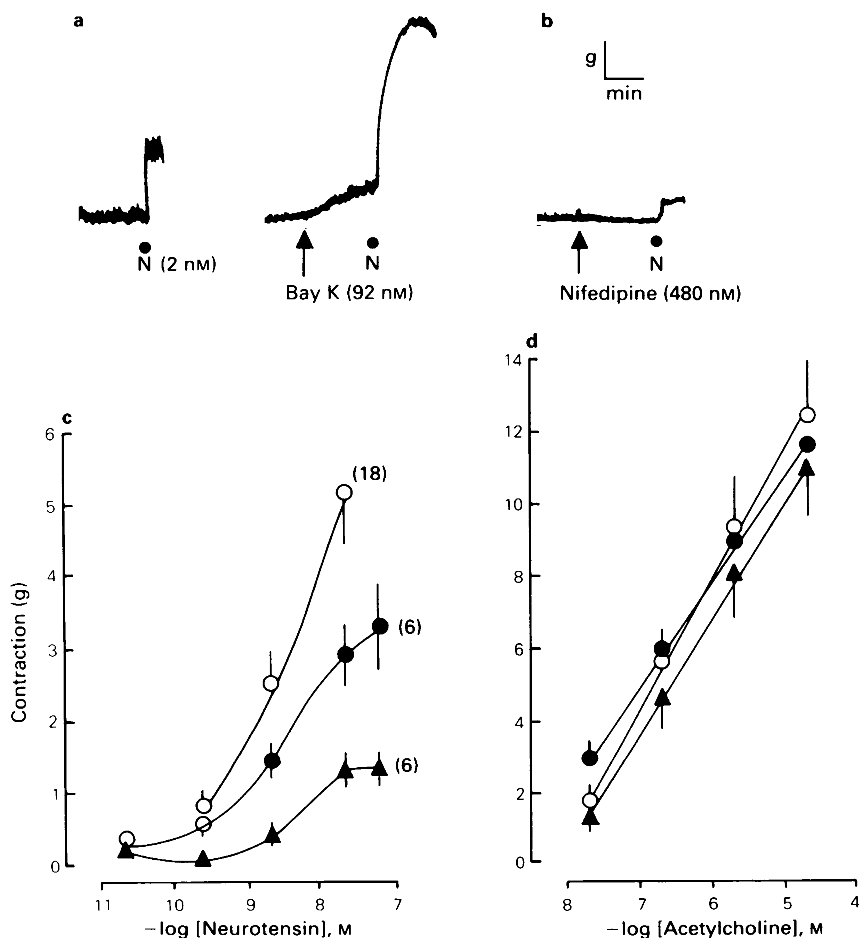

Figure 6 Neurotensin and acetylcholine concentration-response curves obtained in the presence of nifedipine or Bay K 8644. Rat fundus preparations were tested with neurotensin (c) and acetylcholine (d) in the absence and in the presence of either 480 nM nifedipine (▲) or 92 nM Bay K 8644 (○); control (●). Symbols indicate the mean muscular tension (g) generated; vertical lines show s.e.mean. Separate preparations were used to examine the effect of neurotensin or acetylcholine. (a) and (b) show representative tracings of the isometric contractions induced with 2 nM neurotensin (N) in the presence of either of the 1,4-dihydropyridines.

