GLP-1 and GIP receptors co-agonists

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In 2030, it is predicted that about 15% of the United States population will be affected by diabetes [1]. Glucagon-like peptide 1 (GLP-1) and glucose-dependent insulintropic peptide (GIP) are incretin hormones that regulate blood glucose. GLP-1 stimulates insulin secretion, regulates glucose levels and promotes reduction of food intake but also induces nausea. GIP stimulates insulin secretion and induces insulin resistance. Dual agonists with balanced activity at both GLP-1R and GIPR have been shown to control glucose levels better than the parent GLP-1 and GIP peptides; they also facilitate larger body weight loss and provide opportunity to minimize nausea through appropriate balance of GLP-1R and GIPR agonism.

GLP-1 based scaffolds for dual GLP-1R and GIPR agonists have been secured through screening of a recombinant peptide library [2]. One of the scaffold included the “AID” central motif (peptide g1681) which, in addition to other modifications to the GLP-1 sequence, allowed for the generation of dual agonists of various potency ratio at GLP-1 and GIP receptors. New peptides were evaluated *in vitro* for their ability to stimulate cAMP release from transfected CHO cells expressing humanGLP-1R and GIPR. Lipid scan of peptide g1681 showed preferential positions for the attachment of this half-life extension modality. The effect of lipid chain length on potency ratio of the dual potency agonist g1681 was also examined. Table 1 shows sequences of most potent dual agonists with a lipid attached in positions 10, 12, 13, 17, 18, 21 or 40. Activation of both GLP-1 and GIP receptors was stronger with the longer lipids attached to g1681: hexanoyl vs palmitoyl.

Table 1: Lipid scan of g1681, the GLP-1R and GIPR co-agonist

Alias	Sequence	hGLP-1R EC ₅₀ (pM)	hGIPR EC ₅₀ (pM)
GLP-1	HAEGT FTSDV SSYLE GQAAK EFAIW LVKGR G	2	>23600
GIP	YAEGT FISDY SIAMD KIHQQ DFNWV LLAQK	>50700	35
g1681	Y-Aib-EGT FTSDL SILKE RQAID EFNWV LLKGG PSSGA PPPS	3	8
g1896	Y-Aib-EGT FTSD- K(PEG₄-Pal) SILKE RQAID EFNWV LLKGG PSSGA PPPS	0.4	1.3
g2017	Y-Aib-EGT FTSDL S- K(PEG₄-Pal) -LKE RQAID EFNWV LLKGG PSSGA PPPS	1.1	1.6
g1897	Y-Aib-EGT FTSDL SI- K(PEG₄-Pal) -KE RQAID EFNWV LLKGG PSSGA PPPS	0.6	2.1
g2021	Y-Aib-EGT FTSDL SILKE R- K(PEG₄-Pal) -AID EFNWV LLKGG PSSGA PPPS	1.0	2.0
g2022	Y-Aib-EGT FTSDL SILKE RQ- K(PEG₄-Pal) -ID EFNWV LLKGG PSSGA PPPS	2.8	2.4
g2024	Y-Aib-EGT FTSDL SILKE RQAID K(PEG₄-Pal) -FVNWV LLKGG PSSGA PPPS	0.7	1.4
g1734	Y-Aib-EGT FTSDL SILKE RQAID EFNWV LLKGG PSSGA PPPS- PEG₄-K(γE-Pal)	1.0	2.0
g2068	Y-Aib-EGT FTSDL SI- K(PEG₄-Hex) -KE RQAID EFNWV LLKGG PSSGA PPPS	20.8	94.1
g1897	Y-Aib-EGT FTSDL SI- K(PEG₄-Pal) -KE RQAID EFNWV LLKGG PSSGA PPPS	0.6	2.1
g2065	Y-Aib-EGT FTSDL SI- K(PEG₄-Stear) -KE RQAID EFNWV LLKGG PSSGA PPPS	1.2	1.7

Like GLP-1 and GIP, dual agonist g1681 was prone to degradation by enzymes in circulation: trypsin, chymotrypsin, DPP-IV, and others. To enhance proteolytic stability of g1681, several unnatural amino acids were incorporated into the sequence and their effect on dual agonism was evaluated. g1726 with αMeLeu13 showed increase of potency on both receptors. G2008 with Nle6 was 15 times more potent on the GIP receptor and g2033 with Aib11 was 12 times more potent on the GLP-1 receptor than the parent co-agonist g1681.

