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Excitatory neurotensin receptors on the smooth muscle of the rat fundus: possible implications in gastric motility

J. Pablo Huidobro-Toro & Andreas Kullak

Laboratory of Pharmacology, Department of Physiological Sciences, Catholic University of Chile, Casilla 114-D, Santiago 1, Chile

1 Picomolar concentrations of neurotensin caused concentration-dependent contractions of the longitudinal musculature of the fundus of the rat stomach. The EC_{50} of neurotensin was approximately 1.5 nM. On a molar basis neurotensin was about 5–10 times more potent than 5-hydroxytryptamine (5-HT) and approximately 80 times as active as acetylcholine in producing similar contractions.

2 Studies with structurally related peptides indicated that whereas the carboxy terminal portion of neurotensin was essential for biological activity, a substantial part of its amino terminus end could be removed without affecting its potency. The EC_{50} for the neurotensin fragment 8–13 was identical to that of neurotensin, however its 1–8 or 1–11 fragments were completely inactive.

3 Tetrodotoxin did not modify the potency of neurotensin or structurally related analogues suggesting that the neurotensin receptor is probably located on the smooth muscle membrane. In addition, the potency of neurotensin in contracting the fundus was not modified by pretreatment with atropine, methysergide or diphenhydramine.

4 Fade to the contractile response of neurotensin was followed by the development of tachyphylaxis; desensitization was concentration-dependent and characterized by a shift in the agonist concentration-response curve to the right and downwards. Desensitization with a priming concentration of neurotensin (approx. EC_{50}) caused a substantial blockade of its excitability.

5 There was cross-desensitization between neurotensin and the contractile activity of neurotensin 8–13 or xenopsin, but not with angiotensin II, bradykinin, substance P, acetylcholine, 5-HT or histamine.

6 Pretreatment of the fundus strip with verapamil 0.3–1 μM antagonized in a concentration-dependent fashion the neurotensin-induced contractions but not the muscular contractions caused by acetylcholine.

7 It is concluded that neurotensin activates a specific excitatory receptor probably located on the cell membrane of the smooth muscles of the rat fundus. In addition, we suggest that this receptor is somehow related to a voltage-dependent calcium channel, sensitive to verapamil.

Introduction

Neurotensin, a basic 13-aminoacid peptide, is another of the growing list of putative neurotransmitters known as the 'brain-gut neuroregulatory peptides' (Gregory, 1982). By means of immunocytochemical methods it has been established that a large percentage of the neurotensin present in the intestines is restricted to the 'N cells' on the epithelia lining the duodenum and the ileum (Helmstaedter, *et al.*, 1977; Polak *et al.*, 1977) with only a small proportion contained in the nerve cells of the myenteric plexus (Schultzberg *et al.*, 1980; Emson *et al.*, 1982).

Although the physiological role of neurotensin in the periphery remains uncertain, plasma neurotensin levels rise sharply following a fatty meal (Blackburn *et al.*, 1978; Mashford *et al.*, 1978; Rosell & Rokaeus, 1979), suggesting that intestinal neurotensin can be released in response to food, particularly fats. It follows that the neurotensin mobilized by the diet may influence gastrointestinal motility promoting fat digestion and absorption. In fact, neurotensin is a potent stimulant of the gastrointestinal musculature: picomoles of neurotensin cause complex muscular

responses on the guinea-pig or rat ileum, that range from inhibition of the spontaneous activity and the intestinal tone to contractions of the ileum and taenia coli (Kitabgi & Freychet, 1978; Kitabgi, 1982; Huidobro-Toro, 1983; Huidobro-Toro & Yoshimura, 1983). The nature of the contractile responses are varied: whereas neurotensin contracts the taenia coli via a tetrodotoxin-resistant mechanism (Huidobro-Toro, 1983), the contraction of the ileum is in part of neuronal origin, blocked by tetrodotoxin (Kitabgi & Freychet, 1979; Zetler, 1980; Huidobro-Toro & Way, 1982). In support of a neuronal mechanism in the neurotensin-induced contractures of the guinea-pig ileum, Williams *et al.* (1979) demonstrated that neurotensin caused a concentration-dependent increase in the rate of cell firing in about 50% of the neurones examined in the myenteric plexus.

As a first step in understanding whether neurotensin may play a role in the control of gastric motility, we investigated the selectivity of neurotensin receptors in the stomach. Previous results in the literature indicated that applications of neurotensin or xenopsin to isolated stomach strips contracted the tissue (Araki *et al.*, 1973; Quirion *et al.*, 1980). The objectives of the present investigation were two fold. First we wanted to establish whether the neurotensin-induced contractile activity was due to the activation of a selective excitatory neurotensin receptor and if the affinity of these receptor sites is compatible with the circulating levels of plasma neurotensin. Second, we wished to explore whether the excitatory neurotensin activity is due to a neuronal mechanism or to the direct activation of receptors located on the smooth muscle cell membranes, and to ascertain its relation to calcium transport mechanisms.

Methods

Adult Sprague Dawley rats (250–350 g) were used throughout. Animals were bred at our local University animal house, where temperature, humidity and light were kept constant. Standard Purina-like chow was allowed *ad libitum* except for the night before the experiment, when rats were fasted.

The rat isolated fundus preparation

Rats were killed by cervical dislocation, the abdomen was rapidly opened by a midline incision and the stomach was dissected from surrounding tissues and place in a Petri dish containing Tyrode solution of the following composition (mM): NaCl 118, KCl 5.4, $CaACl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 23.8 and glucose 11.1. The fundus of the stomach was excised from the antral portion and opened via the larger curvature; one longitudinal strip was cut on each side of the incision (modification of the classical technique developed by Vane, 1957). Thus, one rat stomach provided two fundus strips. These longitudinal segments were placed immediately in a 30 ml tissue bath containing Tyrode solution at 37°C gassed with air in order to maintain a pH of 7.4. Isometric muscular tension was measured with a Grass FT 03C force-displacement transducer connected to a multichannel Grass model 7D polygraph recorder. Preparations were subjected to an initial tension of 1.5 g. Tissues were allowed to equilibrate for 1 h before drug application; during this equilibration interval, the tissues were washed with 30 ml of Tyrode solution every 15 min.

Contractile responses; concentration-response curves

All the agonists studied produced contractions of the longitudinal smooth muscle layers of the rat fundus. To obtain dose-response curves, different concentrations of each agonist were applied to the tissue chambers for about 15 min, the time required for the peak contraction to occur. The tissues were then washed with 90 ml of drug-free Tyrode solution and retested with an increasing concentration of the agonist at 8 min intervals. At least 6 different concentrations of each agonist were used in deriving a concentration-response curve; stepwise 3 fold increases in agonist concentration were routinely used in a range of a thousandfold molar concentration. The contractile activity of each agonist examined was standardized to that of a saturating concentration of acetylcholine (45 μM); concentration-response curves were plotted as a percentage of the maximal acetylcholine contracture. Potency of the agonists was calculated as the concentration of each compound required to cause a 50% maximal tissue contraction (EC_{50}). The EC_{50} value was derived by interpolation from the respective concentration-response curve (Huidobro-Toro, *et al.*, 1984). Each EC_{50} determination was derived from at least 4–6 strips.

