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Release of γ-aminobutyric acid and acetylcholine by neurotensin in guinea-pig ileum

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1 The release of γ-aminobutyric acid (GABA) and acetylcholine (ACh) from the strips of guinea-pig ileum was investigated in the presence of neurotensin.

2 Neurotensin evoked the release of [^{3}H]-GABA from the strips preloaded with [^{3}H]-GABA, and the evoked release was Ca^{2+}-dependent and tetrodotoxin-sensitive.

3 Hexamethonium, scopolamine, [D-Pro^2, D-Trp^{7,9}] substance P and pretreatment with substance P did not alter the neurotensin-evoked release of [^{3}H]-GABA.

4 Pretreatment with neurotensin inhibited the release of [^{3}H]-GABA evoked by neurotensin but not by high K^+, thereby indicating that neurotensin induced a specific desensitization of its own receptor. These observations indicate that neurotensin may stimulate the GABAergic neurone through its own receptor.

5 Neurotensin evoked the release of [^{3}H]-ACh from strips preloaded with [^{3}H]-choline and this release was Ca^{2+}-dependent and tetrodotoxin-sensitive.

6 The evoked release of [^{3}H]-ACh was not affected by hexamethonium, scopolamine and [D-Pro^2, D-Trp^{7,9}] substance P.

7 Bicuculline partly inhibited the neurotensin-evoked release of [^{3}H]-ACh; thus neurotensin seems to induce a release of ACh partly through the release of endogenous GABA.

8 All this evidence indicates that neurotensin induces release of GABA as well as ACh from the myenteric neurones of the guinea-pig ileum.

Introduction

γ-Aminobutyric acid (GABA) is present in the myenteric plexus of the mammalian intestine, where it probably acts as a neurotransmitter (Jessen *et al.*, 1979; Taniyama *et al.*, 1982; Kerr & Krantis, 1983; Erdö, 1985; Tanaka, 1985). The target cells of GABAergic neurones are thought to be postganglionic cholinergic neurones (Kleinrok & Kilbinger, 1983; Taniyama *et al.*, 1983; Yau & Verdun, 1983). Substance P, one of the gut-neuropeptides was found to evoke the release of GABA from the myenteric neurones of small intestine, and the idea was proposed that there is a neuronal circuit (substance P ergic – GABAergic – cholinergic neurones) located within the myenteric plexus (Tanaka & Taniyama, 1985). Neurotensin, one of the biologically active peptides present in the intestine, has been demonstrated by immunohistochemistry to be present in the endocrine cells of the ileal mucosa of guinea-pig (Helmstaedter *et al.*, 1977; Sundler *et al.*, 1977; Schultzberg *et al.*, 1980). There is a report that immunoreactive neurotensin can be detected in a few scattered nerve fibres in the smooth muscle, although no nerve cell bodies are found, in the guinea-pig ileum (Leander *et al.*, 1984). Neurotensin was found to cause stimulatory and/or inhibitory actions on intestinal motility (Kitabgi & Freychet, 1979; Huidobro-Toro & Yoshimura, 1983; Zetler, 1980). The neurotensin-induced contraction appears to be due to the stimulation of cholinergic neurones (Kitabgi & Freychet, 1979; Huidobro-Toro & Yoshimura, 1983; Yau *et al.*, 1983). Electrophysiological studies demonstrated that neurotensin produced either a depolarization or a hyperpolarization of myenteric neurones of the small intestine (Williams *et al.*, 1979). Such pharmacological observations suggest that neurotensin may affect the motility of the intestine through neuronal mechanisms. The present experiments were designed to examine the effect of neurotensin on the release of GABA and ACh in the guinea-pig ileum.

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Methods

Adult guinea-pigs of either sex, weighing between 300 and 500 g, were killed by cervical dislocation. The ilea were immediately excised and cut along the longitudinal axis. A strip about 2 cm in length was prepared 10 cm proximal to the ileocaecal sphincter from each animal. Each ileal strip had intact longitudinal and circular muscles, intramural plexus, and mucosa.