Table 2: Stabilized non-lipidated analogues of g1681

Alias	Sequence	hGLP-1 R EC ₅₀ (pM)	hGIP R EC ₅₀ (pM)
g1681	Y-Aib-EGT FTSDL SILKE RQAID EFNWV LLKGG PSSGA PPPS	3	8
g1726	Y-Aib-EGT FTSDL SI- αMeLeu -KE RQAID EFNWV LLKGG PSSGA PPPS	0.5	1.4
g2008	Y-Aib-EGT Nle -TSDL SILKE RQAID EFNWV LLKGG PSSGA PPPS	28.1	1.8
g2033	Y-Aib-EGT FTSDL Aib -ILKE RQAID EFNWV LLKGG PSSGA PPPS	2.7	31.2

In the sequence of g1681, combination of chosen substitutions with unnatural amino acids and selective lipidation allowed to secure analogues of increased *in vivo* stability and high potency at both GLP-1R and GIPR.

Peptides g1682 and g1736 retained high potency on both receptors, while g2007 showed somewhat lower activity at GIP R. The long C18 lipid chain in position 13 substantially diminished potency at both receptors, in comparison to potency of g2007 with the shorter C16 lipid chain.

Table 3: Lipidated analogues of g1681 with unnatural amino acids in the sequence

Alias	Sequence	hGLP-1R EC ₅₀ (pM)	hGIPR EC ₅₀ (pM)
g1681	Y-Aib-EGT FTSDL SILKE RQAID EFVNW LLKGG PSSGA PPPS	3	8
g1682	Y-Aib-EGT FTSDL SILKE RQAID EFVN- α MePhe LLKGG PSSGA PPPS- K(γ E-Pal)	7.1	6.3
g1736	Y-Aib-EGT FTSD- K(γ E-Pal) SILKE RQAID E- α MePhe-VN- α MePhe LLKGG PSSGA PPPS	2.2	8.0
g2006	Y-Aib-EGT FTSDL SI-K(γ E-Stearyl)-KE RQAID E- α MePhe-VN- α MePhe LLKGG	321.0	90.0
g2007	Y-Aib-EGT FTSDL SI-K(PEG ₄ -Pal)-KE RQAID E- α MePhe-VN- α MePhe LLKGG	5.0	11.0

Several dual agonist peptides were equipotent or more potent than GLP-1 at the insulinoma cell line (INS-1E). To isolate response of each receptor to the dual agonist peptides, these compounds were also tested at knock-out cell lines. Table 4 shows assay data confirmation that our novel peptides were balanced dual agonists of similar potency in WT and KO cell lines.

Table 4: INS1-E cell assay; EC₅₀ (pM)

Alias	INS-1E	WT CONTROL	INS-1 832/3 GLP-1R KO	GIPR KO
GLP-1	33.3	31.3	-	30.4
GIP	718.6	614.0	387.0	42300
g1681	33.0	17.5	25.1	14.5
g1682	57.0	24.2	17.0	68.6
g1736	18.0	23.7	23.1	15.7

In this study, lipidated dual agonists at GLP-1R and GIPR were secured; they displayed high potency at both receptors and enhanced proteolytic stability. These new peptides could be used as lead compounds for the development of peptide therapeutics.

Potent and balanced GLP-1R and GIPR dual agonists are reported to be under scrutiny not only for the treatment of diabetes and obesity but also in search for novel treatments for Alzheimer disease, a condition believed to be stemmed from insulin de-sensitisation in the brain [3].

References

1. Rowley WR, Bezold C, Arikian Y, Byrne E, Krohe S. Popul. Health Manag. 2017; 20(1): 6-12.
2. Sermadiras I, Ravn P, Bednarek MA, Suckow A, Papworth M, Bernard E. WO2016/131893A1.
3. Hölscher C. Neuropharmacology 2018; 136: 251-259.