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Gastrointestinal neurotensin receptors: lack of modulation by thyrotropin releasing hormone

M. Veronica Donoso, J. Pablo Huidobro-Toro*, *Laboratory of Pharmacology, Department of Physiological Sciences, Faculty of Biological Sciences, Catholic University of Chile, Casilla 114-D, Santiago 1, Chile*

To examine whether thyrotropin releasing hormone (TRH) antagonized gastrointestinal neurotensin receptors in isolated segments of the rat fundus, duodenum and ileum, tissues were superfused, mounted and the isometric tension recorded. Picomoles of neurotensin caused concentration-dependent contractions of the fundus and relaxation of the smooth muscles of the small intestine. Preincubation with 1–10 μM TRH failed to antagonize the activity of neurotensin but potentiated neurotensin-induced relaxation of the ileum. Pretreatment of the tissues with 0·6 μM of neuropeptide fragment 1–11, also failed to block the neurotensin-induced effects but produced a significant potentiation of the relaxant action of neurotensin.

Since the isolation of neurotensin from the small intestine (Carraway et al 1978; Hammer et al 1980), it has been hypothesized that this basic 13-amino acid peptide (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu OH) participates in the regulation of motility and secretion in the gastrointestinal tract. Evidence from two independent investigations supports the notion that neurotensin may function as a physiological regulator in the gastrointestinal system. Neurotensin is potent in contracting the fundus (Quirion et al 1980a) and relaxing small intestine in animals (Kitabgi 1982). On a molar basis, neurotensin is about 50 times as potent as acetylcholine (Kullak & Huidobro-Toro 1983) and 100 times as potent as noradrenaline or adrenaline when causing relaxation of the ileum and colon of the guinea pig or the rabbit (Kitabgi & Vincent 1981; Huidobro-Toro & Yoshimura 1983). Several reports have shown that a meal rich in lipids, or a liquid lipid diet sharply increases blood level of neurotensin (Rosell & Rokaeus 1979; Rosell 1982). The hypothesis that under the appropriate physiological stimuli, diet for example, the intestinal neurotensin stores are mobilized to modify the motility of the intestines via the activation of selective neurotensin receptors distributed along the gastrointestinal tract is supported by these findings.

To prove the existence of specific neurotensin receptors a selective neurotensin receptor blocking agent is required. Although Quirion et al (1980b) and Rioux et al (1980) found that the D-Trp^{11}-neurotensin analogue reduced neurotensin-induced relaxations of isolated blood vessels under certain conditions, this peptide is neither a potent, nor a universal, neurotensin receptor antagonist. Recent reports suggest that thyrotropin releasing hormone (TRH, pGlu-His-Pro NH_2) blocks the neurotensin-induced analgesic response in rodents (Osbarh et al 1981; Hernández et al 1984) and so the aim of this investigation was to examine whether TRH antagonized neurotensin-induced muscular responses in isolated preparations of the gastrointestinal tract. In addition the activity of two neurotensin analogues (neurotensin fragment 1-11 and D-Trp^{11}-neurotensin) as putative gastrointestinal neurotensin receptor antagonists was studied.

* Correspondence.

Materials and methods

Animals and tissue preparation. Adult Sprague Dawley rats (250–280 g) were killed by cervical dislocation and the abdomen opened along a midline incision to the stomach and intestines. Strips of duodenum and ileum were dissected and mounted in a double jacketed superfusion bath and maintained at 37 °C with oxygenated Tyrode solution (Huidobro-Toro & Miranda 1981) and maintained at a tension of ca 1 g. Longitudinal fundus strips were obtained by dissection of the stomach along the larger curvature and mounting the tissues under conditions identical to those of the intestinal segments. Isometric tension was recorded using a Grass FT 03C force displacement transducer connected to a Grass multichannel polygraph. The tissues were equilibrated for at least 1 h, under in-vitro conditions before the drugs were added.

Quantification of drug responses; estimation of drug potency. Increasing concentrations of neurotensin were added to the baths at 8 min intervals and the drugs left in contact with the tissues until the maximal responses were attained, from these responses the concentration-response curves were obtained. The contractile effect of neurotensin in the fundus was quantified as the g of peak tension developed. Interpolation of each concentration-response curve gave the EC50 (concentration causing half maximal contraction). To quantify the relaxant action of the neuropeptide, the duodenum, or ileum, was first made to contract with 55 nM acetylcholine; after 40 s when the muscle maintained 4–5 g of active tension, graded concentrations of neurotensin or other agonists were added. The inhibitory responses were quantified as the g of relaxation induced by neurotensin (see recordings in Fig. 1). The concentration of neurotensin causing half maximal inhibition (IC50) was derived by interpolation from each of the neurotensin-response curves obtained.