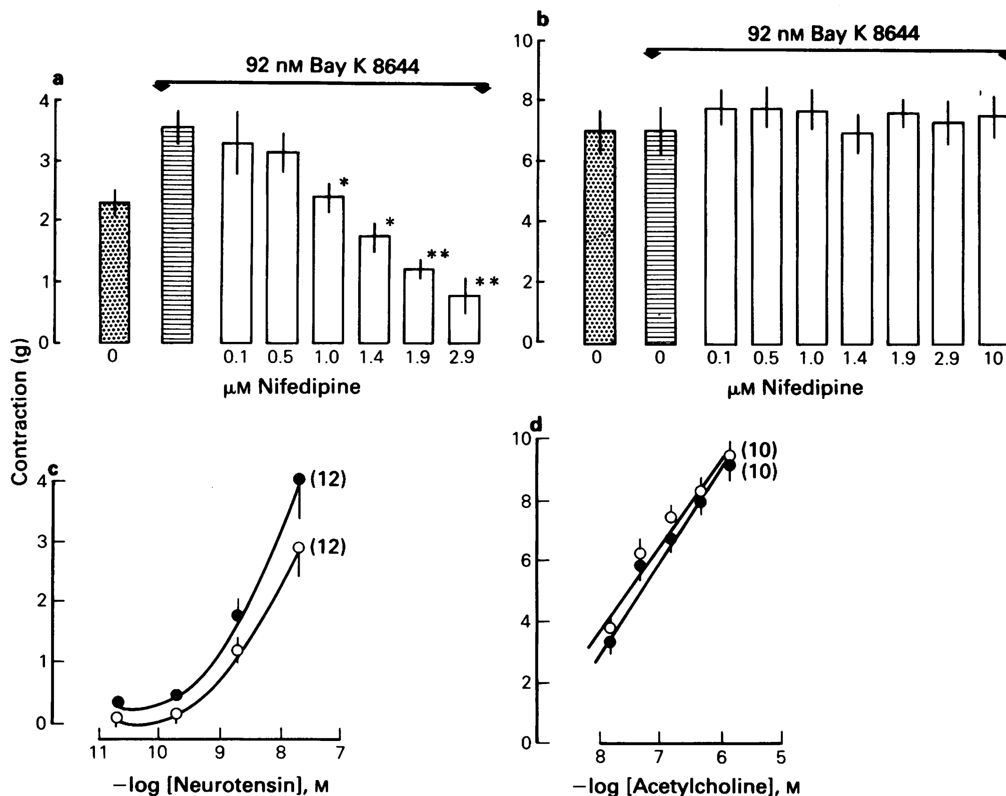

Figure 7 Blockade of the Bay K 8644-induced neurotensin potentiation by nifedipine. (a) Contractile effect generated by the application of 2 nM neurotensin; (b) addition of 50 nM acetylcholine. Control preparation (stippled column); hatched columns represent the effect of neurotensin (a) or acetylcholine (b) obtained in the presence of 92 nM Bay K 8644 alone. Open columns represent muscle contraction generated by either neurotensin or acetylcholine in the presence of 92 nM Bay K 8644 plus graded concentrations of nifedipine. Columns indicate the mean tension (g); vertical lines show s.e.mean obtained in six separate fundus preparations. *$P < 0.05$ in relation to the effect of neurotensin plus Bay K 8644; **$P < 0.01$. (c) Neurotensin and (d) acetylcholine-response curves in the absence (●) and in the presence of 92 nM Bay K 8644 plus 1900 nM nifedipine (○).

blockers may be viewed as an effect occuring distal to the neurotensin receptor. As discussed previously, the neurotensin-induced contractions of the fundus do not involve neuronal activity since the contractions are resistant to tetrodotoxin (Huidobro-Toro & Kullak, 1985). Thus, the lack of contractile response to neurotensin in a medium low in external calcium or with blockade of the calcium channels probably reflects a failure at the level of the membrane signal transduction mechanism activated by the neurotensin receptor. It follows that neurotensin, in contrast to ACh, cannot mobilize intracellular calcium pools to initiate a contractile event in this muscle.

In support of our view, Huidobro-Toro & Kullak (1985) demonstrated that the neurotensin-induced contractions were antagonized by verapamil. We have now extended our studies to demonstrate that three structurally-unrelated groups of calcium channel blocking drugs, such as diltiazem, methoxyverapamil or nifedipine, all antagonized in a non-surmountable fashion the neurotensin-induced contractions without altering to a similar extent the muscarinic effect of ACh. In addition, we have further expanded our arguments by demonstrating that a structural analogue of nifedipine, compound Bay K 8644, a modified dihydropyridine, potentiates the action of neurotensin. This compound has been recently demonstrated to promote calcium influx via a nifedipine-sensitive mechanism in a variety of biological systems (Schramm et al., 1983; Janis et al., 1984; Taira et al., 1985). Present results clearly show that this is also the case in the rat fundus: preincubation with Bay K 8644 causes a concentration-dependent potentiation of the neurotensin-induced contractile responses. The Bay K 8644-induced potentiation of the neurotensin responses was blocked by nifedipine. However, it

should be noted that Bay K 8644 does not increase all smooth muscle contractility unspecifically, since in this bioassay for example, the muscarinic effect of ACh is little affected by the presence of Bay K 8644.

