

The Effect Of Glycosylation On The Potency Of Pramlintide, An Anti-Diabetic Drug

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Pramlintide is an analogue of amylin, a 37 amino acid glucoregulatory hormone (1). Pramlintide is currently approved by the FDA as an adjunct to insulin therapy for diabetes and its anorexigenic properties have also led to the investigation of its potential as an obesity treatment (1). Pramlintide is currently limited as a therapy as it must be administered by subcutaneous injection, three times daily, with no possibility of co-formulation with insulin (1). Therefore, extending the half-life of pramlintide to minimise the number of daily injections would be desirable. This could be achieved through peptide modifications. Glycosylation is one promising modification that protects peptides against degradation, oxidation, precipitation and denaturation (2). This study aimed to investigate whether glycosylation, although possibly pharmacokinetically beneficial, would affect pramlintide potency at its receptors.

N-acetylglucosamine (GlcNAc) was introduced to the amide nitrogens of the six asparagine residues in pramlintide: Asn3, Asn14, Asn21, Asn22, Asn31 and Asn35. The pentasaccharide

Man₃GlcNAc₂ (penta) or undecasaccharide (NeuAcGalGlcNAcMan)₂ManGlcNAc₂ (SG) was then added to each of these residues enzymatically. Using a paired experimental design, these pramlintides were screened at receptors which amylin activates. The GlcNAc peptides were screened at calcitonin receptor subtype (a) (CT_(a)), amylin receptor 1 AMY_{1(a)} and the amylin receptor 3 (AMY_{3(a)}). The other peptides were screened only at the AMY_{1(a)} receptor. Cos 7 cells were transiently transfected with the receptors (3). The potency of each pramlintide was determined by measuring the cAMP produced by receptor stimulation in an Alphascreen assay (3).

The GlcNAc pramlintides had similar potency to unmodified pramlintide at all receptors, although there were small (<5) fold reductions for Asn3 and Asn14 GlcNAc pramlintides. For the penta and SG pramlintides, significant reductions in potency were observed for Asn3, Asn14 and Asn22 (Table 1). The greatest reduction was for Asn3 SG pramlintide, where potency was reduced by ~40-fold compared to unmodified pramlintide. Penta or SG modification did not significantly affect pramlintide potency at Asn21, Asn31 or Asn35.

Table 1 Potency (pEC₅₀) of pramlintides at the AMY_{1(a)} receptor. Values are mean ± SEM for four independent experiments. Statistical significance was assessed by paired t-tests vs unmodified pramlintide; * *P*<0.05, ** *P*<0.01, *** *P*<0.001.

	Asn3	Asn14	Asn21	Asn22	Asn31	Asn35
Pram	9.21±0.27	9.30±0.29	9.30±0.29	9.25±0.26	9.44±0.20	9.32±0.19

Pent a	8.10±0.11**	7.99±0.33*	9.22±0.21	8.58±0.27* **	9.25±0.13	9.21±0.30
Pram	9.52±0.16	9.26±0.17	9.06±0.12	9.06±0.12	9.06±0.12	8.89±0.12
SG	7.89±0.11**	8.55±0.20*	8.62±0.20	8.17±0.11*	8.77±0.12	9.17±0.18

Glycosylation of pramlintide at positions 3, 14 and 22 appears to interfere with receptor activation. In contrast, glycosylation of positions 21, 31 and 35 did not affect peptide activity. This suggests that glycosylation of these residues may be a good strategy to improve pramlintide's pharmacokinetics.

(1) Younk LM et al, Expert Opin Pharmacother 12:1439, 2011

(2) Sola RJ & Griebenow K, J Pharm Sci 98:1223, 2009

(3) Gingell JJ et al, Peptides 31:1400, 2010