Contractile activity of synthetic neurotensin and related polypeptides on guinea-pig ileum

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The discovery of a tridecapeptide, neurotensin in bovine hypothalamus was made by Carraway & Leeman (1974, 1975a) who found that neurotensin induced hypotension in the rat and caused contraction of various isolated smooth muscle preparations, while producing relaxation of the rat duodenum. Subsequently, Carraway & Leeman (1975b) synthesized this physiologically important principle by the solid phase technique (Merrifield, 1963) and demonstrated that the synthetic product was chemically and biologically indistinguishable from the isolated native neurotensin. However, so far no studies have attempted to correlate the amino-acid sequence of neurotensin with biological activity. Besides neurotensin, Yajima, Fujii & others (1974) and Yajima, Kitagawa & others (1975) synthesized five shorter chain peptides related to neurotensin amino-acid sequences of which are shown in Table 1. These were derived, according to the trifluoromethanesulphonic acid procedure of Yajima & others (1974), from synthetic intermediates of neurotensin (Yajima & others, 1975), respectively. In this paper, the biological activity of these analogues was compared for their ability to contract guinea-pig ileum.

Fresh sections of guinea-pig ileum were suspended in a 20 or 40 ml bath containing modified Tyrode solution gassed with air and maintained at 29 ± 1°. The modified Tyrode solution was composed (mM) of NaCl 136·9, KCl 2·7, $CaCl_2$ 0·9, $MgCl_2$ 2·1, NaH_2PO_4 0·4, $NaHCO_3$ 11·9, and glucose 5·6. Muscle contraction was recorded isontonically with a writing lever on a smoked drum. The load applied to the tissue was approximately 1·5 g which allowed the preparation to develop sufficient contraction and tone. The peptides tested were dissolved in saline solution which was previously boiled for 3 min and bubbled with nitrogen-gas for 1 min, and were kept in a siliconized tube at 3° for up to one week before being added to the organ bath.

The typical contraction induced by neurotensin, together with that induced by acetylcholine is shown in Fig. 1. Application of neurotensin in concentrations higher than 10^{-8} M increased both tone and motility of the ileum. Thereafter the tone gradually decreased but the motility was sustained until the bath was washed out with modified Tyrode solution. The response was not affected by previous addition of scopolamine, cyproheptadine, diphenhydramine, tolazoline and propranolol. Similar results were obtained with other

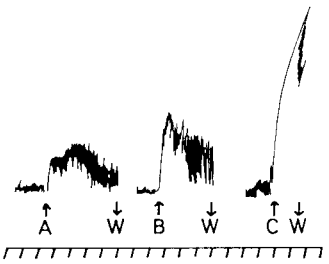

FIG. 1. Effect of synthetic neurotensin on guinea-pig ileum. A—neurotensin 10^{-8}M, B—neurotensin 2×10^{-8} M, C—acetylcholine $5{\cdot}5 \times 10^{-9}$ M, W—wash.

Table 2. *The relative contractile activity of synthetic neurotensin and related polypeptides on guinea-pig ileum.*

neurotensin	1
NT_{2-13}	0.726 (0.613 – 0.859)
NT_{4-13}	0.588 (0.536 – 0.632)
NT_{5-13}	0.352 (0.296 – 0.416)
NT_{6-13}	0.212 (0.178 – 0.251)
NT_{8-13}	0.208 (0.170 – 0.255)

The data are assessed statistically by 4 point assay procedure.
The relative activity is expressed on a molar basis.
Figures in parentheses are 95% confidence intervals.

Table 1. *Amino-acid sequences of synthetic neurotensin and related polypeptides.*

Neurotensin	pyrGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH
NT_{2-13}	H-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH
NT_{4-13}	H-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH
HT_{5-13}	H-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH
NT_{6-13}	H-Lys-Pro-Arg-Arg-Pro-Tyr-Ilr-Leu-OH
NT_{8-13}	H-Arg-Arg-Pro-Tyr-Ile-Leu-OH

† Correspondence.

neurotensin analogues. The relative biological activity towards contraction of guinea-pig ileum was assessed using a 4 point assay procedure. The results are shown in Table 2.

The contractile activity was reduced by removing the *N*-terminal amino-acid but the shortest chain peptide NT_{8-13} still showed activity corresponding to one fifth of that of neurotensin. This suggests that the amino-acid in the *C*-terminal region seems to be more important for the contractile activity than that in the *N*-terminal region. The most interesting fact is that there is a chemical resemblance between NT_{6-13} and xenopsin, pyrGlu-Gly-Lys-Arg-Pro-Trp-Ile-Leu-OH, which was recently isolated from the frog skin (Araki, Tachibana & others, 1973). Xenopsin was found to have a moderate contractile activity on guinea-pig ileum with the threshold dose of $1{\cdot}25 - 6{\cdot}25 \times 10^{-5}$ M (Araki & others, 1973). In this experiment, both NT_{8-13} and NT_{6-13} produced considerable contraction on guinea-pig ileum at a dose of 2×10^{-7} M. Therefore our results suggest that Arg_8 and/or Tyr in *C*-terminal region are necessary for the contractile activity of neurotensin on guinea-pig ileum.

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The adhesion of film coatings to tablet surfaces-measurement on biconvex tablets

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The adhesion of a film coating to a tablet substrate has recently been quantified by measuring the force required to remove the film from a known area of the tablet surface using a specially designed tensile testing apparatus (Fisher & Rowe, 1976). In an attempt to obtain a direct measure of the adhesive forces the instrument was designed to remove the film normal to the tablet surface. This implies that the technique is only applicable to flat-faced tablets since during the testing of film-coated biconvex tablets, there is progressive removal of the film from around the edges of the tablet to the centre. This paper examines the possibility of using the apparatus in the measurement of the adhesion of film coatings to biconvex tablets.

Four sizes of tablets (6·25, 7·94, 10·0 and 11·11 mm diameter, biconvex and flat-faced) were prepared by compressing a standard placebo granule consisting of lactose, starch and magnesium stearate using an instrumented tablet machine (Type F3 Manesty Machines Ltd). To minimize porosity changes in the tablets all were compressed at a constant compression pressure of 200 MPa. The tablets were coated with a film formulation consisting of a mixture of four parts hydroxypropyl methylcellulose and one part ethylcellulose (Grade N7 Hercules Powder Co. Ltd.) with 20% w/w glycerol as plasticizer, applied as a 2·5% w/v solution dissolved in a dichloromethane–methanol (70 : 30% v/v) solvent mixture using either a 6 inch diameter Wurster column or 24 inch Accelacota Manesty Machines Ltd). To obtain a range of coatings with varying adhesions, three grades of hydroxypropyl methylcellulose were used, Pharmacoat 603, Pharmacoat 606 (Shinetsu Chemical Co. Ltd., Japan) and Methocel HG60 viscosity 50 (Dow Chemical Co., U.S.A.). The thickness of the film was approximately 30–40 μm and the tablets were stored at room temperature and 50° R.H. for two weeks before testing. Ten tablets were used for each measurement and the mean and standard deviation calculated. The work done in removing the film was calculated from the areas under the traces obtained from the ultraviolet recorder.

In all the experiments performed (Table 1) the forces required to remove the film from biconvex tablets were consistently lower than those required to remove the same film from the corresponding flat-