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Gastrointestinal neurotensin receptors: contribution of the aromatic hydroxyl group in position 11 to peptide potency

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Neurotensin structural analogues on tyrosine[11] were tested *in vitro* to determine their ability to contract the fundus or relax the intestine. The rank order of potency was: neurotensin > [Phe[11]]-neurotensin > [D-Tyr[11]]-neurotensin > [D-Phe[11]]-neurotensin. All peptides behaved as full agonists. It is concluded that tyrosine[11] is part of the neurotensin pharmacophore; the hydroxyl group increases the affinity not the intrinsic activity of the peptide at the receptor.

Introduction Based on the anatomical localization of neurotensin (*p*-Glu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-LeuOH) along the gastrointestinal tract (Helmstaedter *et al.*, 1977; Schultzberg *et al.*, 1980) and on the observation that picomoles of the peptide greatly modify the tone of the intestinal muscles (Kitabgi, 1982; Huidobro-Toro & Zhu, 1984; Huidobro-Toro & Kullak, 1985), several investigators have suggested that neurotensin participates in the control of gastrointestinal motility. Consistent with such a physiological role, recent biochemical experiments have demonstrated that neurotensin binds to a selective receptor site in the fundus (Kitabgi *et al.*, 1984). In support of the existence of a selective receptor site, studies with structural analogues indicate stringent requirements for receptor activation establishing that the 8–13 fragment retains full biological activity (Quirion *et al.*, 1980a,b). We now report on the contribution of the aromatic hydroxyl group in tyrosine[11] with regard to potency for contraction of the fundus or relaxation of the intestine.

Methods Isolated fundus or small intestinal strips of the rat were prepared as detailed by Donoso & Huidobro-Toro (1985). Tissues were superfused in a 30 ml organ bath with Tyrode solution at 37°C and aerated to maintain a pH close to 7.4. Changes in isometric muscular tension were recorded with a force displacement transducer (FT 03C) coupled to a Grass physiograph.

Neurotensin concentration-response curves were obtained non-cumulatively by adding increasing concentrations of peptides to the bath chambers. The duodenum was contracted with 0.55 μM acetylcholine and the ileum with 0.05 μM acetylcholine prior to the addition of neurotensin (see recordings in Figure 1). The activity on the fundus was quantified as g of tension developed; the inhibitory response of the intestine as g of relaxation of the acetylcholine-induced contractions. The median effective concentrations were calculated by interpolation from each concentration-response curve. Results are expressed as the median effective concentration ± s.e.

Results Neurotensin contracts the smooth muscles of the rat fundus, but relaxes the small intestine (Figure 1); the median effective concentration in the fundus was 1.4 ± 0.2 nM; whereas in the duodenum and ileum it was 4.8 ± 0.9 and 0.8 ± 0.1 nM respectively. A diasteroisomer, [D-Tyr[11]]-neurotensin, proved markedly less potent in all three bioassays. The neurotensin/[D-Tyr[11]]-neurotensin potency ratio was not the same in all three tissues; the most significant change in potency was demonstrated in the duodenum followed by the ileum, and the fundus. Although it may appear from Figure 1a that this peptide behaves as a partial agonist in the fundus, this was not the case. In separate series of experiments, the maximal tension developed in response to [D-Tyr[11]]-neurotensin was not significantly different from that achieved by neurotensin.

[Phe[11]]-neurotensin was less potent than neurotensin in contracting the fundus (10.0 ± 1.3 nM) or relaxing the duodenum and ileum (31.0 ± 4.4 and 2.2 ± 0.2 nM respectively). The neurotensin/[Phe[11]]-neurotensin potency ratio ranged between 0.14–0.36. [D-Phe[11]]-neurotensin behaved as a full agonist although it proved remarkably less potent in all three tissues (Figure 1); the median effective concentration of the peptide was approximately 16.4 ± 0.6 μM in the

