Radiosynthesis of New Radio Neurotensin (8-13) Analogues.

D. Terriere*, K. Chavatte*, M. Ceusters**, D. Tourwé** and J. Mertens*

VUB-Cyclotron*, Laarbeeklaan 103, 1090 Brussel, Belgium.

Department Org. Chem. V.U.B**, Pleinlaan 2, 1050 Brussel, Belgium.

SUMMARY.

Two types of radiolabelled neurotensin(8-13) analogues have been synthesised. For radioiodination 2- and 4-bromophenyl-acetyl and 2- and 4-bromobenzoyl Arg^8 substituted neurotensin(8-13) were used as substrates for radiolabelling carried out by the Cu^{1+} assisted non-isotopic nucleophilic exchange with a labelling yield ranging from 55 to 85% depending on the position of the bromo atom in the phenyl ring. For labelling with ^{111}In, DTPA was substituted as chelating group on the Arg^8 position of neurotensin(8-13). Labelling was achieved in kit-conditions with a yield of 98%. The non carrier added peptide analogue was recovered by HPLC with a purity > 99% and a specific activity of at least 370 TBq/mmol. The inhibition constant values for the binding of [^{3}H]neurotensin to guinea pig fore brain membranes were of the order of 7.5 nM for the halophenyl compounds and 6.5 nM for the DTPA substituted analogue.

Key words : Neurotensin(8-13), Radioiodination, Halophenyl-NT(8-13), ^{111}In-DTPA-NT(8-13)

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INTRODUCTION.

Neurotensin (NT,Glu^{1}-Leu-Tyr-Glu-Asn-Lys-Pro-Arg^{8}-Arg-Pro-Tyr-Ile-Ileu^{13}) is a linear tridecapeptide that was originally isolated from the bovine hypothalamus (1). NT is found in high concentrations in the ileum and the hypoyhalamus and has a broad range of biological effects including hypotension, analgesia, gut contraction, and increase of vascular permeability. NT and its receptors were also found in several tumor cells such as small cell lung carcinoma, human colon carcinoma and human meningiomas.

Structure-activity studies have shown that the C-terminal hexapeptide of neurotensin NT(8-13) is the minimal fragment required for biological activity (2). Radiolabeled NT(8-13) may be an attractive vector for tumor targetting, a strategy already succesfully explored for the somatostatin analogue octreotide. Taking into account that many tumor types do not express somatostatin receptors, NT analogues may be complementary tools for diagnosis and therapy. We now report results on the development of NT(8-13) analogues which are labelled with ^{123}I and ^{111}In, suitable for SPECT.

MATERIALS AND METHODS.

HPLC equipment :

Analytical : The equipment consists of a reodyne injector (50 μl loop), a Hitachi 655A pump and L-6000 II controller, a 655A variable wavelength UV monitor at 254 nm, a NaI(Tl) detector (Harshaw QS) and appropiate electronics (Canberra), a D2000 Chromato integrator Hitachi and an Ankersmidt R40 one-channel recorder.

Quality control was achieved on a Vydac C18 250x4.6 mm Alltech column with H_20/MeOH/ACN//TFA : 80/5/15//0.1 (v/v) mixture as eluent at a flow rate of 1 ml/min.

Semi-preparative : Rheodyne injector (2.5 ml loop), a Waters M6000A pump provided with a semi-preparative pumphead, a Waters Lambdamax UV-480 monotor at 254 nm, A NaI(Tl) detector (Harshaw QS) connected to Ortec electronics, a HP 3580 and a Intersmat ICR-18 integrator.

A H_20/ACN//TFA : 14/86//0.1 v/v mixture of pH 1.9 was used as eleuent at a flow rate of 6 ml/min on a Vydac C18 (10μ) 250x10 mm Alltech column.

Synthesis of the peptide analogues, precursors for radiolabelling.

Neurotensin(8-13) (Arg-Arg-Pro-Tyr-Ile-Leu) was prepared using the solid phase synthesis method, on a standard Merrifield resin, with BOC main chain protection, as described previously (3).

The bromobenzoyl and bromophenylacetyl NT(8-13) analogues were obtained by coupling of the corresponding phenyl entities to the N-terminus of Arg^8 using dicyclohexylcarbodiimide /hydroxybenzotriazole as coupling agents (4).

The DTPA-NT(8-13) was obtained by coupling DTPA-tetra-T-butylester to the peptide with TBTU/hydroxybenzotriazole to the resin-bound peptide (5).

After cleavage of the peptide from the resin using HF_{liq} , the peptides were purified using HPLC conditions described above.

The synthesis of both the precursors was previously described in detail (5).

The radioiodination of the phenyl substituted NT(8-13) analogues.

Stock solution : To 1 mg $SnSO_4$, 25 mg 2,5-dihydroxybenzoic acid and 35 mg citric acid, 500 μl glacial acetic acid and 4500 μl of water were added.

Copper solution : 32,5 mg $CuSO_4.5H_20$ was dissolved in 10 ml of water.

Reaction : To 1 mg of the precursor dissolved in 10μl 100% AcOH were added 500 μl stock solution and 60 μl of the copper solution. After flushing with N_2 for 5 min. the radioiodine solution was added and the reaction mixture was heated at 140°C for 1 hour.

Radiosynthesis of non carrier added (n.c.a.) ^{111}In-DTPA-NT(8-13).

Labelling was performed in a kit-formulation.

Kit composition :10 μg DTPA-NT(8-13) , 4.96 mg trisodiumcitrate, 0.37 mg citric acid, 10 mg inositol and 2 mg 2,5-dihydroxybenzoic acid.