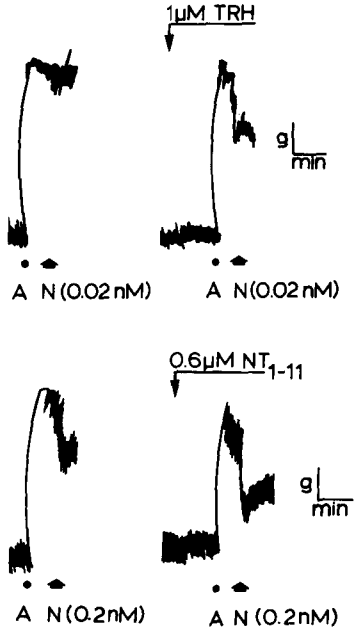

FIG. 1. Isometric muscular recording from the rat ileum. At the dots, application of 55 nM acetylcholine (A) to contract the tissues; at the arrows, addition of neurotensin (N). In the case of the experiment with thyrotropin releasing hormone (TRH), shown in the upper panel, the tissues were challenged with 0·02 nM N. In the lower panel, the effect of 0·2 nM N was studied before and after preincubation with 0·6 μM neurotensin 1-11. At the concentrations tested, neither TRH nor neurotensin 1-11 produced significant changes in the ileum contractility.

Statistics. The experimental design was such that neurotensin-response curves were obtained from the response of the same tissue before and after drug treatment and the paired Student's *t*-test was used to evaluate changes in the EC50. Significance was $P < 0{\cdot}05$.

Drugs. Neurotensin and neurotensin 1–11 as the triacetate salts, noradrenaline bitartrate, adenosine 5'-monophosphate and acetylcholine hydrochloride were purchased from Sigma Chemical Co. (MO, USA). TRH was obtained from Beckman (CA, USA). The sample of D-Trp^{11}-neurotensin was a kind gift of Dr S. St Pierre, University of Sherbrooke, Canada.

Results

TRH at a concentration of 1 μM failed to antagonize the neurotensin-induced muscular contractions in the rat gastrointestinal tract. As shown in Fig. 2, TRH did not significantly modify the excitatory activity of neurotensin on the rat fundus or its inhibitory activity on the duodenum. In the ileum, where neurotensin caused the most potent inhibition of the smooth muscle tension, TRH potentiated neurotensin-induced smooth muscle relaxation. TRH pretreatment displaced the neuropeptide concentration-response curve to the left (Fig. 2), in a parallel fashion, increasing the potency of the neuropeptide. The polygraph tracing (Fig. 1), shows that although 0·02 nM neurotensin in a naive preparation proved inactive, after pretreatment with 1 μM TRH there was a significant and reproducible relaxation. The action of TRH on the rat ileum was selective, and TRH did not potentiate the inhibition produced by noradrenaline or adenosine 5'-monophosphate (Table 1).

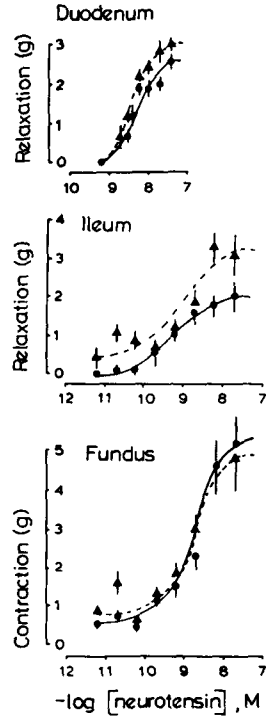

FIG. 2. Effect of TRH on the neurotensin-induced muscular activity in the rat gastrointestinal tract. Neurotensin-response curves were derived from rat fundus, duodenum and ileum in the absence (●) and in the presence (▲) of 1 μM TRH. Symbols represent the mean values, bars the s.e. Twelve separate fundus preparations were tested, eight of which were re-examined in the presence of TRH. In the case of the duodenum and ileum, the results were obtained from 6 separate rat preparations.