An explanation compatible with these findings argues in favour of the notion that the activation of the neurotensin receptor (probably localized on the smooth muscle membrane: Donoso & Huidobro-Toro, 1985; Huidobro-Toro & Kullak, 1985) is coupled to the opening of calcium channels sensitive to allosteric modulation by the dihydropyridines and related drugs. Thus, calcium channel blockers inhibit the neurotensin effect by reducing the influx of calcium following receptor activation. However, in the presence of Bay K 8644, the influx of calcium mobilized by the neurotensin receptor is facilitated. Nifedipine blocks the Bay K 8644-induced potentiation by blocking the influx of calcium caused by neurotensin and potentiated by Bay K 8644. The selectivity of Bay K 8644 in the fundus is demonstrated by the fact that the contractile activity of ACh is not significantly altered either by nifedipine or Bay K 8644. Consistent with this finding, some authors have explained that the main source of calcium for the muscarinic contractile responses in visceral smooth muscles is not necessarily of extracellular origin, as in the case of neurotensin, but that part of it may originate from intracellular stores (Ishizawa & Miyazaki, 1977; Bolton, 1979; Shinohara & Kosaka, 1984). In this connection, the physiology of muscarinic responses has been hypothesized to occur by degradation of membrane phospholipids via activation of phospholipase C which generates inositol triphosphate and diacylglycerol (Brown & Brown Masters, 1984; Berridge & Irvine, 1984). It is currently accepted according to this hypothesis that phosphorylated inositol mobilizes intracellularly stored calcium ions from the reticulum, sites not sensitive to the action of 1,4-dihydropyridines. That the muscarinic responses to ACh are only minimally attenuated by the channel blockers may be interpreted as an indication that the intracellular calcium pools must be sufficiently large to overcome the blockade of a fraction of the channels. However, it is possible that massive concentration of these drugs will eventually also abolish muscarinic responses.

With regard to selective alterations of the phasic or tonic components of the neurotensin-induced contractions of the fundus (Golenhofen & Wegner, 1975) either by changes in external calcium concentration or by the channel blockers, it is difficult to state differences except to say that the total contractile capacity of neurotensin was markedly attenuated. However, in the case of the contractile activity of ACh in the ileum, a response that is not as well maintained as in the case of the fundus, we frequently observed that omission of calcium in the buffer or the addition of calcium entry blockers to the superfusion media, modified to a large extent the tonic but not the phasic component of the contraction (Kullak & Huidobro-Toro, unpublished observations). It was not possible to assess this particular effect of neurotensin in the ileum, however, since neurotensin causes muscle relaxation of this tissue (Kitabgi, 1982; Huidobro-Toro & Yoshimura, 1983; Huidobro-Toro & Zhu, 1984).

In conclusion, it is our view that the neurotensin receptors in the rat fundus are coupled to calcium channels, sensitive to nifedipine and related drugs whereas the muscarinic receptors are not linked to the same channels. Based on the present results, it is our working hypothesis that the activation of the neurotensin-receptors of the fundus causes an influx of calcium ions via voltage-sensitive channels. Present results illustrate the usefulness of the gastrointestinal isolated preparations as model systems for the study of drug-receptor interactions, and exploration of the intracellular mechanisms caused by receptor activation.

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References

BERRIDGE, M.J. & IRVINE, R.F. (1984). Inositol triphosphate, a novel second messenger in cellular signal transduction. *Nature*, **312**, 315–321.

BOLTON, T.B. (1979). Mechanism of action of transmitters and other substances on smooth muscle. *Physiol. Rev.*, **59**, 606–718.

BROWN, J.H. & BROWN MASTERS, S. (1984). Does phosphoinositide hydrolysis mediate "inhibitory" as well as "excitatory" muscarinic responses? *Trends Pharmac. Sci.*, **5**, 417–419.