Effect of some receptor blocking agents on the neurotensin-induced contractile activity

To examine whether the contractile effect of neurotensin was related to the release of endogenous acetylcholine, 5-hydroxytryptamine (5-HT) or histamine, concentration-response curves for neurotensin were obtained in the absence and in the presence of atropine (0.26 μM), methysergide (0.29 μM) or diphenhydramine (0.21 μM), added directly to the tissue chambers 2 min before the respective agonist. The EC_{50}s of neurotensin and the agonists were determined in the same preparation before and after the application of each antagonist. All these experiments were

repeated on at least 3–4 separate fundus strips; each tissue was used to examine the effect of a single antagonist.

Metabolism studies

Since the contractile activity induced by neurotensin in the fundus was not sustained but faded with time, we investigated next whether fade was due to tissue metabolism of the peptide. For this experiment, fundus strips were incubated with 2, 5.9 or 20 nM neurotensin for 10 min. Aliquots of the superfusion media (0.9 ml) were withdrawn from the tissue bath 0.1, 1, 3, 5 and 10 min following peptide application to the chamber. Aliquots were kept on ice and bioassayed on a previously calibrated preparation. The aliquots were supposed to contain 0.059, 0.2 and 0.59 nM neurotensin respectively at time zero. The contractile responses induced by the sample aliquots were matched to the tissue calibration curve by expressing the tension achieved as a percentage of the maximal acetylcholine contracture. Results of these experiments are expressed as the percentage of the acetylcholine maximal response induced by each of the sample aliquots. Each of these experiments was repeated on 4 occasions, each time in triplicate. In a parallel series of experiments, the metabolism of xenopsin was examined by incubating tissues with 10 or 30 nM xenopsin. The procedure used to perform the experiment was identical to that described for neurotensin. Results show the polygraph tracing of the isometric tension recording and the contractile activity of the different samples.

Desensitization protocols

Desensitization was used to study receptor specificity. To induce neurotensin desensitization, we used a protocol previously examined by Huidobro-Toro & Yoshimura (1983). In essence, two concentrations of neurotensin or structurally related analogues were used: a priming concentration was followed 10 min later (without washing the tissue) by a second application of the same or higher concentration of neurotensin or its analogues. Neurotensin, neurotensin 8–13 and xenopsin concentration-response curves were obtained in naive preparations and in tissues previously desensitized. Different desensitizing concentrations of the peptides were examined.

To test for cross-desensitization, fundus strips were made tachyphylactic to neurotensin 1.59 nM; 10 min later the tissues were tested with neurotensin structural analogues, angiotensin II, bradykinin, bombesin, substance P, acetylcholine, 5-HT or histamine. Results show concentration-response curves or the EC_{50} derived from these plots in desensitized and non-desensitized preparations. Each concentration-response curve was derived in the same tissue before and after the neurotensin-induced desensitization.

Effects of tetrodotoxin, apamin and verapamil on the neurotensin and acetylcholine-induced fundus contractions

Neurotensin concentration-response curves were obtained before and after a 2 min pre-incubation with tetrodotoxin 100 nM, or with verapamil 0.3–1.0 μM. In the case of apamin, the tissues were incubated with 0.1 μM of the bee venom for 3 min. Eight separate fundus preparations were used in each protocol; results compare the action of neurotensin and related peptide analogues with and without tetrodotoxin, apamin or verapamil. As a control test for the action of these drugs, simulataneous experiments were carried out with acetylcholine.

Statistical analysis

The paired two tail Student's t test was used to compare the concentrations of agonists required to cause 50% contractile activity (EC_{50}) before and after drug treatment. A P value less than 0.05 was considered significant.

Drugs and chemicals

Neurotensin, neurotensin fragments 1–8, 1–11, 8–13, xenopsin, angiotensin II, bradykinin, bombesin, substance P, acetylcholine chloride, histamine dihydrochloride, 5-hydroxtryptamine (serotonin) creatinine sulphate complex, tetrodotoxin, apamin, atropine sulphate and diphenhydramine hydrochloride were all purchased from Sigma Chemical Co. (St. Louis, MO). Methysergide sulphamate was a present from Sandoz, verapamil (commercial ampoule of 2.5 mg ml^{-1}) was obtained from Knoll Pharmaceuticals. The peptides, tetrodotoxin and apamin, were stored at − 40°C (1 μg μl^{-1} aliquots) for at least a year without significant loss in biological activity as assessed by the rat fundus, vas deferens and ileum bioassays. Drug concentrations refer to the final molarity of the bases or peptides. Reagents for the preparation of the Tyrode solution were all analytical grade purchased from Merck (Darmstad, Germany).

Results

Contractile activity of neurotensin; structural requirements for receptor activation

The application of 2–40 pmol of neurotensin caused a consistent and reproducible, concentration-dependent contraction of the rat fundus. The neurotensin-in-

duced muscular contraction was not sustained but faded to a considerable degree within minutes of its application. When compared to acetylcholine, the neurotensin concentration-response curve was shifted to the left about 80 fold; neurotensin reached saturation at about 50% of the maximal acetylcholine contracture. Figure 1 illustrates concentration-response curves for neurotensin, acetylcholine and a variety of other agonists; it can be observed that neurotensin is about 5–10 fold more potent than 5-HT and at least 5,000 times more active than histamine. In relation to other neuropeptides active on the rat fundus, xenopsin (a structural analogue derived from *Xenopus*), was about twice as active as neurotensin and reached a slightly higher saturation. Angiotensin II is a potent peptide to contract the fundus, with