Measurement of [^{3}H]-GABA release

The strip of ileum was incubated with [^{3}H]-GABA (5×10^{-8} M) for 60 min, washed in Krebs-Ringer solution of the following composition (in mmol l^{-1}): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.19, $NaHCO_3$ 25.0, KH_2PO_4 1.18 and glucose 11 for 30 min, mounted in the apparatus, and superfused at 1.2 ml min^{-1} with the same solution. The incubation and perfusion medium used was Krebs-Ringer solution containing aminooxyacetic acid (10 μM) to prevent the metabolism of [^{3}H]-GABA (Baxter & Roberts, 1961) and β-alanine (1 mM) to prevent the glial uptake of [^{3}H]-GABA (Schon & Kelly, 1975), gassed with 95% O_2:5% CO_2, and maintained at 35°–37°C. Superfusates were collected at 30 s intervals, and the radioactivity was counted in a liquid scintillation counter. Experiments were started 60 min after the spontaneous release of tritium had approached a plateau. At the end of experiment, the tissue was dissolved in Soluene and radioactivity was measured in a scintillation counter. To determine the proportion of unchanged GABA in the tritium release of the superfusates, 100 μl of superfusates with added unlabelled GABA were subjected to high voltage electrophoresis on Whatman 3-mm chromatography paper, as described by Taniyama *et al.* (1982). Even 120 min after the experiment was started, 93.2 ± 3.8% ($n = 10$) and 92.7 ± 4.7% ($n = 10$) of the total radioactivity in the superfusates from stimulated and non-stimulated samples were associated with unchanged [^{3}H]-GABA, respectively. Accordingly, the total radioactivity in the superfusate was taken as the amount of [^{3}H]-GABA.

Measurement of [^{3}H]-acetylcholine release

The strips were incubated with [^{3}H]-choline (2×10^{-7} M) in Krebs-Ringer solution. After washing in the fresh medium, strips were mounted in the apparatus and superfused at 1.2 ml min^{-1} with the same solution. The perfusion medium was Krebs-Ringer solution containing hemicholinium-3 to prevent the reuptake of choline formed from ACh, and gassed with 95% O_2:5% CO_2. The superfusates were collected at 1 min intervals, and the radioactivity was counted in a liquid scintillation counter. At the end of the experiment the tissue was dissolved in Soluene and the radioactivity was measured in a scintillation counter. Extraction and separation of [^{3}H]-ACh and [^{3}H]-choline was carried out by the method of Potter & Murphy (1967). The superfusates were collected in 1 ml of 3-heptanon-tetraphenylboron (10 mg ml^{-1}) on ice. [^{3}H]-ACh and [^{3}H]-choline were extracted with 1 N HCl, dried and dissolved in 1 N formic acid and acetone (15:85; v/v). The samples were then subjected to electrophoresis to separate ACh and choline. The spots were identified with iodine and the radioactivity was determined in a liquid scintillation spectrometer. The proportion of [^{3}H]-ACh to total tritium in the superfusates collected before and during the stimulation by neurotensin was 44.5 ± 3.8% ($n = 7$) and 78.9 ± 4.2% ($n = 7$), respectively. The total radioactivity in the superfusates from neurotensin-stimulated preparations was considered to approximate the amount of [^{3}H]-ACh and therefore was denoted as [^{3}H]-ACh release.

Calculation

The release of tritium was represented as the fractional rate obtained by dividing the amount of tritium in the superfusate by the respective amount of tritium in the tissue. The tritium content of the tissue at each period was calculated by adding cumulatively the amount of each fractional tritium efflux, to the tritium content of the tissue at the end of the experiment. Data were analysed by Student's t test and a P value of 0.05 or less was considered statistically significant.

Drugs and chemicals

Substances used were as follows: [^{3}H]-γ-aminobutyric acid ([^{3}H]-GABA, 57 Ci $mmol^{-1}$) and [^{3}H]-choline (60 Ci $mmol^{-1}$) (Amersham), aminooxyacetic acid, bicuculline and hexamethonium bromide (Sigma), hemicholinium-3 (Aldrich), tetrodotoxin (Sankyo), ethyleneglycol-*bis* (β-aminoethyl-ether) N,N'-tetra-acetic acid (EGTA), β-alanine and scopolamine hydrobromide (Nakarai), neurotensin and [D-Pro^2,D-Trp^{7,9}] substance P (Peptide Institute) and Soluene (Packard). Other chemicals used were of reagent grade.