For details of the potency of neurotensin see Tables 1 and 2. Increasing the concentration of TRH to 10 μM did not substantially affect the results observed with 1 μM TRH. No antagonism was observed in the rat ileum, rather some degree of potentiation was in evidence, as demonstrated by the finding that the neurotensin IC50 decreased from 0·44 ± 0·08 to 0·30 ± 0·08 nM (n = 4).

In a separate series of experiments, the pretreatment of gastric or intestinal strips with neurotensin analogues was examined. Preincubation of gut segments with 0·02 or 0·6 μM neurotensin 1-11 did not block the neurotensin

responses (Table 2); but caused a significant potentiation of the inhibitory action of neurotensin on the ileum. Neurotensin 1-11 proved to be inactive in concentrations up to 1 μM.

In a further series of experiments D-Trp[11]-neurotensin was examined. Results indicate that while in the ileum, 2 nM neurotensin caused 3·3 ± 0·12 g of relaxation, in tissues pretreated with 19 nM D-Trp[11]-neurotensin, the relaxation increased to 4·0 ± 1·1 g ($P < 0·05$, n = 3). In the rat fundus preincubation of the tissues with 19 nM D-Trp[11]-neurotensin tended to decrease the contractile action of 6 nM neurotensin (from 2·8 ± 0·8 to 2·1 ± 0·6 g; $P > 0·05$, n = 3). D-Trp[11]-neurotensin proved inactive at 20 nM; much larger concentrations were required before a modest degree of neurotensin-like activity was seen.

Table 1. Effect of thyrotropin releasing hormone (TRH) on the inhibitory potency of neurotensin and other agonists.

	Ileum IC50 (nM), mean ± s.e.	
	Before	After 1 μM TRH
Neurotensin	0·76 ± 0·09	0·57 ± 0·09 n = 11
Noradrenaline	55·0 ± 7·5	43·6 ± 7·1 n = 6
Adenosine 5'-monophosphate	1840 ± 630	2060 ± 470 n = 6

Table 2. Effect of pretreatment with the inactive neurotensin fragment 1-11 on the potency of neurotensin.

	nM (mean ± s.e.)	
	Fundus EC50	Ileum IC50
I. Control	0·98 ± 0·08 (4)	0·65 ± 0·05 (6)
+ 0·02 μM neurotensin 1-11	1·01 ± 0·15 (4)	0·89 ± 0·18 (6)
II. Control	1·03 ± 0·12 (3)	1·03 ± 0·03 (3)
+ 0·6 μM neurotensin 1-11	1·70 ± 0·30 (3)	0·34 ± 0·08* (3)

Numbers in parentheses denote the times the experiment was repeated in separate preparations.
* $P < 0·01$, paired Student's t-test.

Discussion

The idea that TRH might function as a gastrointestinal neurotensin antagonist or modulator was attractive because of the need to find a neurotensin blocking drug and also because TRH itself is biosynthesized and released from gastrointestinal tissues (Leppäluoto et al 1978). Were TRH to be an antagonist acting at the neurotensin receptor level or by modification of a local regulatory mechanism, the interaction would be of interest. Unlike the analgesic action of neurotensin its gastrointestinal effects, excitatory or inhibitory, were not altered by TRH. It is unlikely that TRH-induced blockade of neurotensin analgesia occurred at the receptor level; this interaction probably reflects an event occurring at a site distal to the receptor. TRH also antagonized only neurotensin-induced analgesia, not the hypothermia or the hyperglycaemia it induced (Hernández et al 1984). The lack of neurotensin blockade by two of its closely related structural analogues, suggests that these peptides lack affinity for the neurotensin receptors and in view of the structural requirement for neurotensin receptor occupation (Leeman & Carraway 1982; Donoso & Huidobro-Toro 1984), it seems unlikely that TRH could antagonize the analgesic action of neurotensin via blockade of the receptor as other neuropeptide sites with similar properties can be found in the gastrointestinal system. Alternative explanations could be that multiple neurotensin receptors are involved, but this is premature.

