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How to separate THC's good from the bad? The molecular basis underlying cross-talk in CB_1-5-HT_{2a} receptors heteromers

J. Botta[1], A. Cordomí[2], L. Bibič[1], A. Bondar[3], J. Lazar[3], L. Pardo[2], P. J. McCormick[4]. [1]University of East Anglia, Norwich, United Kingdom, [2]Laboratori de Medicina Computacional, Unitat de Bioestadística, Facultad de Medicina, Universitat Autònoma de Barcelona, Bellaterra, Spain, [3]Laboratory of Cell Biology, Institute of Nanobiology and Structural Biology, Nové Hrady, Czech Republic, [4]Dept. Of Veterinary Pre-Clinical Studies, University of Surrey, Guildford, United Kingdom.

Introduction: CB_1R-5-HT_{2A}R heteromers have been recently identified as an alternative target through which the cognitive deficits induced by THC can be dissociated from its desired analgesic properties (1). However, little is known about the rules governing these hetero-receptor interactions. Questions include: cooperativity between dimers, minimal functional units responsible of downstream signalling, oligomerization interfaces and the molecular basis underlying cross-talk through dimers. Due to the interest in addressing some of these conditions, we investigated the molecular interactions involved in receptor activation and/or G protein recruitment in CB_1R-5-HT_{2A}R heteromer-mediated cross-talk and cross-antagonism. Altogether, our findings provide functional and structural insights into this novel target, a key step towards making GPCRs heteromers druggable.

Method: site-directed mutagenesis was performed as described by Liu and Naismith (2). ERK1/2 phosphorylation levels were assessed using AlphaLISA® technology. Calcium release and cAMP production where measured using the GCamP6s and pGloSensor™-22F biosensors, respectively. G protein coupled CB_1R and 5-HT_{2A}R models were built using the CB_1R (PDB 5TGZ), 5-HT_{2B}R (PDB 4IB4) and active β_2-AR (PDB 3SN6) as templates. 5-HT_{2A}R-mediated non-canonical Gi coupling was visualized by two-photon polarization microscopy (3) and we applied the recently developed NanoBiT® system to study heteromerization and β-arrestin2 recruitment.

Results: in HEK293 cells co-expressing 5-HT_{2A}R-CB_1R heteromers, 5-HT_{2A}R activation promotes the coupling of non-canonical $G_{i/o}$ proteins to the complexes (Fig.1A). This class switch requires both receptors in an active-like conformation, as inhibition of cAMP production does not occur in the presence of either CB_1R nor 5-HT_{2A}R non-functional mutants. Likewise, increasing CB_1R:5-HT_{2A}R ratios reduce 5-HT_{2A}R-mediated calcium release only in the presence of functional CB_1 receptors (Fig.1B). In addition, 5-HT_{2A}R-CB_1R heteromerisation provide a compensatory mechanism at the level of ERK1/2 phosphorylation signaling, as 5-HT_{2A}R and CB_1R non-functional mutants rescue its signaling properties when interacting with WT protomers.

Conclusions: our mutagenesis results illustrate different mechanisms driving CB_1R and 5-HT_{2A}R receptor activation. Furthermore, cross-talk at the level of cAMP signaling and 5-HT2AR-mediated Gi recruitment to CB1R-5HT2AR heteromers require the presence of two functional protomers, arguing against receptor trans-activation and suggesting a quaternary structure of a GPCR tetramer in complex with two Gi proteins. In addition, the mechanism underlying cross-talk differs between pERK1/2 and cAMP signaling, most likely due to the role of different effectors; G proteins and arrestins.

References:

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3. Lazar J et al. (2011). Nat Methods **8(8)**: 684-690.