Results

Neurotensin-evoked release of [^{3}H]-GABA

The fractional rate of spontaneous release of [^{3}H]-GABA from the strips preloaded with [^{3}H]-GABA (which approached a fairly constant level after 60 min superfusion) was 0.00412 ± 0.00012 min^{-1} (mean-

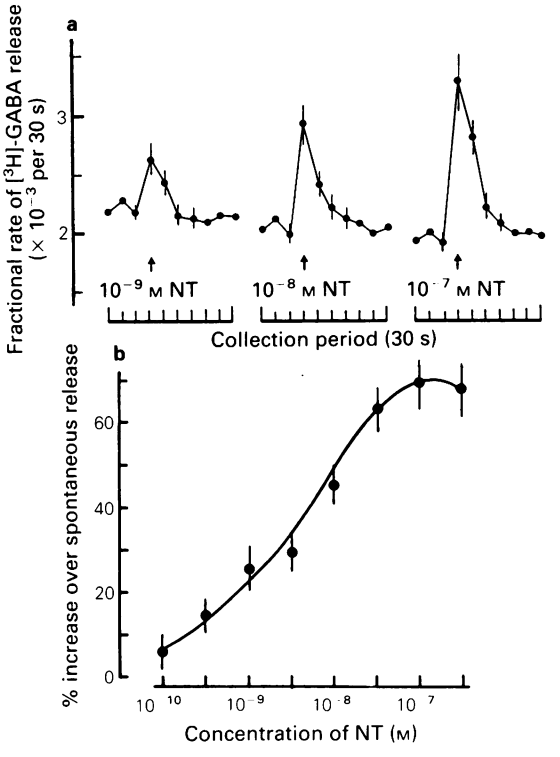

Figure 1 Neurotensin (NT)-evoked release of [^{3}H]-γ-aminobutyric acid ([^{3}H]-GABA). (a) Typical experiments showing [^{3}H]-GABA release. (b) Dose-response curve for neurotensin. Neurotensin was perfused for 30 s at 30 min intervals. Each point represents the mean from 5 animals with s.e.mean shown by vertical lines.

± s.e. for 30 determinations). The experiments were started after 60 min superfusion. The release of [^{3}H]-GABA was increased by perfusion with neurotensin at 10^{-8} M for 30 s. When 10^{-8} M neurotensin was applied for 30 s successively, three times at 30 min intervals to the same preparation, the evoked release of [^{3}H]-GABA declined only slightly from the first to third application of neurotensin. The effect of neurotensin on [^{3}H]-GABA release was then examined in the same preparation. Neurotensin, at concentrations ranging from 3×10^{-10} M to 10^{-7} M evoked the release of [^{3}H]-GABA, in a dose-dependent manner (Figure 1). Perfusion with the Ca^{2+}-free medium containing EGTA (10^{-3} M) and medium containing tetrodotoxin (3×10^{-7} M) prevented the neurotensin (10^{-8} M)-evoked release of [^{3}H]-GABA (Table 1), indicating that the [^{3}H]-GABA released originated from the nerve terminals. Hexamethonium at 3×10^{-4} M and scopolamine at 10^{-6} M did not affect the neurotensin (10^{-8} M)-evoked release of [^{3}H]-GABA (Table 1). A substance P antagonist, (D-Pro^2, D-Trp^{7,9}) substance P at 10^{-6} M also failed to alter the neurotensin (10^{-8} M)-evoked release of [^{3}H]-GABA (Table 1). When desensitization to substance P at 10^{-8} M was induced by continuous perfusion with substance P for 30 min, neurotensin (10^{-8} M)-evoked release of [^{3}H]-GABA was inhibited by only 10% (Table 1).

Desensitization to neurotensin was observed by a 30 min exposure of the preparation to neurotensin. When neurotensin (10^{-8} M) was present in the superfusion medium, a second addition of the same concentration of neurotensin failed to induce any response and the response to high K^+ (3×10^{-2} M) remained without change (Figure 2).