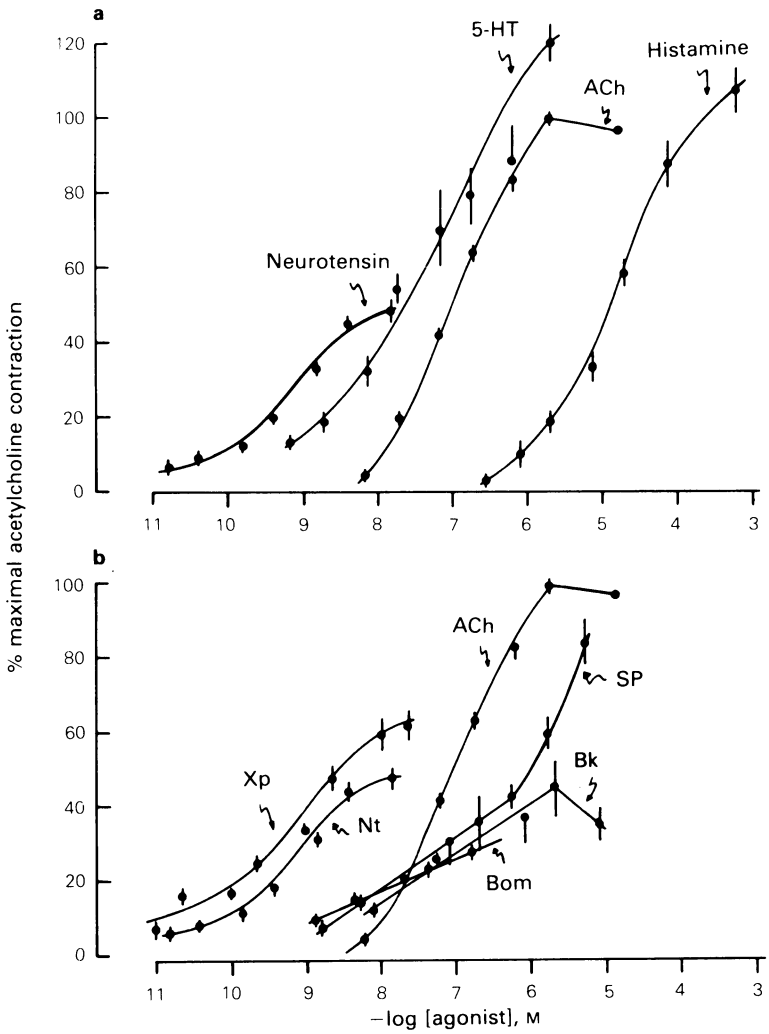

Figure 1 Concentration-response curves to neurotensin, other neuropeptides and agonists on the rat fundus preparation. Agonists were added to the tissue bath and peak contractions were recorded. Concentration-response curves were derived for each agonist; effects are expressed as a percentage of a maximal response to acetylcholine. A calibration curve for acetylcholine is shown in (a) and (b). Symbols denote the mean average of the muscular response, bars indicate s.e. Each concentration-response curve was derived from at least 4 separate fundus preparations. abbreviations used as follows: 5-HT, 5-hydroxytryptamine; ACh, acetylcholine; Xp, xenopsin; Nt neurotensin; Bom, bombesin; Bk, bradykinin; SP, substance P.

Table 1 Potency of neurotensin, analogues and peptide fragments in contracting the rat fundus

		n	EC_{50} (nM) $\bar{x} \pm$ s.e.
Neurotensin		19	1.58 ± 0.18
Xenopsin		20	1.36 ± 0.15
Neurotensin	1–11	8	> 100*
Neurotensin	1–8	8	> 100*
Neurotensin	8–13	20	1.29 ± 0.20

*No contractile response was observed to the application of 100 nM of each peptide.

an EC_{50} value close to that of neurotensin. Substance P, bradykinin and bombesin contracted the rat fundus, but proved to be significantly less potent than neurotensin. For details of agonists EC_{50} values see Table 3.

In relation to the structural requisites for receptor activation, it is apparent that the terminal carboxy group of the peptide plays a key role in receptor action. Removal of the last two amino acids results in a complete loss of biological activity. Interestingly, the residues at the amino terminus can be removed without significant loss in potency. Table 1 shows that the neurotensin fragments 8–13 is as active as the native peptide. In addition, xenopsin a 7-amino acid structural analogue of the carboxy terminus end of neurotensin, is as active as neurotensin.

Effects of some antagonists

The contractile action of neurotensin was not blocked by pretreatment of the tissues with atropine, methysergide or diphenhydramine. Atropine at a concentration that antagonized 30 fold the action of acetylcholine did not significantly modify the EC_{50} of neurotensin. Likewise, methysergide reduced by approximately 20 fold the potency of 5-HT but did not alter significantly the potency of the peptide. In a similar manner, diphenhydramine blocked histamine-induced contractures but did not modify significantly the neurotensin EC_{50}. Results of these experiments are summarized in Table 2.

Neurotensin fade; lack of metabolism

The neurotensin-induced contraction was not sustained; it faded within minutes of incubation with the fundus strips. Figure 2 shows the fading time-course for 20 nM neurotensin or 30 nM xenopsin. As can be appreciated from Figure 2, after a 10 min incubation of the tissues with either 2 and 20 nM neurotensin or 10 and 30 nM xenopsin at a time of considerable fade of the contractile responses, approximately the same contractile activity was demonstrated in the aliquots of the superfusion media as that found after a 10 s incubation period. These results suggest that the muscular fade was not necessarily related to metabolism of the peptides to inactive peptide fragments.

Neurotensin desensitization; specificity of drug action

(a) *Autodesensitization*: fade to the contractile action of neurotensin was followed by considerable reduction of the smooth muscle sensitivity to a second application of the neuropeptide. The recording in Figure 3a shows fading to neurotensin 5.9 nM and the almost complete lack of activity to a second similar application of the peptide 10 min later. Desensitization was proportional to the priming concentration; pretreatment of the tissues with 0.059 nM neurotensin shifted the neurotensin concentration response-curve to the right in a parallel fashion. Increasing the desensitizing

Table 2 Resistance of the neurotensin activity on rat fundus to blockade by atropine, methysergide and diphenhydramine

Antagonist		n	EC_{50} (nM) $\bar{x} \pm$ s.e. *before*	*after blocker*
Atropine 0.26 μM	Acetylcholine	4	105 ± 4.87	3227 ± 372.3*
	Neurotensin	4	1.81 ± 0.35	0.92 ± 0.09
Methysergide 0.29 μM	5-HT	3	5.42 ± 0.20	100.0 ± 0.0*
	Neurotensin	3	0.95 ± 0.24	1.47 ± 0.11
Diphenhydramine	Histamine	4	6927 ± 267.9	27400 ± 6290*
0.21 μM	Neurotensin	4	1.16 ± 0.30	1.91 ± 0.49

* $P < 0.01$ as compared to the EC_{50} before application of the antagonist.

