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SYMPOSIUM

Optimization of the Native Glucagon Sequence for Medicinal Purposes

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Abstract

Background:

Glucagon is a life-saving medication used in the treatment of hypoglycemia. It possesses poor solubility in aqueous buffers at or near physiological pH values. At low and high pH, at which the peptide can be formulated to concentrations of a milligram or more per milliliter, the chemical integrity of the hormone is limited, as evidenced by the formation of multiple degradation-related peptides. Consequently, the commercial preparation is provided as a lyophilized solid with an acidic diluent and directions for rendering it soluble at the time of use. Any unused material is recommended for disposal immediately after initial use.

Methods:

A set of glucagon analogs was prepared by solid-phase peptide synthesis to explore the identification of a glucagon analog with enhanced solubility and chemical stability at physiological pH. The physical properties of the peptide analogs were studied by solubility determination, high-performance chromatography, and mass spectral analysis. The biochemical properties were determined in engineered human embryonic kidney cell line 293 (HEK293) cells that overexpressed either the human glucagon or glucagon-like peptide-1 (GLP-1) receptors linked to a luciferase reporter gene.

Results:

We observed the previously characterized formation of glucagon degradation products upon incubation of the peptide in dilute acid for extended periods or elevated temperature. Lowering the isoelectric point of the hormone through the substitution of asparagine-28 with aspartic acid significantly increased the solubility at physiological pH. Similarly, the C-terminal extension (Cex) of the hormone with an exendin-based, 10-residue, C-terminal sequence yielded a peptide of dramatically enhanced solubility. These two glucagon analogs, D28 and Cex, maintained high potency and selectivity for the glucagon receptor relative to GLP-1 receptor.

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Abbreviations: (Asn) asparagine, (Asp) aspartic acid, (Boc) tert-butyloxycarbonyl, (cAMP) cyclic adenosine monophosphate, (Cex) C-terminal extension (GPSSGAPPPS), (D28) glucagonD28, (EC50) effective concentration, (GCGR) glucagon receptor, (GLP-1) glucagon-like peptide-1, (glucagonCex) glucagonGPSSGAPPPS, (HCl) hydrochloric acid, (HEK293) human embryonic kidney cell line 293, (HF) hydrogen fluoride, (HPLC) high-performance liquid chromatography, (LC-MS) liquid chromatography coupled with mass spectrometry, (MS) mass spectrometry, (PBS) phosphate-buffered saline, (pI) isoeclectric point, (T1DM) type 1 diabetes mellitus, (Thr) threonine, (Trp) tryptophan, (Tris) tris(hydroxymethyl)aminomethane, (TFA) trifluoroacetic acid

Keywords: glucagon, glucagonCex, glucagonD28, isoelectric point, solubility, stability

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Abstract cont.

Conclusions:

Glucagon presents unique structural challenges to the identification of an analog of high biological activity and selectivity that also possesses sufficient aqueous solubility and stability such that it might be developed as a ready-to-use medicine. The glucagon analogs D28 and Cex demonstrated all of the chemical, physical, and biochemical properties supportive of further study as potential clinical candidates for treatment of hypoglycemia.

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