Although it is not fully understood why, or how, TRH potentiates the action of neurotensin in the ileum and not the duodenum, we suggest that its increased potency in the presence of TRH may be due, in part, to a decrease in the rate of metabolism of the peptide in the superfusion bath. Consistent with this, it could be argued that TRH or neurotensin 1-11 are substrates of ileum peptidases and as such compete with neurotensin for enzymatic hydrolysis. One such enzyme could be prolylendopeptidase, which is known to catabolize both neurotensin and TRH (Wilk 1983). Since prolylendopeptidase prefers small peptide substrates, metabolism of neurotensin could be substantially reduced in the presence of high concentrations of TRH or of the neuropeptide analogue. In support of this, TRH potentiated only the inhibitory activity of neurotensin not that of noradrenaline or adenosine 5'-monophosphate.

Although little investigation into the relation of the biochemical nature of neurotensin receptor(s) in the gastrointestinal tract has been done, recent work by Goedert et al (1984) demonstrates that [^{3}H]neurotensin binds to an apparent single population of sites. In spite of the high non-specific labelling, these receptors are predominantly located in the smooth muscle layers of the guinea-pig ileum, particularly the circular layer. These binding studies corroborate the findings of Donoso & Huidobro-Toro (1984) who presented evidence in favour of muscular distribution of the neurotensin receptors as opposed to neuronal localization.

In conclusion, neither TRH, D-Trp[11]-neurotensin nor neurotensin 1-11, antagonized the gastrointestinal effects of neurotensin, suggesting that all these peptides lack affinity for the neurotensin receptors located in the rat digestive system.

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Cerebrospinal fluid uptake and peripheral distribution of centrally acting drugs: relation to lipid solubility

HERMANN R. OCHS*, DAVID J. GREENBLATT‡, DARRELL R. ABERNETHY‡§, RAINER M. ARENDT†¶, JOACHIM GERLOFF†, WOLFGANG EICHELKRAUT†, NORBERT HAHN†, *Medizinische Universitätsklinik D-5300 Bonn-Venusberg, and †The Department of Experimental Surgery, University of Bonn, West Germany; and ‡The Division of Clinical Pharmacology, Tufts–New England Medical Center, Boston, USA.*

In an anaesthetized dog model, serum kinetics and CSF entry were determined after i.v. administration of the following 8 drugs: salicylic acid (as acetylsalicylic acid), antipyrine, acetaminophen (paracetamol), lidocaine (lignocaine), trimipramine, amitriptyline, haloperidol, and imipramine. Kinetic variables were evaluated in relation to in-vitro lipophilicity, measured by the reverse-phase high-pressure liquid chromatographic (HPLC) retention index. After correction for individual values of serum binding (determined as the CSF: serum ratio at equilibrium), in-vivo volume of distribution was highly correlated with HPLC retention ($r = 0{\cdot}92$). Conversely, the time of peak CSF concentration and the CSF entry half-life were negatively correlated with HPLC retention ($r = -0{\cdot}83$ and $-0{\cdot}63$, respectively). Thus lipophilicity is a physiochemical property which has an influence on the peripheral distribution of drugs as well as their rate of entry into CSF.

For many drugs, the time-course and intensity of their pharmacodynamic action depends upon the time course of concentrations at the receptor site mediating pharmacological activity. This in turn depends not only upon the rate and extent of distribution to the tissue at which the drug is active, but also on drug uptake into other tissues which serve as storage or depot sites. For centrally acting drugs, the rate of entry into brain and the rate and extent of peripheral distribution may be determinants with as much influence on the time course of action as the rates of elimination or clearance (Coutinho et al 1970; Oldendorf et al 1972; Ramsey et al 1979; Caccia et al 1980a, b; Howerton et al 1983; Arendt et al 1983a). Alterations in physiological state, such as obesity, may profoundly influence the distribution and elimination half-life of drugs without altering total metabolic clearance (Abernethy et al 1981a, 1982a; Abernethy & Greenblatt 1982).

Extent of lipid solubility has been proposed to explain variations within and between individuals in the rate and extent of drug distribution (Arendt et al 1983a, b; Toon & Rowland 1983; Greenblatt et al 1983a). We have evaluated the rate of entry into cerebrospinal fluid (CSF), as well as the extent of peripheral distribution, of some drugs varying widely in lipid solubility. All of them have actions on the central nervous system.

Methods

Determination of lipophilicity in-vitro. The high pressure liquid chromatographic (HPLC) retention index (Arendt et al 1983a; Greenblatt et al 1983a; Arendt & Greenblatt 1984) was used to determine lipophilicity of

* Correspondence.
§ Current address: Baylor College of Medicine, Houston, TX, USA.
¶ Current address: Klinikum Grosshadern, Munich.

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