NTS1 AND NTS2 DIVERSITY DELINEATED BY MOLECULAR MODELLING DRIVES THE SYNTHESIS OF NEW SELECTIVE NEUROTENSIN ANALOGUES

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Introduction

Neurotensin is a tridecapeptide first isolated by Caraway and Leeman (1973) from bovine hypotalami.(1) NT exerts a wide range of biological functions including hypothermic(2), analgesic(3) and antipsychotic properties(4). Its activity is related to the binding with different receptors (NTSs) belonging either to the superfamily of the G-protein-coupled receptors (NTS1 and NTS2) or to the family of sortilin receptors (NTS3)(5). NTS2 receptor is an important target for the analgesic effect of NT analogues since it has been demonstrated recently its implication in pain modulation(6). For this reason and for a better understanding of its physiological role, there is an urgent need to provide further information on the structure activity relationship of the receptor-ligand interaction to develop new selective analogues.

Results and Discussion

The recently crystallized rNTS1 receptor bound to its agonist peptide Neurotensin 8-13(7) gave the structural basis for NTS1 targeting. In this work, starting from these structural data, we built models for both hNTS1 and hNTS2 receptors bound to the NT[8-13] peptide and we were able to observe some discrepancies in terms of interaction. Interestingly, the positively charged Arg^{212} and the negatively charged Glu^{179} residues were aligned on the initial sequence alignment used for the homology modelling step, and were therefore located at the same position of the extracellular loop 2 of the two receptors, at the entry of the binding site (Figure 1). Based on this observation we chose to replace the Tyr^{11} with the basic amino acid lysine for the preparation of compound 1 (JMV 5836) and with aspartic acid for compound 2 (JMV 5839 and glutamic acid for compound 3 (JMV 5963). We also prepared compound 4 (JMV 5965) in which a lysine replaces the Tyr^{11} and the TMSAla residue replaces the C-terminal leucine since we have recently demonstrated that this modification not only increased the binding affinity compared to the NT native peptide but it produced analgesia in vivo in experimental models of acute and persistent pain.(8)

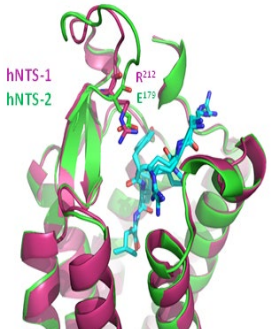

Figure 1. Localization of the two R212 and E179 residues in the extra-cellular loop 2 of hNTS1 (in magenta) and hNTS2 (in green) receptors, respectively.

We evaluated the ability of the NT[8-13] derivatives to inhibit the binding of ^{125}I-Tyr^3-NT on membranes prepared from cells stably expressing either hNTS1 or hNTS2 receptors and results are shown in table 1. The replacement of the residue at position 11 resulted in a decreased binding affinity for both hNTS1 and hNTS2, as compared to the native NT[8-13] peptide but in the case of compounds 1 and 4, a gain of selectivity toward hNTS2 was observed as it was expected from molecular modelling results.

Table 1. Binding potencies of the reference compound NT[8-13] and NT analogues.

compd	sequence	IC_{50} binding (nM)		selectivity
		hNTS1	hNTS2	hNTS1/ hNTS2
NT[8-13]	H-Arg-Arg-Pro-Tyr-Ile-Leu-OH	1.21 ± 0.06	7.46 ± 2.47	0.16
1	H-Lys-Lys-Pro-Lys-Ile-Leu-OH	6426 ± 858	297 ± 82.6	21.6
2	H-Lys-Lys-Pro-Asp-Ile-Leu-OH	>10,000	4753 ± 840	nd[a]
3	H-Lys-Lys-Pro-Glu-Ile-Leu-OH	>10,000	1824 ± 420	nd[a]
4	H-Lys-Lys-Pro-Lys-Ile-TMSAla-OH	752 ± 90.8	76 ± 29.5	9.89

[a] *Selectivity for these compounds is not determinable since binding on hNTS1 is greater than 10,000 nM.*

Once the binding results were determined, in order to confirm our hypothesis, we decided to express a mutated version of NTS1 receptor in order to verify if the affinity of our ligand could be restored. We then evaluated the affinity of compounds 1 and 4 on the hNTS1-R212E mutant by competition with ^{125}I-Tyr^3-NT. As shown in table 2, the binding affinity was increased for compounds 1 and 4 when tested on hNTS1-R212E. Compound 1 showed the most important gain in binding affinity with a 28-folds increase, whereas compound 4 displays a moderate affinity gain of 5-folds.

Table 2. Binding potencies of 1 and 4 toward the wild-type hNTS1 and mutated hNTS1-R212E receptors.

		IC_{50} binding (nM)		Affinity gain
compd	sequence	hNTS1-WT	hNTS1-R212E	R212E / WT
NT[8-13]	H-Arg-Arg-Pro-Tyr-Ile-Leu-OH	1.21 ± 0.06	5.32 ± 0.85	- 4.4
1	H-Lys-Lys-Pro-Lys-Ile-Leu-OH	6426 ± 858	226 ± 35.9	28.5
4	H-Lys-Lys-Pro-Lys-Ile-TMSAla-OH	752 ± 90.8	144 ± 19.4	5.22

These results indicate that the ionic interaction influence the binding affinity and plays a very important role in the selectivity towards the hNTS2 receptor.

References

01. Carraway R., Leeman S.E., J. Biol. Chem., 1973, 248(19), 6854-6861.
02. Dubuc I, Costentin, J., Doulut, S., Rodriguez, M., Martinez, J., Kitabgi, P. Eur. J. Pharmacol., 1992, 219(2), 327-329.
03. Nemeroff C.B., Osbahr A.J., Ervin G.N., Prange A.J., Jr. Proc. Natl. Acad. Sci. 1979, 76(10), 5368-5371.
04. Skoog K.M., Cain S.T., Nemeroff C.B. Neuropharmacology, 1986, 25(7), 777-782.
05. Kleczkowska P., Lipkowski A.W., Eur. J. Pharmacol., 2013, 716(1-3), 54-60.
06. Tetreault P., et al. FASEB J, 2013, 27(9), 3741-3752.
07. White J.F., et al. Nature, 2012, 490(7421), 508-513.
08. Fanelli R, et al. J. Med. Chem. 2015, 58(19), 7785-7795.