To the kit 1.1 ml 0.02 N HCl and $^{111}InCl_3$ in 0.02 N HCl were added. After 30 minutes at room temperature the reaction mixture was diluted with 1 ml of Semi-prep. eluent and injected on the semi-prep. HPLC. The radiolabelled peptide was collected at 25.5 min and the collected eluent was diluted with an equal volume of doubly-destilled water. The ^{111}In-DTPA-NT(8-13) was pre-concentrated on a Baker Bond Octadecyl 100 mg mini-column and recovered in 1 ml of EtOH/PBS-buffer - 50/50 (v/v) pH 7.4 (PBS buffer : 0.14 M NaCl, 19 mM Na_2HPO_4, 2.4 mM NaH_2PO_4).

Drug competition.

Tissue preparation (6): Pirbright guinea-pigs (250-300g) were sacrificed by decapitation. The brains were rapidly removed and the fore-brain area was

dissected. Tissue samples were homogenised in 15 ml of Tris-HCl buffer (50 mM, pH = 7.4) with a Ultraturrax homogenizer. The homogenate was centrifuged at 16000 RPM for 10 min in a refrigerated Sorvall centrifuge (Du Pont Instruments). The pellet was twice re-homogenised and re-centrifuged as described above. The final pellet was suspended in a Tris-HCl buffer in a dilution of 12.5 mg of original wet weight of tissue/ml.

[^{3}H]Neurotensin binding. (6): [^{3}H]Neurotensin binding was performed in 0.5 ml Tris-HCl incubation buffer (50 mM Tris-HCl pH 7.4, 0.5 mM o-phenanthroline, 1 mM EDTA , 0.1 % BSA) containing 10 mg of homogenate and 1 nM [^{3}H]Neurotensin. Incubation was run at 25°C for 30 min , 4 ml of ice-cold Tris-HCl buffer (50 mM, pH 7.4) was added and followed by rapid filtration, under reduced pressure, through Whatman GF/B glass fibre filters, using a 40-well Multividor (Janssen Scientific Instruments). The filters were rinsed twice with 4 ml ice-cold Tris-HCl buffer and placed in plastic vials with 4 ml of Instagel Gold Scintillation fluid (Packard).Afer 2 hours the radioactivity was counted in a Packard Scintillation spectrometer.

Before use, the filters were soaked for 2 hours in 0.1% of polyethylenimine to reduce adsorption of the peptide to the filters. IC_{50} values obtained from the drug competition experiments were calculated using the pseudo Hill-plot (7).

RESULTS AND DISCUSSION.

Radiosynthesis.

Radioiodinated NT(8-13) analogues.

The Cu(I)-assisted nucleophilic non isotopic displacement reaction on brominated

precursor molecules in reducing and acidic conditions was used for the radioiodination of the phenyl substituted NT(8-13) analogues.(4,8). The formation of 10-20% of radioiodinated side products was observed. The advantages of this method are that the brominated precursor can be prepared by standard peptide synthesis methods without selectivity problems and that the radioiodine can be introduced at any position determined by the position of the bromine atoms.

Table 1. shows the labelling yields obtained for the phenylacetyl and benzoyl analogues. As it is known that the carbonyl group activates the nucleophilic exchange on ortho and para position of the substituted benzoyl group , one should expect a higher yield for the Br-benzoyl-NT(8-13) analogues than for Br-phenylacetyl-NT(8-13) analogues. Nevertheless the 2-Br-phenylacetyl-NT(8-13) shows a yield comparable to that obtained with the 2-Br-benzoyl compound. It is supposed this is due to a weak chelating interaction of the carbonyl with Cu^{1+}, thus facilitating the catalytic interaction with the ortho-bromo on the phenacyl group (4,5).

Brominated precursor	Labelling Yield %
2-Br-phenylacetyl-NT(8-13)	84
4-Br-phenylacetyl-NT(8-13)	54
2-Br-benzoyl-NT(8-13)	86
4-Br-benzoyl-NT(8-13)	55
4-Br-benzoyl-NT(8-13) 2 mg	71

Table 1 : Labelling yield dependence for the different phenyl groups substituted on NT(8-13).

^{111}In-DTPA-NT(8-13).

For labelling with ^{111}In, DTPA was substituted as chelating group on the Arg^{8} position of neurotensin(8-13) as described above in an almost quantitative manner. An earlier described method (9) based on monoactivation of one carboxylic acid using one molar equivalent of dicyclohexylamine and 1-hydroxybenzotriazole did not yield reproducible results. Therefore the proposed method was preferred.

The labelling is achieved in kit-conditions with an average labelling yield of more than 98%. The overall yield of the n.c.a. ^{111}In labelled NT(8-13) is about 75% with a radiochemical purity of at least 99% and a specific activity > 370 TBq/mmol.

Drug Competition.

Table 2 shows the Ki values, derived from the IC_{50} values, obtained for the different neurotensin analogues.

Peptide	Ki (nM)
Neurotensin	4.9
H-NT(8-13)	1
Acetyl-NT(8-13)	6.8
2-Br-phenylacetyl-NT(8-13)	7.8
4-Br-phenylacetyl-NT(8-13)	11.8
2-Br-benzoyl-NT(8-13)	7.5
4-Br-benzoyl-NT(8-13)	7
DTPA-NT(8-13) Substrate ^{111}In labelling.	6.5

Table 2 : Ki values of the different NT(8-13) analogues for the inhibition of [^{3}H]neurotensin binding performed in guinea pig forebrain homogenate (Kd [^{3}H]neurotensin 7.1 nM).

Substitution of a halophenyl group or a complexing group on the Arg^8 of NT(8-13) hardly changes the affinity for neurotensin receptor sites as compared to the original neurotensin.

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