Importance of conformational differences between wild-type and phosphomimetic variants of GPCR C-terminal parts for the interaction with their physiological partner arrestin

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Résumé

G-Protein-Coupled Receptors (GPCRs) are membrane proteins involved in signal transduction. Their signaling is involved in numerous biological functions and their dysfunctions are linked to several pathologies. Consequently, GPCRs are the target of one-third of current clinical drugs. Upon activation, the C-terminal part of GPCR is phosphorylated by specific kinases (GRKs) allowing subsequent binding of arrestin proteins.[1] GRKs 2/3 and 5/6 are the two families of kinases which phosphorylate non-visual GPCRs at different sites of their C-terminus. The resulting pattern of phosphorylation dictates the conformation of arrestin and therefore downstream cellular events (Figure 1).[2] The impact of GPCR C-terminus phosphorylation on its conformation and interaction with arrestin is not well described in the literature due to its disordered nature.
The aim of my thesis project consisted in characterizing the conformational changes of the receptor C-terminal part upon GRKs phosphorylation and the impact on the interaction with its physiological partner, β-arrestin1. We have studied three different class A receptors, which are, on one hand, important therapeutic targets, and on the other hand, exhibit different type of interactions toward their arrestin partner[3].

The characterization by Nuclear Magnetic Resonance (NMR) of these three truncated GPCR C-termini in their wild-type and phosphomimetic forms allowed us to compare their content in secondary structures in order to better understand the structural impact of phosphorylation. We have shown that the three C-termini undergo a structural transition, which is located in the β-arrestin1 binding region.

We used a divide-and-conquer strategy to decipher, at residual resolution, the structural perturbation upon phosphorylation and/or arrestin binding. These results will be placed in a membrane-like environment using a full-length receptor reconstituted in nanodisks. In a long term, a better understanding of the GPCR-arrestin signaling mechanism could help to disentangle it from the G signaling pathways, and thus, to develop new therapeutics drugs without side effects.

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