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Role of Gà C-terminal helix for binding affinity to muscarinic receptors

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Introduction: GPCRs typically couple to a certain G protein family. Our aim was to get a better insight into the general mechanism of how receptors choose their cognate G protein. Today's crystal structures give new evidence that the C-terminus of the G protein is mainly involved in GPCR - G protein coupling (1,2) which might therefore be responsible for the G protein subtype selectivity.

Methods: The M_2 and the M_3 muscarinic receptor were used as a model system to investigate this binding site because they are closely related to each other but still bind to two different G protein classes. For the counterpart, chimeric Gqo and Goq subunits were created, in which the last few amino acids of the C-terminal helix were exchanged with the respective ones of the other G protein class. The relative binding affinities to the receptor were compared with the native Gq and Go subunits by using a HEK293T cell-based assay, based on Fluorescence Resonance Energy Transfer (FRET) similar to (3). We suggested that the stability of the ternary complex (reflected by the off kinetics after stimulation with Acetylcholine) is associated with the binding affinity to the receptor under GTP free conditions. Therefore, the cells were permeabilized to wash out the nucleotides.

Results: We found out that the dissociation kinetics of the chimeric subunits coupling to M_3 receptors were similar to the native subunits (k_{off} Gqo 0.0023 ± 0.0015 s^{-1}, Gq 0.0029 ± 0.0007 s^{-1} and k_{off} Goq 0.056 ± 0.005 s^{-1}, Go 0.046 ± 0.002 s^{-1}, unpaired t-tests, $n \geq 4$). In contrast, only the native Go but not Gqo, Goq and Gq were able to bind to M_2 receptors ($n \geq 6$).

Conclusion: The C-terminal helix of the G protein seems not to be the crucial part in generating the high-affinity binding to the M_3 receptor. As the C-terminus is still relevant for some receptors (e.g. M_2) we propose that different structures of the G protein contribute together to the overall binding affinity, which varies among different GPCRs.

References:

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