Influence of arginine mimetics on the biological effects of NT(8-13) analogues

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Introduction

Neurotensin(NT) is a 13 amino acid containing neuropeptide expressed in both central nervous system (CNS) and periphery (gastrointestinal tract and the cardiovascular system). The biological effect is result from specific interaction of the peptide with three different receptors (NTS1, NTS2 and NTS3/sortilin). The basic sequence for modifications was its C-terminal hexapeptide NT(8-13) (Arg⁸-Arg⁹-Pro¹⁰-Tyr¹¹-Ile¹²-Leu¹³). Structure-activity studies on NT show that this fragment is equipotent or even more potent than the native NT(1-13) in binding to the NT receptor, while maintaining the same biological and pharmacological properties.

Besides their numerous central and peripheral functions it was reported that NTRs are over expressed in various human tumours. NT peptides are known to influence cell proliferation and pass through blood-brain barrier. Like many other neuropeptides, the main drawback in the use of NTs is extremely short half-life as a result of their rapid degradation by peptidases. To overcome this problem, various neurotensin analogues were synthesized.

Herein, we present the synthesis and biological properties of a small peptide library of the new neurotensin ligands 1-9 incorporating arginine mimetics (Figure 1).

Figure 1: NT(1-13) analogues

Results and Discussion

Structurally, NT(8-13) exhibits the so-called “hot spots” that are suitable for further modification. Structure-activity studies on NT(8-13) shown that the last C-terminal residue of the peptide could not be removed or amidated without a complete loss of biological activity. The two guanidino groups of Arg⁸-Arg⁹, the hydroxy-phenyl group of Tyr¹¹, the side chains of Ile¹²-Leu¹³ and the carboxylic group of Leu¹³ are the functional groups of NT(8-13). The Arg⁹ appeared to be more critical to the activity of NT(8-13) than was Arg⁸. Furthermore, it was well known that the cleavage sites are located between Arg⁸-Arg⁹, Pro¹⁰-Tyr¹¹ and Tyr¹¹-Ile¹².

Previously, we replaced the terminal arginine units with its non-proteinogenic analogue canavanine (Cav) [1]. These modification resulted in the discovery of enzymatically and hydrolytic (in different values of pH, available physiologically) stable and more potent analogues [2, 3].

To learn more about the biological activity of NT(8-13), we envisioned the synthesis of target peptides 1-6 (Figure 1) incorporating shorter homologues of arginine - α -amino-4-guanidino-butyric acid (Agh), and α -amino-3-guanidino-propionic acid (Agp). Further replacement of the Tyr¹¹ with Phe(pF) was also achieved (peptides 7-8). All peptides were synthesized using standard solid-phase protocol by the Fmoc/^tBu strategy.

Elongation of the peptide chain was done by repetitive cycles starting from commercially available Leu-2-CT Resin. The crude peptides were precipitated into cold diethyl ether. Then, the precipitate was dissolved in 10% CH³COOH and desalted by gel filtration on a Sephadex G25.

The yields of each product were over 60%. HPLC and CE (Figure 2) analysis revealed a purity of >95% for all peptides.

Figure 2: Electropherogram of NT(8-13), analogue 9; Chromatographic conditions: Effective capillary length: 340/407 mm, silica fused; BGE: 20 mM Tris, 5 mM H₃PO₄, 50 mM SDS, pH 8,6, U = 25KV, I = 25µA, T 23oC, UV 200 nm

The biological activity of the new NT(8-13) analogues is currently under investigation. Received preliminary data from MTT assay of the parent molecule tested in a wide concentration range (2 - 0.03 mM) on MCF-7 (breast cancer cells) and 3T3 (non-cancerous cells) after 72 hours, showed slight influence on viability over both 'normal' and cancer cell lines.

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