Table 1 Effects of various agents on the spontaneous and neurotensin-evoked release of [^{3}H]-γ-aminobutyric acid ([^{3}H]-GABA)

		Fractional rate of [^{3}H]-GABA release ($\times 10^{-3}$ per 30 s)		$\frac{b}{a} \times 100$
		Spontaneous (a)	*Evoked (b)*	
None (control)	(5)	1.981 ± 0.091	2.904 ± 0.188	146.6
Ca^{2+}-free medium containing 10^{-3} M EGTA	(4)	1.990 ± 0.185	2.075 ± 0.139*	104.0*
Tetrodotoxin 3×10^{-7} M	(4)	1.987 ± 0.204	2.112 ± 0.196*	106.1*
Hexamethonium 3×10^{-4} M	(4)	1.972 ± 0.208	2.908 ± 0.192	147.5
Scopolamine 10^{-6} M	(4)	1.974 ± 0.158	2.952 ± 0.147	149.5
[D-Pro^2,D-Trp^{7,9}] substance P 10^{-6} M	(4)	1.985 ± 0.179	2.814 ± 0.181	141.8
Substance P 10^{-8} M	(5)	2.057 ± 0.188	2.913 ± 0.203	141.6

The fractional rate of the spontaneous and neurotensin-evoked [^{3}H]-GABA release was measured after 120 min superfusion. Neurotensin (10^{-8} M) was included in the perfusion medium for 30 s. Ca^{2+}-free medium containing EGTA (10^{-3} M) and tetrodotoxin were applied 15 min, and hexamethonium, scopolamine and (D-Pro^2,D-Trp^{7,9}) substance P were applied 10 min before and during addition of neurotensin. Substance P was applied 30 min before and during the addition of neurotensin. Values are means ± s.e.mean for the number of experiments shown in parentheses. *Significance of difference from the control value was calculated by Student's t test, at the <0.05 level of probability.

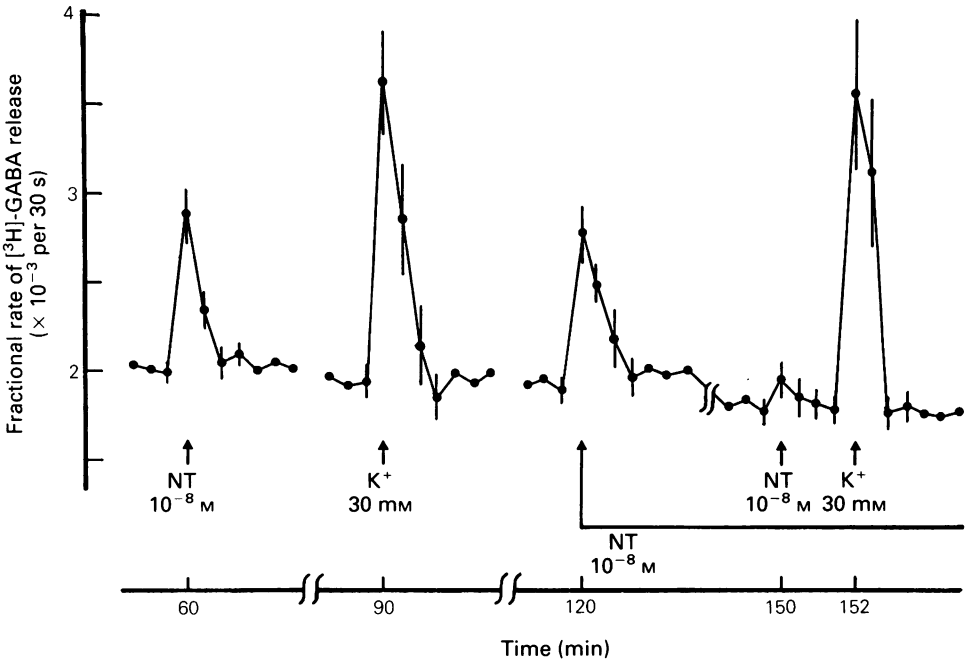

Figure 2 Desensitization of neurotensin (NT)-evoked release of [^{3}H]-γ-aminobutyric acid ([^{3}H]-GABA). Neurotensin (10^{-8} M) and high KCl (3×10^{-2} M) were perfused fo 30 s at 30 min intervals. Each point represents the mean from 5 animals with s.e.mean shown by vertical lines.