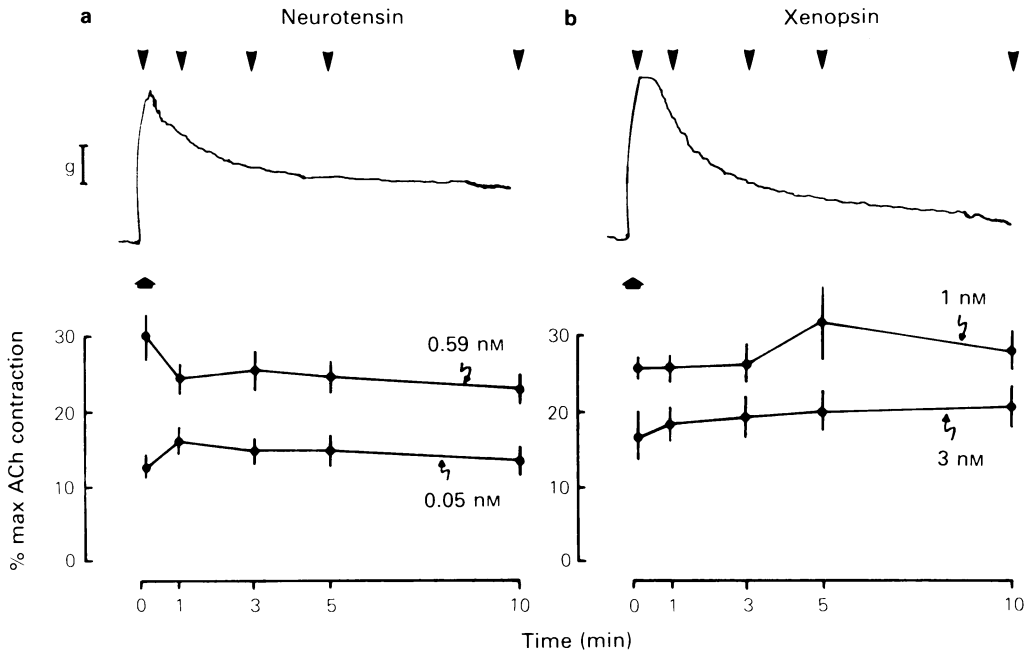

Figure 2 Fade and metabolism of the contractile effect of neurotensin and xenopsin. (a) Rat fundus preparation: 20 nM neurotensin (1 μg) was added at the wide arrow. Aliquots of the superfusion media (0.9 ml, that should contain 30 ng neurotensin, equivalent to 0.59 nM) were sampled at 10 s, 1, 2, 5 and 10 min as indicated by the narrow arrows at the top of the tracing and bioassayed on a naive, previously calibrated preparation. Results of the contractile activity of the sample aliquots are expressed as a percentage of the maximal tension developed by the tissue to acetycholine. Top polygraph tracing presents the muscular tension developed by 20 nM neurotensin. In a parallel, separate preparation, 2 nM neurotensin was added; aliquots were obtained and bioassayed under identical conditions (0.05 nM neurotensin). Four separate experiments were conducted and samples were run in triplicates. In (b), an identical experiment was performed with 30 nM xenopsin (0.9 ml samples were withdrawn that should contain 30 ng xenopsin, equivalent to 1 nM xenopsin). A parallel experiment was performed using 10 nM xenopsin (sample aliquots of 0.3 nM). Each xenopsin experiment was repeated twice; samples were bioassayed in triplicate.

concentration to 0.19 nM, a concentration of the peptide causing 10–15% of its maximal contractile response, shifted the neurotensin response curve in a non-parallel fashion. As shown in Figure 3, 1.9 nM neurotensin caused more than a 75 fold reduction in the neurotensin sensitivity, markedly changing the slope of the response curve. Further increases produced even larger displacements of the neurotensin response curve.

Similar desensitization kinetics were observed when studying the contractile activity of the neurotensin fragment 8–13 or when testing xenopsin. Figure 3 shows that desensitization in all cases was proportional to the priming concentration of the peptides; pretreatment of the fundus with 12.1 nM neurotensin 8–13 resulted in a 75 fold shift of its response curve to the right in a non-parallel fashion. Likewise, preincubation of the fundus preparation with 3 nM xenopsin produced an approximately 75 fold increase in the EC_{50} of xenopsin; increasing the priming concentration to 10 nM resulted in a marked non-parallel displacement of the agonist-response curve.

(b) *Cross desensitization*: tachyphylaxis to neurotensin was only extended to its structurally related peptide analogue but not to the action of the other agonists examined. Figure 4 shows that there was cross-desensitization between neurotensin and xenopsin. Similarly, desensitization with xenopsin produced a state of reduced neurotensin excitability. It must be emphasized that preincubation of the tissues with 1.9 nM neurotensin produced a larger displacement of the agonists concentration-response curve to the right than after incubation with 1.9 nM xenopsin. Whereas the magnitude of the cross desensitization was about the same for both agents, the displacement of the curve

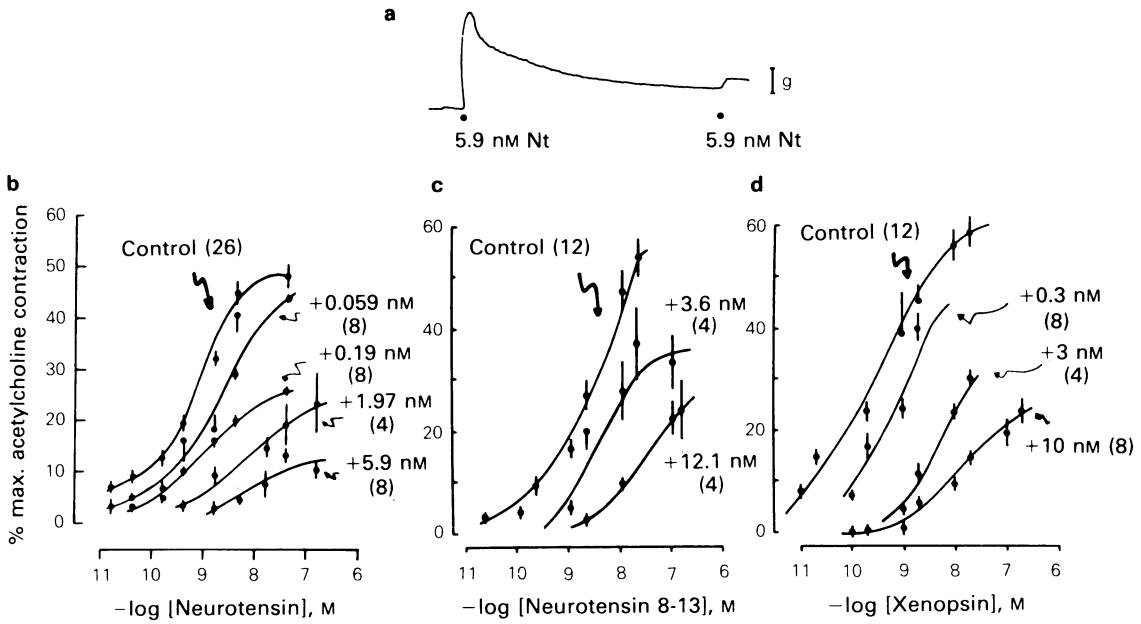

Figure 3 Desensitization of rat fundus to the contractile action of neurotensin and structurally related peptides: (a) polygraph recording showing fade and desensitization to application of 5.9 nM neurotensin (Nt). Concentration-response curves are shown to the excitatory action of neurotensin (b), neurotensin fragment 8–13 (c) and xenopsin (d). Control curves were obtained in tissues before desensitization with the concentration of the peptide specified under each curve. The protocol used for desensitization is exemplified in (a): a desensitizing concentration of each peptide was followed 10 min later by a test to perform the respective concentration-response curve. Symbols represent the mean contraction; bars show s.e.mean.

