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Monitoring trafficking of angiotensin and chemokine receptor complexes using bioluminescence resonance energy transfer (BRET)

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Introduction: The formation of heteromers between different G protein-coupled receptors (GPCRs) leads to the generation of complexes with unique pharmacological profiles. For example, the heteromer between the angiotensin II type 1 (AT_1) receptor and the chemokine (C-C motif) receptor (CCR2) results in a complex that has apparent altered $G\alpha_i$ coupling and enhanced β-arrestin2 recruitment (1), however the trafficking profile of the heteromer has not been investigated.

Method: This study aimed to investigate the trafficking of angiotensin and chemokine receptors, as well as heteromers of these receptors using the Receptor-Heteromer Investigation Technology (1) on the Bioluminescence Resonance Energy Transfer (BRET) platform (2). Receptor trafficking profiles were generated through monitoring the proximity between luciferase (Rluc8) tagged receptors and fluorophore (Venus) tagged membrane associated compartment markers. Coexpression of the second, untagged receptor allowed comparison of the trafficking profiles of the monomeric and heteromeric receptors. Data are given as mean ligand-induced BRET ratio ± SEM (n = 3 experiments performed in triplicate).

Results: In comparison to CCR2-Rluc8 expressed alone, coexpression of the AT_1 receptor resulted in a reduced level of internalisation of CCR2-Rluc8 away from the Venus-Kras plasma membrane marker upon dual receptor activation (-0.65 ± 0.04 for CCR2-Rluc8 vs. -0.05 ± 0.03 for CCR2-Rluc8 + AT_1receptor). Additionally, dual receptor activation resulted in reduced trafficking of CCR2-Rluc8 into Venus-Rab5 early endosomes (0.11 ± 0.01 for CCR2-Rluc8 vs. 0.01 ± 0.01 for CCR2-Rluc8 + AT_1 receptor) and Venus-Rab7 late endosomes (0.084 ± 0.002 for CCR2-Rluc8 vs. 0.017 ± 0.012 for CCR2-Rluc8 + AT_1 receptor).

Conclusion: Coexpression of the AT_1 receptor and CCR2 results in altered trafficking profiles upon agonist activation.

References:

1. Ayoub MA et al. (2015). *PloS One* **10(**3), e0119803.

2. Tiulpakov A et al. (2016). *Molecular Endocrinology*, **30**(8), 889–904.