Structural and pharmacological insights into orphan GPCR, GPR151 with G protein couplings

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GPR151 is a class A orphan G protein-coupled receptor (GPCR) that has been implicated in a variety of biological processes, including neuropathic pain, metabolism, and inflammation. It is a potential target for the development of novel drugs to treat neuropathic pain. However, the molecular mechanisms underlying GPR151 function are poorly understood because its endogenous ligands have not been identified. We generated constructs of GPR151 with different epitope tags and fusion proteins to monitor receptor expression, to enable biophysical assessment of G protein coupling in mammalian cells, and for expression and purification in insect cells. Semi-permeabilized HEK293 cells in the presence and absence of $GDP_{\beta}S$ were used to assess constitutive coupling of G protein subtypes via bioluminescence resonance energy transfer (BRET) between GPR151-Rluc8, $G\beta_1$-$Venus_{1/2}$, and $G\gamma_2$-$Venus_{1/2}$ when co-expressed with individual Ga subunits. Consistent with previous reports, the greatest constitutive G protein recruitment was observed with members of the Gαi/o family, but with greatest window observed for $G\alpha_oA$ and $G\alpha_oB$. This was further validated using the TruPath G protein activation assay. We have also confirmed robust expression and detergent solubilization of GPR151, and individual $G\alpha_i$ and $G\alpha_o$ subunits, together with $G\beta_1\gamma_2$ insect cells. Current experiments are investigating the ability of co-expression of GPR151 with different Gαi/o proteins to support formation of stable active complexes that could be used for structure determination.