was larger for neurotensin. Similar results can also be observed from Figure 3; independent of agonist potency, neurotensin-induced the most desensitization as compared to xenopsin or neurotensin 8–13. The specificity of desensitization was demonstrated by the fact that under conditions of markedly reduced sensitivity to neurotensin, the tissues remained fully sensitive if not more sensitive to angiotensin II, bradykinin, substance P, acetylcholine, 5-HT or histamine. Table 3 summarizes the results of this series of experiments. Whereas the potency of neurotensin was decreased 75 fold after a 10 min preincubation with the

Table 3 Specificity of the neurotensin-induced desensitization in rat fundus

	n	Agonist EC_{50} (nM) $\bar{x} \pm$ s.e. *before*	*After desensitization*	*Ratio*[a]
Neurotensin	4	1.5 ± 0.4	105.2 ± 12.3*	75.0**
Angiotensin II	6	2.3 ± 0.4	2.3 ± 0.7	1.0
Bradykinin	6	258.0 ± 133.7	85.6 ± 13.5	0.33
Substance P	6	827.5 ± 246.7	217.0 ± 23.6	0.26*
Acetylcholine	4	146.2 ± 10.8	89.4 ± 13.3	0.61
5-HT	6	17.5 ± 1.7	24.2 ± 5.2	1.38
Histamine	8	9312.0 ± 996.0	2345.0 ± 320.0	0.25*

[a] Ratio refers to the EC_{50} after/before desensitization.
** $P < 0.01$ as compared to the EC_{50} prior to desensitization, indicating the condition of desensitization.
* $P < 0.05$, establishing a significant increase of agonist activity.

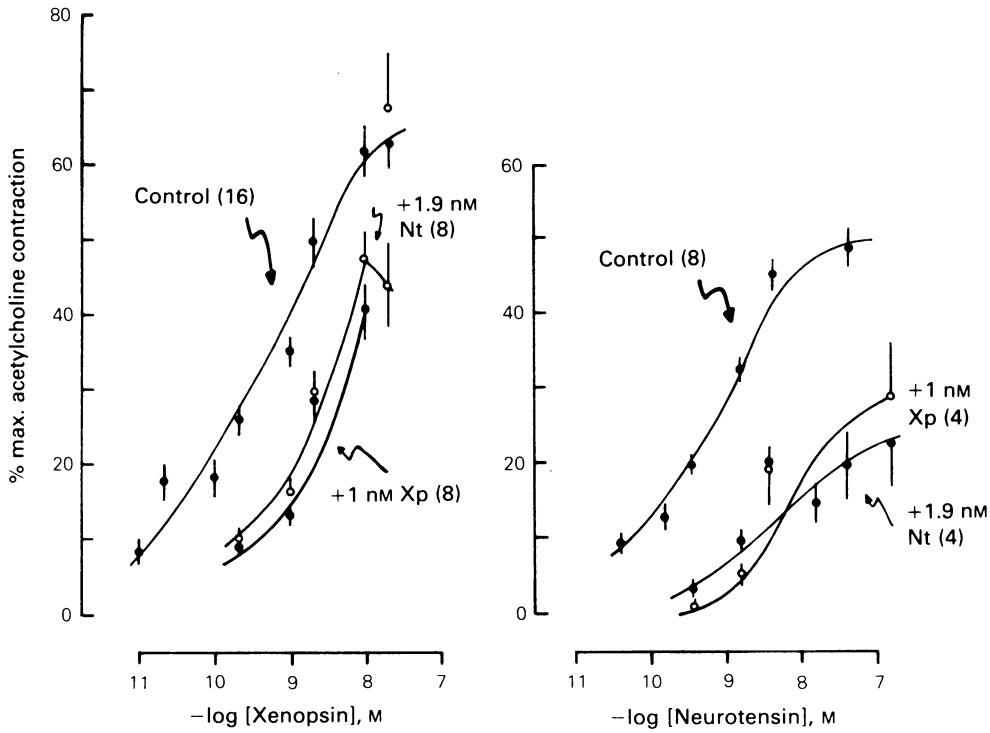

Figure 4 Xenopsin and neurotensin desensitization: induction of cross tachyphylaxis in rat fundus. Xenopsin and neurotensin concentration-response curves were obtained in control, naive tissues, and in the same preparations following either desensitization with 1.9 nM neurotensin (Nt) or 1 nM xenopsin (Xp). The priming concentrations were applied for 10 min. Each preparation was used to test the action of only one agonist. Symbols represent the mean values; bars show s.e.mean.

peptide, the potency of the other agonists remained essentially unaltered (Table 3 and Figure 5). In the case of substance P and histamine, a 4 fold potentiation of the responses occurred which was statistically significant.

Effect of ionic channel blocking drugs

To investigate whether the contractile effect of neurotensin is sensitive to the application of tetrodotoxin, apamin or verapamil, the activity of neurotensin was tested in the presence of these drugs.

(a) *Tetrodotoxin*: the application of tetrodotoxin 100 nM did not modify the potency of neurotensin or related peptides; in fact, the EC_{50} of neurotensin before treatment with tetrodotoxin was 0.76 ± 0.13 nM ($n = 3$), a value that was not significantly different from 0.84 ± 0.22 ($n = 3$) obtained in the presence of this toxin. Figure 6 shows concentration-response curves for neurotensin 8–13 and xenopsin in the absence and presence of 0.1 μM tetrodotoxin. The effectiviness of tetrodotoxin in blocking nerve conduction was tested on the muscular contraction of the rat vas deferens induced by nerve stimulation or the guinea-pig ileum bioassay (Huidobro-Toro *et al.*, 1984).

(b) *Apamin*: pretreatment of the fundus strips with apamin 100 nM did not antagonize the contractile activity of neurotensin or acetylcholine. On the contrary, as shown in Figure 7, apamin caused a slight potentiation of the neurotensin responses, shifting the concentration-response curve to the left and increasing by about 20% the maximum tension produced by the neuropeptide ($P < 0.05$). Apamin did not significantly modify the potency of acetylcholine (Figure 7).