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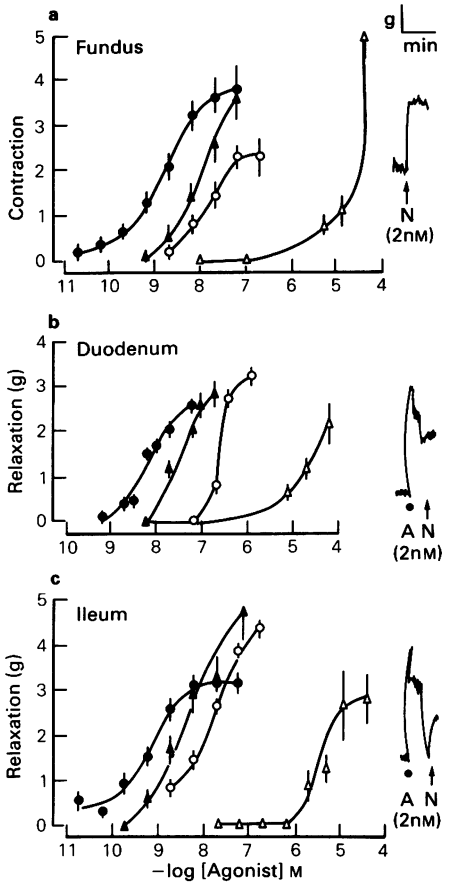

Figure1 Concentration-response curves of neurotensin and structural analogues on the fundus (a), duodenum (b) and ileum (c) of the rat. Neurotensin (NT) curves represent the composite of all the response curves performed prior to the testing of each analogue and average 15 separate preparations in the fundus, 12 in the duodenum and 19 in the ileum. Symbols denote the mean values; bars the s.e.: (●) neurotensin; (○) [D-Tyr^{11}]-neurotensin; (▲) [Phe^{11}]-neurotensin; (△) [D-Phe^{11}]-neurotensin.

fundus, $14.3 \pm 1.8\,\mu$M in the duodenum, and $3.9 \pm 0.6\,\mu$M in the ileum. Interestingly, the relation between the potency ratio of neurotensin/[D-Tyr^{11}]-neurotensin and [Phe^{11}]-neurotensin/[D-Phe^{11}]-neurotensin was only 10 for the duodenum but about 100 for the fundus and ileum.

Discussion Three main conclusions can be derived from this study. In the first place, the hydroxyl group of the tyrosine^{11} residue must play a significant role in the formation of the neurotensin receptor complex. The importance of this OH group seems particularly evident in the fundus and duodenum. The potency ratio between neurotensin/[Phe^{11}]-neurotensin in the ileum is 0.36 as compared to 0.14–0.15 in the other tissues. The lack of the hydroxyl group or the incorrect spatial orientation of the aromatic ring decreases peptide potency but not maximal effect. This finding implies that this particular OH group increases mainly the affinity rather than the intrinsic activity of the peptide at the receptor. In this connection, we tested inactive concentrations of [D-Tyr^{11}]-neurotensin or [D-Phe^{11}]-neurotensin to see whether they antagonized the effects of neurotensin. Neither peptide showed neurotensin-receptor blocking properties (data not shown).

Second, neurotensin interacts stereospecifically with its receptors, as shown by the very significant drop in potency when comparing the activity of neurotensin versus [D-Tyr^{11}]-neurotensin. Interestingly, the degree of stereospecificity of the phenylalanine^{11} substituted diasteroisomers is larger than that of the tyrosine^{11} diastereoisomers, stressing the importance of the aromatic hydroxyl group.

Finally, these analogues followed the same order of potency in all three bioassays, indicating that the recognition sites for neurotensin causing either contraction or relaxation have similar structural requirements. This conclusion is supported by the work of Quirion *et al.* (1980a,b), Rioux *et al.* (1980) and Kitabgi (1982).

The hydroxyl group in position 11 may serve to stabilize neurotensin with its receptor, perhaps via a hydrogen bond. In the absence of such an interaction, larger concentrations of the analogues are needed to obtain full agonist activity. Further work is required to discover whether the variations in potency of the peptides in the tissues examined reflect differences in receptor topology, modalities of neurotensin-receptor interactions or pharmacokinetic variables. It is unlikely that metabolism contributes to potency since the D-substituted peptides are known to be more resistant to enzymatic hydrolysis. There is, as yet, no solid evidence for more than one type of neurotensin receptor although this possibility warrants further investigation.

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