CHAPTER 5

SYNTHETIC PEPTIDE AS A TOOL FOR BIOASSESSMENT
5 Introduction

Neurotensin (NT) is a tridecapeptide whose sequence is Glu-Leu-Tyr-Glu-lys-pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH. It was originally isolated from calf hypothalamus by Caraway and Leeman in 1973. It has a wide spectrum of pharmacological action including production of hypotension, gut contraction increased vascular permeability, hypoglycemia and hyper glycogonemea. Like many other neuropeptide, it fulfills a dual function of neurotransmitter or neuromodulator in the nervous system and of local hormone in the periphery. NT is a neuromodulator of dopamine transmission and of anterior pituitary hormone secretion and exerts potent hypothermic and analgesic effects in the brain.

Like all neuropeptides, neurotensin (NT) is synthesized as part of a larger precursor which also contains neuromedin (NN), a six aminoacid neurotensin like peptide (1). NT and NN are located in the C-terminal domain of the precursor (pro-NT/NN), where they are flanked and separated by Lys-Arg sequences. A fourth dibasic sequences preceding an N N-like sequences is present in the N-terminal domain.

Neurotensin and somatostatin inhibit glucose by modulating release of insulin but no action was reported on glucogen by pancreatic islets (2). In vitro studies of neurotensin on the endocrine
pancreas shows at low glucose concentration for short period stimulates insulin, glucagons and somatostatin release (3).

Administration of neurotensin to dogs resulted in rises in circulating blood glucose, glucagons and insulin levels, the rise in glucagons being more pronounced than that in insulin. Infusion of somatostatin along with neurotensin suppressed glucagons and insulin responses to neurotensin and prevented the rise in blood glucose levels. These results suggest that the hyperglycemia seen after neurotensin is due to neurotensin stimulation of glucagons release over insulin release (4). Williams, G. et al. (5) reported that neurotensin concentration was found to be reduced in hypothalamus in genetically obese diabetic rat. Neurotensin levels in pancreas were increased in diabetic animals. Daily administration of insulin to diabetic rats completely reversed this effect, and pancreatic neurotensin levels in these animals return to control values (6). Concerning its neuromodulatory role, the possibility of subtle and complex modulation of the dopaminergic system offers the exciting possibility of a new treatment strategy for certain psychotic disorders (7,8,9,10).

This chapter deals with the quantifications of neurotensin isolated from brain stem, cerebral cortex and hypothalamus of controlled and diabetic rats. For this, neurotensin was synthesised on
GDMA crosslinked PMMA support and HPLC peak areas were determined corresponding to various concentrations of synthesised neurotensin. Then a standard working curve was constructed as shown in figure (5.1), taking different concentrations of synthesised neurotensin along the X-axis and corresponding HPLC peak areas along the Y-axis.

![Graph showing concentration vs peak area](image)

**Fig. 5.1**

### 5.1 Methodology and Interpretation

As pointed out earlier, this chapter deals with the quantification of neurotensin isolated from brain stem, cerebral cortex and hypothalamus of controlled and diabetic rats using a standard
working curve as shown in figure 5.1. The isolation, purification and HPLC profile of neurotensin from controlled and diabetic rats were done as explained below.

5.1.1 Isolation of native neurotensin from controlled and diabetic rats

Wistar rats of ~200g body weight were used for all experiments. They were housed in separate cages under 12-hour dark period. The animals were maintained on standard food pellets and water ad libitum. Diabetes was induced by a single intra-femoral dose of streptozotocin (STZ) prepared in citrate buffer, pH 4.5. The animals were sacrificed by decapitation, the brain regions were dissected out according to Glowninski and Iversen. Tissues were homogenized in 0.4N perchloric acid and centrifuged at 2500g for 10 minutes and the supernatent was filtered and lyophilized.

5.1.2 Purification of isolated lyophilized crude native neurotensin

Lyophilized crude peptides were subjected to gel filtration on Sephadex G-15(200x4cm column; eluted with 0.1M ammonium acetate, pH 8.6). The next purification step was an ion-exchange chromatography on carboxy methyl cellulose(CM-5C whatman). Ammonium acetate elution gradients were used with initial and final buffer concentrations chosen as a function of the calculated
electrical charge of the peptide at neutral PH. Column dimensions were 25 × 2.5 cm and a three vessel gradient forming device (kontess) was used. Elution profiles were monitored by measuring absorbance at 230nm, with a Beckman DB-GT spectrophotometer connected to a Gilson Sampler.

Quantities of peptides were calculated from the mean residue value of stable aminoacids after acid hydrolysis (6M, Hcl. 20h.1100°C) of an aliquot and aminoacid analysis by a Beckman1200c. Enzymatic digestion involved incubation with papain and then with peptidase and then with imidopeptidase. Reverse-phase high performance liquid chromatography was performed on a Waters Associates instrument model 204. Columns were either c18/ Bondaple. Two homologous columns were fitted in series and run with 10mM triethyl ammonium phosphate (PH3)/Mehanol (50/50,v/v) at acetonitrile (74/26,v/v) at 1.5ml/min. Peptides were dissolved in the eluting buffer and about 20nmol were injected. Column effluents were continuously monitored by absorbance at 230nm.

5.1.3 Concentration of neurotensin isolated from brainstem of controlled and diabetic rats

Neurotensin was isolated from brain stem of controlled and diabetic rats and purified as explained in 5.2.1 and 5.2.2. The HPLC profiles of neurotensin isolated from 10mg of brainstem of
controlled and diabetic rats are shown in the figure 5.2 and 5.3., respectively. The peak area was found 3623 units for controlled rat and 2520 units for diabetic rat.

The concentrations of neurotensin corresponding to these peak area were determined with the aid of the working curve, fig 5.1, and found 0.57636 and 0.400891 respectively.

Fig. 5.2

Fig. 5.3
5.1.4 Concentration of neurotensin isolated from cerebral cortex of controlled and diabetic rats

Neurotensin was isolated from cerebral cortex of controlled and diabetic rats and purified as explained 5.2.1 and 5.2.2. The HPLC profiles of neurotensin isolated from 10mg of cerebral cortex of controlled and diabetic rats are shown in the figure 5.4 and 5.5 respectively. The peak area was found 3237 units for controlled and 4939 units for diabetic rat. The concentrations of neurotensin corresponding to these peak areas were determined with the help of the working curve, fig 5.1, and found 0.514954 and 0.785714 respectively.

Fig. 5.4
5.1.5 Concentration of neurotensin isolated from hypothalamus of controlled and diabetic rats

Neurotensin was isolated from hypothalamus of controlled and diabetic rats and purified as explained in 5.2.1. and 5.2.2. The HPLC profiles of neurotensin isolated from 10mg of hypothalamus of controlled and diabetic rats are shown in the figure 5.6 and 5.7, respectively.
The peak area was found 7720 units for controlled rat and 2994 units for diabetic rat. The concentration of neurotensin corresponding to these peak areas were determined using the working curve, figure 5.1, and found 1.228126 and 0.476297 respectively.
References