(c) *Verapamil*: preincubation of the fundus with verapamil 0.3–1 μM antagonized in a concentration-dependent fashion the neurotensin contractions but

not the acetylcholine-induced contractile responses. As shown in Figure 8, the nature of the neurotensin blockade appeared to be non-competitive since verapamil decreased the maximal tension achieved by the peptide. In contrast, the contractions induced by acetylcholine were unchanged (Figure 8).

Discussion

Receptor blocking agents have played an important role in the pharmacological characterization of biological receptors. In the absence of these agents, it has been difficult to ascertain whether the action of a given drug is due to the selective interaction at a specific receptor site. In the case of neurotensin, no such antagonist is available as yet; in spite of this limitation we developed in the present investigation three arguments to substantiate the notion that the contractile activity of the peptide is due to a selective-neurotensin receptor site. Structural modifications in the neurotensin molecule resulted in marked changes in biological activity, indicating the need for a particular peptide conformation for receptor activation. Second, neurotensin showed cross-desensitization only to structural analogues of the neuropeptide and not to other agonists. Thirdly, cholinoceptor, 5-HT or histamine receptor blocking agents did not modify the potency of neurotensin.

In relation to the structural prerequisites for receptor activation, it is evident that the first seven amin-acids at the amino terminal end of neurotensin are not essential, at least to induce contractile activity. In constrast, the carboxy end residues are all necessary to maintain potency. Deletion of the last one or two amino acids resulted in a very significant drop in activity on the fundus assay (Quirion *et al.*, 1980) or in the binding potency to purified cell membranes (Kitabgi *et al.*, 1980). The potent biological activity of xenopsin can be explained by the fact that this natural peptide analogue conserves unaltered the carboxy end-sequence of the peptide. Consistent with the present findings, Leeman & Carraway (1982) suggested that the active site of the neurotensin receptor recognizes essentially the 8–13 sequence of the peptide.

Desensitization is an interesting though old argument in terms of receptor characterization (Schild, 1973). Although the mechanisms of homologous desensitization remain largely unknown, desensitization can be used as an experimental tool in receptor studies (Huidobro-Toro & Yoshimura, 1983; Huidobro-Toro & Zhu, 1984). The rationale for this experimental strategy is based on the fact that upon prolonged or repeated exposures to neurotensin, the tissue loses excitability only to structurally related compounds or congeners. In fact, after neurotensin tissue desensitization, tachyphylaxis was found to extend only to neurotensin 8–13 or xenopsin but not to the action of angiotensin II, bradykinin, substance P, bombesin, acetylcholine, 5-HT or histamine. Thus, it must be accepted that the selectivity of desensitization reflects a receptor activated process, supporting our notion of the existence of neurotensin-specific receptor sites. Curiously, although the agonist potency of neurotensin, xenopsin or neurotensin 8–13 are

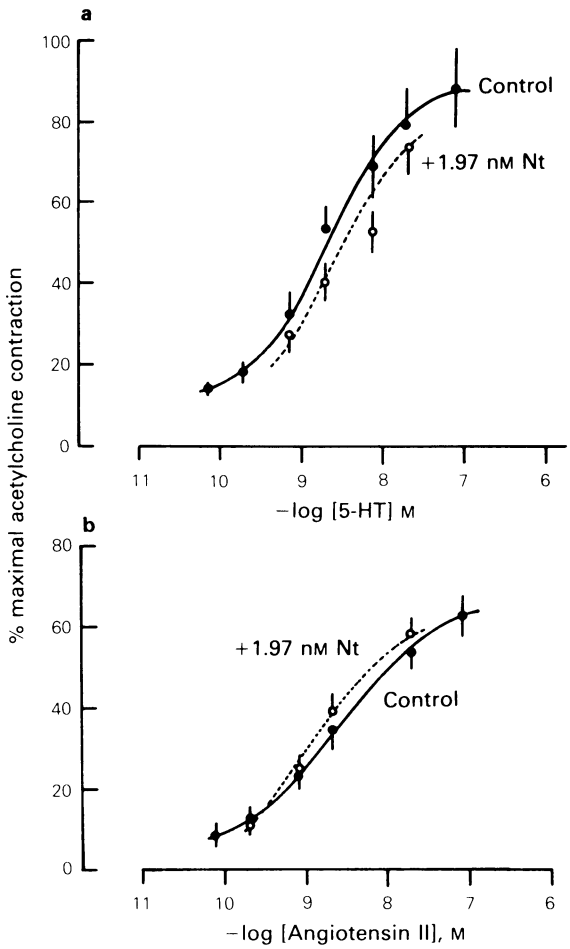

Figure 5 Specificity of the neurotensin desensitization of rat fundus; lack of cross tachyphylaxis to 5-hydroxytryptamine (5-HT) or angiotensin II. Concentration-response curves to the excitatory action of 5-HT (a) and angiotensin II (b) were performed prior to (Control, solid line) and following tissue desensitization with 1.97 nM neurotensin (Nt, broken line). Results are expressed as a percentage of the maximal acetylcholine contracture. Points represent the mean contractile effect, bars show s.e.mean (n = 8 for each experiment).

