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Unraveling the complex G protein interaction signatures of Free Fatty Acid Receptors using BRET ER/K biosensors

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Introduction: It is clear that many GPCRs interact with multiple different Gα proteins. Intermolecular BRET biosensors have therefore been used to assess the full range of G proteins activated by a given receptor. A key limitation is that expression levels of receptors and signaling proteins involved will impact sensor readouts. We have generated a range of expression level independent BRET sensors based on described FRET sensors (1) and used these to assess G protein coupling to free fatty acid (FFA) family of GPCRs (2).

Methods: Sensors were generating by cloning the GPCR fused at its C terminus to mNeonGreen, a 10nm ER/K linker (1), Nanoluciferase (NLUC), and peptide corresponding to the final 28 a.a. specific Gα's. Sensors for 10 different Gα's, and one control (lacking any Gα) were generated for each GPCR. Sensors were expressed in HEK293 cells and BRET monitored after NLUC substrate addition, before and for 2 min after ligand addition. Area under the ligand response curve (AUC) was calculated to determine responses for each GPCR-Gα combination.

Results: The biosensor approach was validated using GPCRs classically associated with Gs (β_2-AR), Gi/o (D_2), Gq/11 (FFA1), and G12/13 (GPR35) (each $n \geq 3$). In each case, sensors reported significant increases ($p<0.05$) in AUC for members of the classically associated Gα family. Extending these studies to two short chain fatty acid receptors, FFA2 (n=3) and FFA3 (n=3), demonstrated that activation by the endogenous ligand, propionate (C3), resulted in significant increases ($p<0.05$) in AUC for all Gα's other than Gs for FFA2 for each member of the Gi/o family as well as for G12 for FFA3. An FFA2 allosteric agonist, AZ1729 (3), produced significant ($p<0.05$) AUC responses only for Gi1/2, Gi3 and G12 sensors. Co-addition of C3 and AZ1729, resulted in a synergistic increase to Gi1/2, Gi3, and G12 AUC compared with C3 (10.3-fold, 7.2-fold and 10.30-fold) or AZ1729 (7.3-fold, 9.6-fold, 5.7-fold) alone. Activation of the long chain fatty acid receptor, FFA4, by endogenous ligand, α-linolenic acid, or by various synthetic agonists (each $n \geq 3$) (2), resulted in significant AUC increases ($p<0.05$) for all Gα's exept G12 and G13.

Conclusion: These studies demonstrate the complex GPCR-G protein coupling profiles of the FFA family of GPCRs using novel BRET ER/K biosensors.

References

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