CARRAWAY, R. & LEEMAN, S.E. (1973). The isolation of a new hypotensive peptide, neurotensin, from bovine hypothalami. *J. biol. Chem.*, **248**, 6854–6861.

CARRAWAY, R. & LEEMAN, S.E. (1976). Characterization of radioimmunoassayable neurotensin in the rat. *J. biol. Chem.*, **251**, 7045–7052.

DONOSO, M.V. & HUIDOBRO-TORO, J.P. (1985). Gastrointestinal neurotensin receptors: lack of modulation by thyrotropin releasing hormone. *J. Pharm. Pharmac.*, **37**, 425–428.

GOLENHOFEN, K. & WEGNER, H. (1975). Spike-free activation mechanism in smooth muscle of guinea-pig stomach. *Pfluegers Arch.*, **354**, 29–37.

HAMMER, R.A., LEEMAN, S.E., CARRAWAY, R. &

WILLIAMS, R.H. (1980). Isolation of human intestinal neurotensin. *J. biol. Chem.*, **255**, 2476–2480.
HUIDOBRO-TORO, J.P. & KULLAK, A. (1985). Excitatory neurotensin receptors on the smooth muscle of the rat fundus: possible implications in gastric motility. *Br. J. Pharmac.*, **84**, 897–910.
HUIDOBRO-TORO, J.P. & YOSHIMURA, K. (1983). Pharmacological characterization of the inhibitory effects of neurotensin on the rabbit ileum myenteric plexus preparation. *Br. J. Pharmac.*, **80**, 645–653.
HUIDOBRO-TORO, J.P. & ZHU, Y. (1984). Neurotensin receptors on the ileum of the guinea-pig: evidence for the coexistence of inhibitory and excitatory receptors. *Eur. J. Pharmac.*, **102**, 237–251.
ISHIZAWA, M. & MIYAZAKI, E. (1977). Calcium and the contractile response to prostaglandin in the smooth muscle of guinea-pig stomach. *Experientia*, **33**, 376–377.
JANIS, R.A., SERMIENTO, J.G., MAURER, S.C., BOLGER, G.T. & TRIGGLE, D.J. (1984). Characteristics of the binding of (3H)-nitrendipine to rabbit ventricular membranes: modification by other Ca^{++} channel antagonist and by the Ca^{++} channel agonist Bay K 8644. *J. Pharmac. exp. Ther.*, **231**, 8–15.
KITABGI, P. (1982). Effects of neurotensin on intestinal smooth muscle: application to the study of structure activity relationships. *Ann. N.Y. Acad. Sci.*, **400**, 37–55.
KITABGI, P., CARRAWAY, R. & LEEMAN, S.E. (1976). Isolation of a tridicapeptide from bovine intestinal tissue and its partial characterization as neurotensin. *J. biol. Chem.*, **251**, 7053–7058.
MASHFORD, M.L., NILSSON, G., RÖAEUS, A. & ROSELL, S. (1978). The effect of food ingestion on circulating neurotensin-like immunoreactivity (NTLI) in the human. *Acta physiol. scand.*, **104**, 244–246.
ROSELL, S. & RÖKAEUS, A. (1979). The effect of ingestion of aminoacids, glucosa and fat on circulating neurotensin-like immunoreactivity (NTLI) in man. *Acta physiol. scand.*, **107**, 263–267.
SCHRAMM, M., THOMAS, G., TOWART, R. & FRANCKOWIAK. G. (1983). Novel dihydropyridines with positive inotropic action through activation of Ca^{++} channels. *Nature*, **303**, 535–537.
SHINOHARA, Y. & KOSAKA, I. (1984). Contribution of intracellular stored calcium to contractile activation in contractures of stomach circular muscle of *Bufo vulgaris formosus*. *Jap. J. Physiol.*, **34**, 443–455.
TAIRA, N., WADA, Y. & SATOH, K. (1985). Is antagonism by Bay K 8644 of the negative dromotropic effect of nifedipine pharmacological? *J. Pharmac. exp. Ther.*, **232**, 244–250.

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