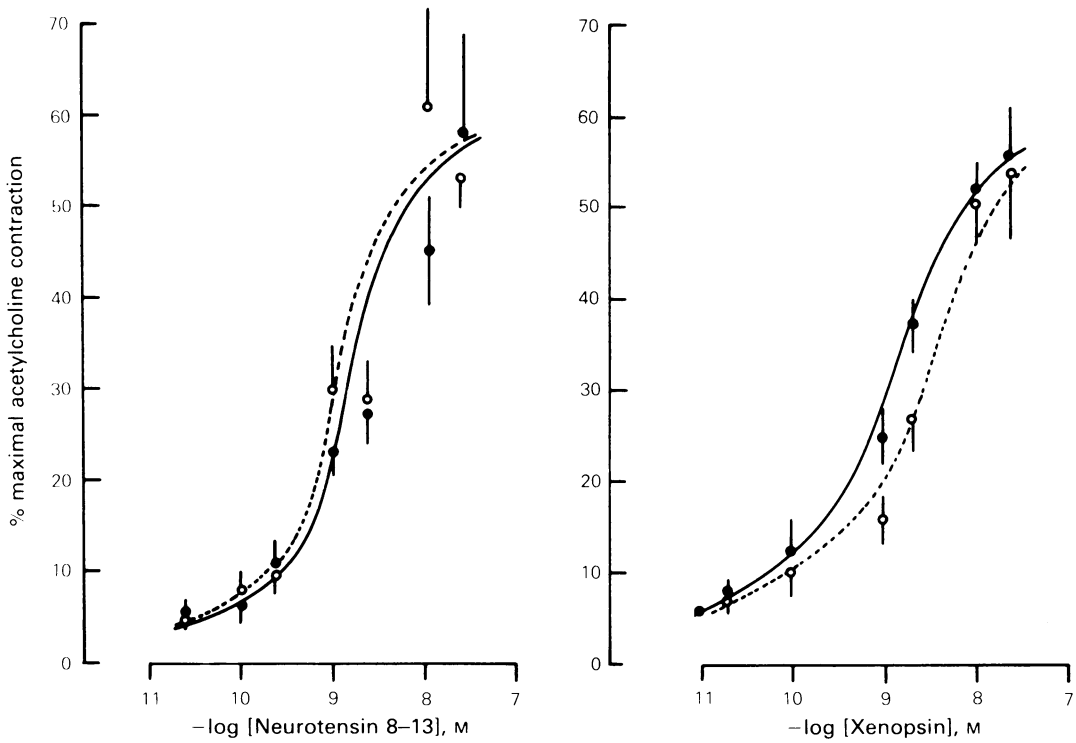

Figure 6 Influence of tetrodotoxin on the contractions induced by neurotensin 8–13 and xenopsin. Concentration-response curves for neurotensin 8–13 and xenopsin were performed in the absence (solid line) and in the presence (broken line) of 0.1 μM tetrodotoxin. The toxin was added to the tissue chambers 2 min before the application of the peptides. Each of these experiments was performed on 8 separate fundus preparations. Symbols denote the mean muscular tension expressed as a percentage of the maximal acetylcholine response, bars denote s.e.mean.

roughly the same, the potency of these compounds in inducing desensitization is not identical. In fact, neurotensin induced a much greater degree of desensitization than xenopsin or the 8–13 neurotensin fragment. The reasons for this difference are not clear at present but could be related to the particular conformation of these peptides, and to the fact that additional binding residues in the neurotensin molecule may influence other receptor-activated properties in addition to agonist potency.

The fact that the contractile activity of neurotensin is not antagonized by atropine, methysergide or an H_1-blocking agent, suggests that neurotensin does not interact at the same receptor site as acetylcholine, 5-HT or histamine and that neurotensin does not release these compounds endogenously on the rat fundus. These results contrast with observations where neurotensin is known to mobilize acetylcholine from neurones in the myenteric plexus (Kitabgi & Freychet, 1978; 1979; Huidobro-Toro & Way, 1982; Huidobro-Toro *et al.*, 1984) or histamine from mast cells (Rossie & Miller, 1982; Oishi *et al.*, 1983) and thereby produce vasodilatation and hypotension (Kerouac *et al.*, 1982). Additional evidence in support of a direct, muscular action of neurotensin on the longitudinal smooth muscles of the rat fundus is provided by the fact that tetrodotoxin did not alter its potency, indicating that the neurotensin-induced contraction is not of neuronal origin as opposed to the contractile action of neurotensin in the guinea-pig ileum where several authors have documented its relation to cholinergic synapses. Thus, it may be suggested that the contractile effect of neurotensin on the rat fundus is due to the activation of receptor sites located directly on the longitudinal smooth muscle probably on the outer membrane. That the activity of neurotensin is confined to the longitudinal muscles of the fundus was indicated in experiments where the antral portion of the stomach was examined. In these preparations no contractile activity of neurotensin could be demonstrated. Likewise, in fundus strips where the contractile activity of circular smooth muscle layers was

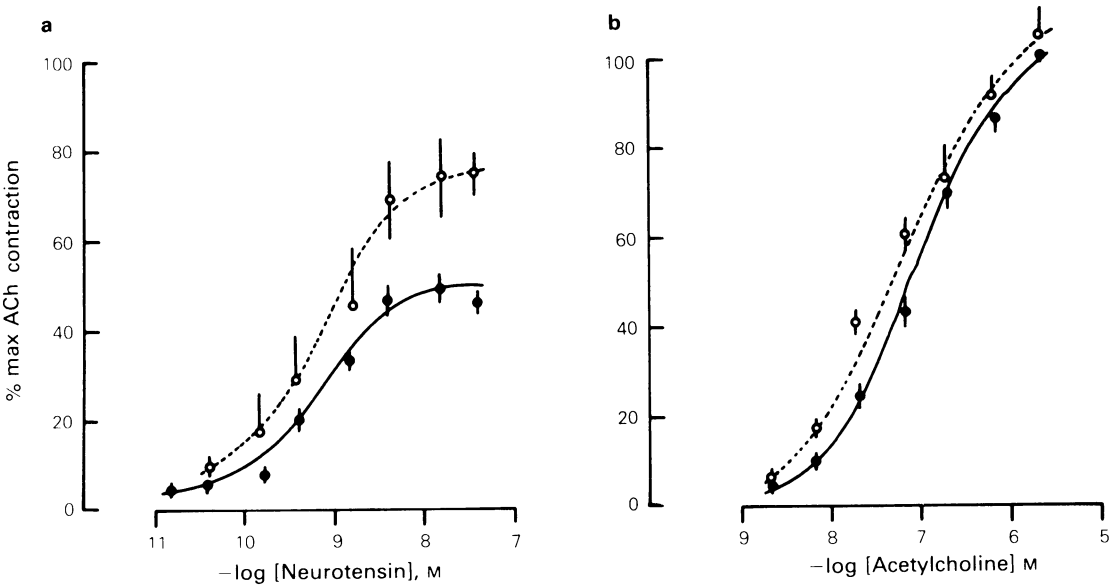

Figure 7 Effect of apamin on the contractions induced by neurotensin and acetylcholine. Concentrations-response curves for the contractile action of neurotensin (a) and acetylcholine (b) were determined in fundus strips prior to (solid line) and 3 min following incubation with 0.1 μM apamin (broken line). Points represent the mean contractile effect and bars the s.e.mean ($n = 6$).

