Glycosylated PACAP Hormones are Neuroprotective in Animal Models of Parkinson’s Disease, Stroke and Traumatic Brain Injury

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**Abstract:** Peptides are a class of biomolecules that play many developmental and regulatory roles *in vivo*, and are critical in maintaining homeostasis. They can act as neurotransmitters, neuromodulators, hormones, and are widely distributed throughout the body in different organ systems in addition to the brain. Peptides have high affinity for their targeted receptors, which results in minimal off-target interactions and reduced side effects. Furthermore, their metabolism typically does not generate toxic by-products. Thus, peptides have immense potential to be viable therapeutic agents for various disorders and syndromes. Unfortunately, peptides are rapidly metabolized, exhibit poor penetration across biological membranes, and are not readily bioavailable.

We previously demonstrated that glycosylation of endogenous opioid neuropeptides enhances their stability and transport properties *in vivo* while retaining potency and efficacy at their original GPCR targets. Our current studies focus on the design, synthesis, and biological evaluation of glycopeptide analogues of the Pituitary Adenylate Cyclase Activating Peptide (PACAP). PACAP is a pituitary hormone that exhibits potent neuroprotective, antiapoptotic, and anti-inflammatory activity. It has been specifically shown to be neuroprotective in animal models of neurological disorders including stroke, traumatic brain injury (TBI) and Parkinson’s disease. PACAP exerts biological effects through three Class B GPCRs, namely PAC1, VPAC1, and VPAC2. We have prepared a small library of glycopeptides containing strategic amino acid substitutions to enhance stability and receptor selectivity. *In vitro* cAMP stimulation assays were performed in 3 separate CHO cell lines expressing either PAC1, VPAC1, or VPAC2 receptors in order to assess functional activity and receptor selectivity. Stability was analyzed in artificial CSF initially, and a subset of drug candidates were analyzed for *in vivo* BBB penetration and stability using microdialysis coupled with mass spectrometry. Selected compounds were then examined for their neuroprotective potential *in vivo* using models for stroke and TBI in mice and for Parkinson’s disease in rats. Overall, enhanced stability, increased penetration of the BBB, and neuroprotective effects have been observed *in vivo*. These analogues represent potential lead compounds for the treatment of neurodegeneration in a variety of contexts.

Research reported here was supported by the National Institute of Neurological Disorders and Stroke of the National Institutes of Health under Award Numbers R01NS091238 and R01NS084941, and by the Michael J. Fox Foundation. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. Additional support has been provided by Phoenix Children’s Hospital and UA Mission support to R.K.R