Table 2 Effects of various agents on the spontaneous and neurotensin-evoked release of [^{3}H]-acetylcholine ([^{3}H]-ACh).

		Fractional rate of [^{3}H]-ACh release ($\times 10^{-3}$ per min)		$\frac{b}{a} \times 100$
		Spontaneous (a)	*Evoked (b)*	
None (control)	(5)	5.430 ± 0.185	7.635 ± 0.244	140.6
Ca^{2+}-free medium containing 10^{-3} M EGTA	(4)	5.260 ± 0.241	5.322 ± 0.327*	101.2*
Tetrodotoxin 3×10^{-7} M	(4)	5.327 ± 0.321	5.760 ± 0.455*	108.1*
Hexamethonium 3×10^{-4} M	(4)	5.305 ± 0.289	7.559 ± 0.352	142.5
Scopolamine 10^{-6} M	(4)	5.266 ± 0.311	7.850 ± 0.352	149.1
[D-Pro^2,D-Trp^{7,9}] substance P 10^{-6} M	(4)	5.263 ± 0.308	7.095 ± 0.416	134.8

The fractional rate of the spontaneous and neurotensin-evoked [^{3}H]-ACh release was measured after 120 min superfusion. Neurotensin (10^{-8} M) was applied for 30 s to the perfusion medium. Ca^{2+}-free medium containing EGTA (10^{-3} M) and tetrodotoxin were applied 15 min and the other agents were applied 10 min before the measurements. Values are mean ± s.e.mean for the number of experiments shown in parentheses. *Significance of difference from the control value was calculated by Student's *t* test, at the <0.05 level of probability.

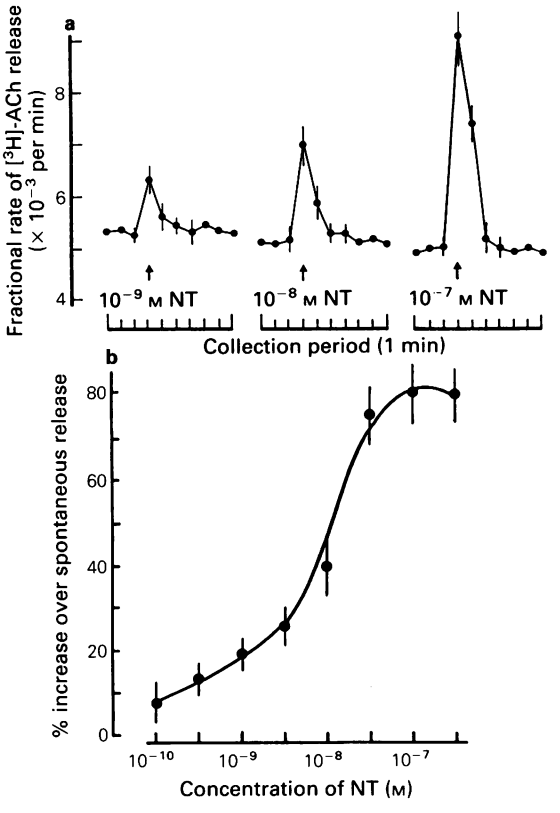

Figure 3 Neurotensin (NT)-evoked release of [^{3}H]-acetylcholine ([^{3}H]-ACh). (a) Typical experiments showing [^{3}H]-ACh release. (b) Dose-response curve for neurotensin. Neurotensin was perfused for 30 s at 30 min intervals. Each point represents the mean from 5 animals with s.e.mean shown by vertical lines.