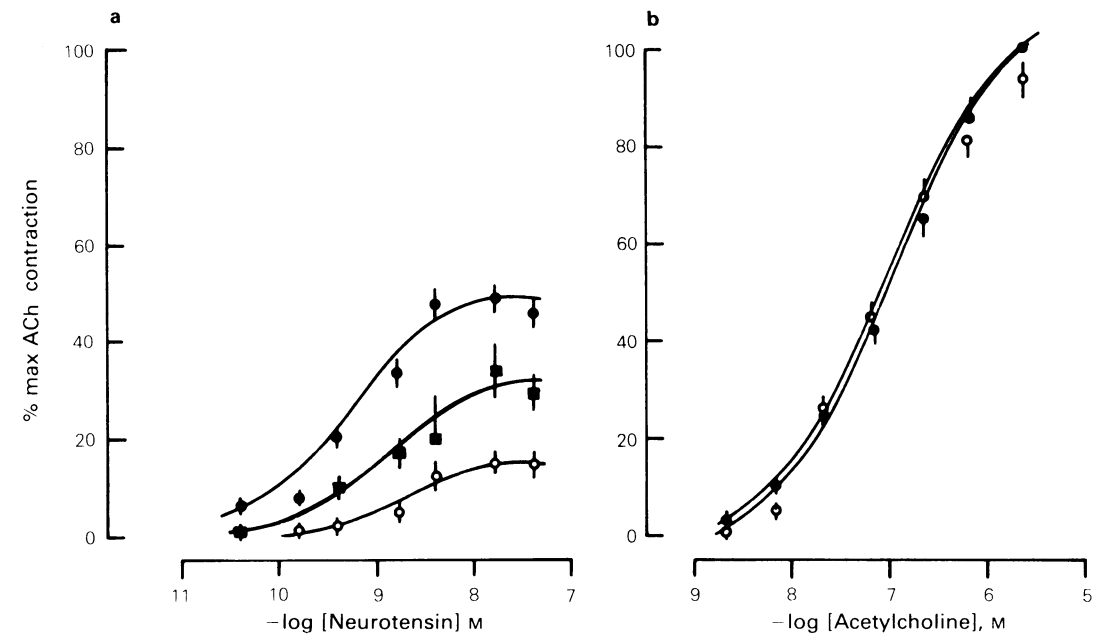

Figure 8 Effect of verapamil on the neurotensin (a) and acetylcholine (b) response of the rat fundus. Concentration-response curves were determined in control, naive, tissues (●) and in the same preparations after a 2 min incubation with either 0.3 μM verapamil (■) or 1 μM verapamil (○). Each concentration of verapamil was tested in 8 separate strips; points represent the mean contractile effect, bars the s.e.mean.

measured no effects of neurotensin were recorded (Kullak, unpublished observations).

An interesting finding derived from this investigation was that the neurotensin-induced contractile activity was antagonised in a concentration-dependent manner by verapamil. The most unexpected result was that the acetylcholine contractions were markedly resistant to the action of this calcium channel antagonist. Additional experiments with nifedipine, or lowering the calcium content of the Tyrode buffer confirmed the selective ability of verapamil to block the neurotensin contractions but not the muscarinic actions (Kullak & Huidobro-Toro, 1984, unpublished observations). The implications of this finding are not as yet clear, but allow us to suggest that the excitatory neurotensin receptor in the rat fundus may be linked to a voltage-dependent calcium channel. Thus, the contraction induced by neurotensin is in marked contrast to the action of acetylcholine on the fundus, which is quite resistant to the lack of external calcium. We next examined the effect of apamin on the neurotensin response since Kitabgi & Vincent (1981) and Huidobro-Toro & Yoshimura (1983) reported that apamin potently antagonized (3–30 nM) the gastrointestinal effects of the peptide, particularly the neurotensin-induced smooth muscle relaxations. The present results clearly demonstrate that this toxin, which blocks K^+ channels activated by calcium (Banks *et al.*, 1979; Jenkinson, 1981) does not reduce the contractile activity of neurotensin on the fundus. On the contrary, our results demonstrate a slight potentiation of the maximal neurotensin activity in the presence of 100 nM apamin. This stimulatory effect was not observed when the contractile effect of acetylcholine was tested in the same preparations; its significance remains to be clarified.

Concerning a putative physiological role for neurotensin in the control of the gut motility, the present results support the existence of selective neurotensin receptors on the fundus. The affinity of these sites as demonstrated by the bioassay is in the high picomolar range but the tissue is sensitive to amounts of the peptide as low as 2–5 pmol. It is appropriate to mention that with our modified Vane technique for setting up the fundus preparation, the EC_{50} of neurotensin was increased some 10 fold, as compared to the EC_{50} values obtained by Quirion *et al.* (1980). However, for the affinity of neurotensin receptors to have physiological significance, it is necessary that the receptor affinity matches that of the circulating plasma levels of the peptide. Recent publications in this area show a great diversity in plasma 'neurotensin-like immunoreactivities' content ranging from 0.004 pmol ml^{-1} (Hammer *et al.*, 1982) to values of about 40–60 pmol ml^{-1} (Rosell & Rokaeus, 1979; Rosell, 1982). Both groups of investigators consistently demonstrated a 4–10 fold increase in the plasma immunoreactivity neurotensin following the ingestion of a meal abundant in fats. Although the present values of agonist potency may seem remote from physiological reality, three considerations are in order. In the first place, the bioassay presents considerable pharmacokinetic barriers to the measurement of a physiologically relevant receptor affinity. Binding assays to purified membranes generally show a 10–100 fold decrease in receptor K_D values as compared to simple bioassays. Second, the half life of neurotensin in the rat circulation is only 30 s (Leeman & Carraway, 1982) indicating a first rate peptide degradation. Third, the value of 40 pmol neurotensin-ml^{-1} was obtained from a systemic sample of blood, suggesting that the concentration in the local gastrointestinal circulation might be much higher. In support of our contention that neurotensin may play a role in gastric motility, Hellstrom *et al.* (1982) reported that neurotensin prolonged the transit time of the chyme in the stomach and the intestines of the rat.

Finally, we would like to comment on some interesting pharmacological properties of the rat fundus as compared to other intestinal bioassays commonly used in the study of this peptide. The guinea-pig ileum is the classical organ on which most of the original characterization of neurotensin was done (see Kitabgi, 1982; Huidobro-Toro & Way, 1982; Huidobro-Toro, 1983). In this tissue neurotensin has complex effects on the motility of the organ, due to the coexistence of inhibitory and excitatory neurotensin-receptor-mediated responses (Huidobro-Toro & Zhu, 1984). Whereas in the ileum of the guinea-pig, neurotensin causes an initial relaxation, the predominant action of the neuropeptide appears to be a neuronally mediated contractile effect; this action is rather species-specific since in the rat, the rabbit and mouse, neurotensin produces essentially relaxation of the ileum (Kitabgi & Freychet, 1978; Huidobro--Toro & Yoshimura, 1983; Donoso & Huidobro-Toro, unpublished). It is in this connection that our interest in the fundus preparation lies. In this tissue the neurotensin activity is only excitatory, with no concomitant inhibitory effects. In addition, the fundus contractions are not sensitive to tetrodotoxin suggesting a muscular location of the receptors as in the taenia coli (Kitabgi *et al.*, 1979; Huidobro-Toro, 1983). Preliminary experiments indicate that neurotensin contracts the fundus of the guinea-pig by a mechanism similar to that found in the rat. The significance of the coupling of the neurotensin excitatory muscular receptor to a voltage-sensitive channel is of interest and is being actively pursued in our laboratory.

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