Structural understanding of tirzepatide on GIPR and GLP-1R

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Approximately 34% of all drugs approved by the US Food and Drug Administration (FDA) target G-protein coupled receptors (GPCRs)[1]. Understanding how ligands and transducer proteins interact with GPCRs and how structural changes and activation are related is crucial for understanding GPCR function at the molecular level. The glucagon-like peptide-1 receptor (GLP-1R) and glucose-dependent insulinotropic polypeptide receptor (GIPR) are class B1 GPCRs that bind incretin hormones and are involved in the control of post-prandial insulin secretion and glycaemic homeostasis. These receptors are validated therapeutic targets for drugs to treat type 2 diabetes (T2D) and obesity. Two groups have published cryogenic electron microscopy (cryo-EM) structures of tirzepatide-bound GLP-1R and GIPR bound to Gs protein and the Gs stabilizing nanobody, Nb35. However, both groups used additional and differing stabilisation strategies during the sample preparation, including the NanoBiT tethering[2] of transducers to the receptor in combination with mutations on GIPR[2], while the other group utilized small-molecule positive allosteric modulators with agonism in addition to a G protein stabilizing single chain antibody fragment (scFv16)[3]. Moreover, the reported interactions in the GLP-1R tirzepatide structures differed and one group reported two conformations of the GLP-1R. These suggest that the stabilization strategies employed may influence the observed peptide:receptor interactions. In the current study, without applying additional stabilization strategies as mentioned above, we determined cryo-EM structures of tirzepatide-bound GLP-1R:dominant negative Gs (DNGs) at 3.0-3.2 Å (3 subclasses) and GIPR:DNGs at 2.4 Å, respectively. This allowed us to analyse the tirzepatide:receptor interactions on both receptors. From the tirzepatide-bound GLP-1R dataset, we filtered out 3 different subclasses: one with a clear tirzepatide density in the ligand-binding pocket (class 00), one with weak peptide and GLP-1R N-terminal domain (NTD) densities with the peptide (modeled as alanine helix) in a slightly different location within the GLP-1R transmembrane domain (TMD) compared to class 1 (class 01), and a class with no peptide nor GLP-1R NTD density resolved (class 02). These classes consisted of ~30%, 24%, and 46% of selected particles. On the other hand, we only resolved a single class from the tirzepatide-bound GIPR dataset where both the peptide and receptor NTD densities were well resolved. Tirzepatide engages with both receptors with similar interaction patterns inside the GLP-1R (Class 00) and GIPR ligand binding pockets but the first tyrosine of tirzepatide was more resolved in the latter case. However, consistent with the ability to resolve different receptor classes, three-dimensional variability analysis (3DVA) indicated that the displacement of the densities of tirzepatide and GLP-1R NTD were greater between extreme frames compared to GIPR suggesting that tirzepatide in the GLP-1R binding pocket is more dynamic than when bound to the GIPR. Taken together, this suggests the engagement of tirzepatide is generally more transient for the GLP-1R than GIPR, which is consistent with higher affinity and potency in cyclic adenosine monophosphate (cAMP) assays at the GIPR relative to the GLP-1R[4].

References:

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