CONFORMATIONAL ANALYSIS OF AGOUTI-RELATED PROTEIN (AGRP)-BASED MELANOCORTIN RECEPTOR ANTAGONIST

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Introduction

Agouti-related protein (AGRP) is one of two known naturally occurring antagonists of G-protein coupled receptors and antagonizes the melanocortin-3 and -4 receptors (MC3R, MC4R). The C-terminal domain of the hAGRP peptide (fragment 87-132) and its shorter analog (fragment 87-120) containing four disulfide bridges have equipotent melanocortin receptor pharmacology as the full length endogenous form of this protein. It has also been identified that the core monocyclic decapeptide hAGRP(109-118) possesses only µM antagonism at the central MC3 and MC4 receptors, and agonist activity at the peripheral MC1R.

In attempts to identify a sequence of minimal length and minimal side chain cyclizations, yet still maintain nM mMC4R pharmacology, bicyclic AGRP-based ligand was designed and pharmacologically characterized. Additionally conformational analysis was performed using 2D NMR techniques.

Experimental

Peptide NMR samples were prepared by dissolving 3 mg of peptide 1 in 600 μL 95% H2O/5% D2O, adjusting the pH to 5.5, and adding DSS as an internal standard. NMR data were collected at 30 °C with 600 and 750 MHz Bruker Avance spectrometers in the Advanced Magnetic Resonance Imaging and Spectroscopy (AMRIS) facility at the University of Florida. Standard proton based 2D NMR data were collected, processed and analyzed.

Results and Discussion

Bicyclic AGRP analog possesses equipotent (within the inherent 3-fold experimental error) binding affinity, but was an 80-fold less potent antagonist than hAGRP(87-132). NMR measurement, computer assisted molecular modeling (CAMM), and cluster analysis of bicyclic hAGRP analogue identified five structural families, two of which are highly populated. Computational docking experiments of this bicyclic hAGRP derivative, using a three-dimensional homology molecular model of the mouse MC4R, was performed in attempts to identify any putative ligand-receptor interaction differences that could be identified to potentially account for the 80-fold difference in antagonist potency.

Conclusions

In this study 22-amino acid bicyclic antagonist possessing equipotent mMC4R binding as compared with the C-terminal hAGRP ligand that possesses five disulfide bridges and 46 amino acids, was identified. NMR conformational studies revealed that the core hAGRP(109-118) region possesses very similar conformation in both C-terminal AGRP and bicyclic analog.

Acknowledgements

This work has been supported by NIH Grants R01DK57080 (C.H.-L.), R01DK64250 (C.H.-L.), 5P41RR016105 (A.S.E.), and an NSF CAREER award (A.S.E.). C.H.-L. is a recipient of an American Diabetes Association Research Award. A.W. is a recipient of an American Heart Association Postdoctoral Fellowship.

References

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