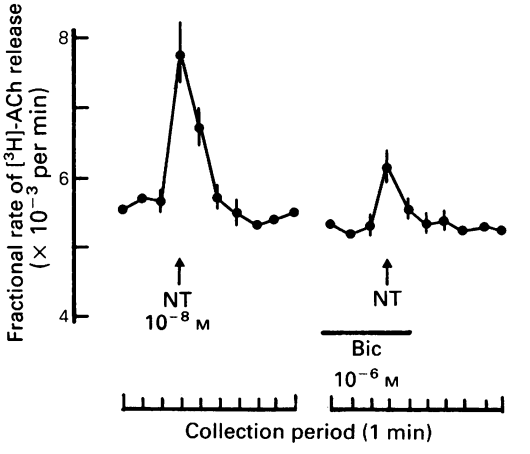

Figure 4 Inhibition by bicuculline (Bic) of neurotensin-evoked release of [^{3}H]-acetylcholine ([^{3}H]-ACh). Neurotensin (NT, 10^{-8} M) was perfused for 30 s. Bicuculline (10^{-6} M) was applied 10 min before and during addition of neurotensin. Each point represents the mean for 5 animals with s.e.mean shown by vertical lines.

Neurotensin-evoked release of [^{3}H]-ACh

The release of [^{3}H]-ACh from the strips preloaded with [^{3}H]-choline was examined in the presence of neurotensin. The fractional rate of the spontaneous release of tritium after 60 min superfusion, was $0.00566 \pm 0.00025\ min^{-1}$ (mean ± s.e. for 30 determinations). When 10^{-8} M neurotensin was applied 3 times to the same preparation at 30 min intervals, there were no significant differences in the percentage increase in [^{3}H]-ACh release by the first to the third applications of neurotensin. Neurotensin (3×10^{-10} M–10^{-7} M) evoked [^{3}H]-ACh release, in a dose-dependent manner (Figure 3). The neurotensin (10^{-8} M)-evoked release of [^{3}H]-ACh was prevented by superfusion with Ca^{2+}-free medium containing EGTA (10^{-3} M) and treatment with tetrodotoxin (3×10^{-7} M), indicating that the released [^{3}H]-ACh is of neuronal origin (Table 2). Hexamethonium (3×10^{-4} M), scopolamine (10^{-6} M) and [D-Pro^2, D-Trp^{7,9}] substance P (10^{-6} M) did not affect the neurotensin (10^{-8} M)-evoked release of [^{3}H]-ACh (Table 2): hence nicotinic, muscarinic and substance P receptors apparently do not participate in the effect of neurotensin on cholinergic neurones. Bicuculline (10^{-6} M), an antagonist for GABA, inhibited the neurotensin (10^{-8} M)-evoked release of [^{3}H]-ACh by 64.1 ± 6.3% (mean ± s.e. for 5 determinations) (Figure 4), without effect on the spontaneous release.

Discussion

We have obtained evidence that neurotensin is a substance which stimulates GABAergic neurones in the guinea-pig enteric nervous system. Neurotensin evoked a dose-dependent, Ca^{2+}-dependent and tetrodotoxin-sensitive release of GABA from the isolated ileum. As hexamethonium and scopolamine were without effect on the neurotensin-evoked release of GABA, nicotinic and muscarinic receptors are probably not involved in the effect of neurotensin on GABAergic neurones. The release of GABA evoked by neurotensin, but not by high K^+ was inhibited in the presence of neurotensin. Since the desensitization to excitatory action of neurotensin is a selective

phenomenon, neurotensin may induce a release of GABA through its own receptor.

The site of action of neurotensin appears to be somatic rather than at the axon terminals of GABAergic neurones, because the tetrodotoxin-resistant release of neurotransmitter is assumed to be due to the direct depolarization of nerve terminals (Alberts *et al.*, 1982; Gonella *et al.*, 1980). Electrophysiological studies with intracellular recording demonstrated that neurotensin depolarized the Type 1 myenteric neurones of guinea-pig ileum (Williams *et al.*, 1979). Neurotensin may stimulate the cell bodies of GABAergic neurones located within the myenteric plexus, as demonstrated immunohistochemically (Saito & Tanaka, 1986). The possibility must be considered that neurotensin stimulates the GABAergic neurone through the other neurones. The contraction of intestine induced by neurotensin is reported to be mediated by the release of substance P (Monier & Kitabgi, 1980), a compound which induces the release of GABA (Tanaka & Taniyama, 1985). However, the release of GABA evoked by neurotensin was reduced slightly, but not significantly during desensitization to substance P, therefore substance P is probably not involved in such an action of neurotensin on GABA release.

Neurotensin evoked a neuronal release of ACh from ileal strips. When the neurotensin-evoked release of [^{3}H]-ACh is calculated according to the proportion of [^{3}H]-ACh to total tritium in the superfusates (as described in the methods section) it is greater than the value shown in Figure 3a. Such an effect of neurotensin induces contraction of the ileum (Kitabgi & Freychet, 1979; Zetler, 1980; Huidobro-Toro & Zhu, 1984). The neurotensin-evoked release of ACh was partly inhibited by bicuculline, an antagonist for GABA. Based on the findings that GABA induced neuronal release of ACh from the myenteric plexus of the guinea-pig ileum (Kleinrok & Kilbinger, 1983; Taniyama *et al.*, 1983; Yau & Verdun, 1983), our results indicate that bicuculline inhibits the binding of endogenous GABA released by neurotensin to the GABA receptor: hence the neurotensin-evoked release of ACh is inhibited. The interaction of neurotensin, GABAergic neurone and cholinergic neurone may occur in the guinea-pig ileum. The action of neurotensin appears to be similar to that of substance P in the intestine (Tanaka & Taniyama, 1985). The maximum amounts of GABA and ACh released by 10^{-7} M neurotensin (approximately 1.7 fold and 1.8 fold the amount released spontaneously) are less than the amounts released by substance P (3.3 fold and 4 fold). Neurotensin seems to be less potent than substance P in the stimulation of GABAergic and cholinergic neurones. Similar differences in potency in depolarizing myenteric neurones between neurotensin and substance P have been shown in electrophysiological studies (Williams *et al.*, 1979). The present results demonstrate that neurotensin applied exogenously induces the release of GABA and ACh through its own receptor. There are differences between the distribution of neurotensin and that of substance P within the intestinal tissue of guinea-pig. Neurotensin is present predominantly in the endocrine cells of mucosa (Helmstaedter *et al.*, 1977; Sundler *et al.*, 1977; Schultzberg *et al.*, 1980), whilst most of the substance P is present in the extramucosal part, mainly in neurones. In view of this localization, it seems unlikely that neurotensin acts directly on the myenteric plexus to evoke the release of GABA and ACh selectively. It is possible that neurotensin acts on the GABAergic neurone and cholinergic neurone indirectly through the release of some intermediary substances.

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References

ALBERTS, P., BARTFAI, T. & STJÄRNE, L. (1982). The effects of atropine on [^{3}H]acetylcholine secretion from guinea-pig myenteric plexus evoked electrically or by high potassium. *J. Physiol.*, **329**, 93–112.

BAXTER, C.F. & ROBERTS, E. (1961). Elevation of γ-aminobutyric acid in brain: selective inhibition of γ-aminobutyric-α-ketoglutamic acid transaminase. *J. biol. Chem.*, **236**, 3287–3294.

ERDÖ, S.L. (1985). Peripheral GABAergic mechanisms. *Trends Pharmac. Sci.*, **7**, 205–208.

GONELLA, J., NIEL, J.P. & ROMAN, C. (1980). Mechanism of the noradrenergic motor control on the lower oesophageal sphincter in the cat. *J. Physiol.*, **306**, 251–260.

HELMSTAEDTER, V., TAUGNER, C.H., FEURLE, G.E. & FORSSMANN, W.G. (1977). Localization of neurotensin-immunoreactive cells in the small intestine of man and various mammals. *Histochemistry*, **53**, 35–41.

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.X. (1984). Neurotensin receptors on the ileum of the guinea-pig: Evidence for the coexistence of inhibitory and excitatory receptors. *Eur. J. Pharmac.*, **102**, 237–250.

JESSEN, K.R., MIRSKY, R., DENNISON, M.E. & BURNSTOCK, G. (1979). GABA may be a neurotransmitter in the vertebrate peripheral nervous system. *Nature*, **281**, 71–74.

KERR, D.I.B. & KRANTIS, A. (1983). Uptake and stimulus-

evoked release of [^{3}H]-γ-aminobutyric acid by myenteric nerves of guinea-pig intestine. *Br. J. Pharmac.*, **78,** 271–276.

KITABGI, P. & FREYCHET, P. (1979). Neurotensin contracts the guinea-pig longitudinal ileal smooth muscle by inducing acetylcholine release. *Eur. J. Pharmac.*, **56,** 403–406.

KLEINROK, A. & KILBINGER, H. (1983). γ-Aminobutyric acid and cholinergic transmission in the guinea-pig ileum. *Naunyn-Schmiedebergs Arch. Pharmac.*, **322,** 216–220.

LEANDER, S., EKMAN, R., UDDMAN, R., SUNDLER, F. & HAKANSON, R. (1984). Neuronal cholecystokinin, gastrin-releasing peptide, neurotensin, and β-endorphin in the intestine of the guinea-pig. *Cell Tiss. Res.* **235,** 521–531.

MONIER, S. & KITABGI, P. (1980). Substance P-induced autodesensitization inhibits atropine resistant, neurotensin-stimulated contractions in guinea pig ileum. *Eur. J. Pharmac.*, **65,** 461–462.

POTTER, L.T. & MURPHY, W. (1967). Electrophoresis of acetylcholine, choline and related compounds. *Biochem. Pharmac.*, **16,** 1386–1388.

SAITO, N. & TANAKA, C. (1986). Immunohistochemical demonstration of GABA containing neurons in the guinea pig ileum using purified GABA antiserum. *Brain Res.*, **376,** 78–84.

SCHON, F. & KELLY, J.S. (1975). Selective uptake of [^{3}H] β-alanine by glia: association with the glial uptake system for GABA. *Brain Res.*, **86,** 243–257.

SCHULTZBERG, M., HOKFELT, T., NILSSON, G., TERENIUS, L., REHFELD, J.F., BROWN, M., ELDE, R., GOLDSTEIN, M. & SAID, S. (1980). Distribution of peptide- and catecholamine-containing neurones in the gastro-intestinal tract of rat and guinea-pig: immunohistochemical studies with antisera to substance P, vasoactive intestinal polypeptide, enkephalins, somatostatin, gastrin/cholecystokinin, neurotensin and dopamine β-hydroxylase. *Neuroscience*, **5,** 689–744.

SUNDLER, F., HAKANSON, R., HAMMER, R., ALUMETS, J., CARRAWAY, R., LEEMAN, S. & ZIMMERMAN, E. (1977). Immunohistochemical localization of neurotensin in endocrine cells of the gut. *Cell Tiss. Res.*, **178,** 313–321. 321.

TANAKA, C. (1985). γ-Aminobutyric acid in peripheral tissues. *Life Sci.*, **37,** 2221–2235.

TANAKA, C. & TANIYAMA, K. (1985). Substance P provoked γ-aminobutyric acid release from the myenteric plexus of the guinea-pig small intestine. *J. Physiol.*, **362,** 319–329.

TANIYAMA, K., KUSUNOKI, M., SAITO, N. & TANAKA, C. (1982). Release of γ-aminobutyric acid from cat colon. *Science*, **217,** 1038–1040.

TANIYAMA, K., KUSUNOKI, M., SAITO, N. & TANAKA, C. (1983). GABA evoked ACh release from isolated guinea pig ileum. *Life Sci.*, **32,** 2349–2353.

WILLIAMS, J.H., KATAYAMA, Y. & NORTH, R.A. (1979). The action of neurotensin on single myenteric neurons. *Eur. J. Pharmac.*, **59,** 181–186.

YAU, W.M. & VERDUN, P.R. (1983). Release of γ-aminobutyric acid from guinea pig myenteric plexus synaptosomes. *Brain Res.*, **278,** 271–273.

YAU, W.M. VERDUN, P.R. & YOUTHER, M.L. (1983). Neurotensin: A modulator of enteric cholinergic neurons in the guinea pig small intestine. *Eur. J. Pharmac.*, **95,** 253–258.

ZETLER, G. (1980). Antagonism of the gut-contracting effects of bombesin and neurotensin by opioid peptides, morphine, atropine or tetrodotoxin. *Pharmacology*, **21,** 348–354.

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