Interaction of the μ-Opioid Receptor with G-proteins and GRK2

Introduction: The initial step in the homologous desensitization of G-protein-coupled receptors is their phosphorylation by one of the G-protein-coupled receptor kinases (GRKs). We demonstrate here measurement of the interaction of GRK2, a ubiquitously expressed GRK, with the μ-opioid receptor (μOR) by FRET and its dependence on agonist efficacy.

Methods: FRET measurements were performed between the μOR tagged with YFP and Gβ, Gγ or GRK2 labelled with Cerulean or mTurquoise as well as between YFP-labelled $G\alpha_{i1}$ subunit and Cerulean-labelled Gβ subunit. All measurements were performed in transiently transfected HEK293 cells which were superfused with 10 μM [D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin (DAMGO) or 30 μM morphine.

Results: HEK293T cells transfected with YFP-tagged μOR, mTurquoise-tagged GRK2 and non-fluorescent hete-rotrimeric G_i,proteins showed a robust increase in FRET upon superfusion with 10 μM DAMGO which was reversible upon agonist washout. The partial agonist morphine (30 μM) also caused an increase in FRET but the amplitude of the FRET signal was only 20.0% (± 3,4%) of that of the prior DAMGO stimu-lation, compared to 84,5% (±3,4%) for DAMGO. GRK2 binds G-protein βγ (Gβγ) subunits, and therefore we aimed to find out how cotransfection of GRK2 affected the interaction of Gβγ with the μOR. However, we could not measure any DAMGO-induced FRET changes between YFP-tagged μOR and Cerulean-tagged $G\beta_1$ or CFP-tagged $G\gamma 2$ in the presence of non-fluorescent $G\alpha_{i1}$ and $G\gamma_2$ or $G\beta_1$ respectively. This was unexpected because we had previously successfully determined interactions between Gβγ and the α_{2A}-adrenergic receptor or the M_3 muscarinic acetylcholine receptor. This lack of FRET was not due to an inability of the tagged Gβγ to interact with the μOR as we could measure DAMGO-induced FRET changes between YFP-tagged $G\alpha_{i1}$ and $G\beta_1\gamma_2$ (Gβ tagged with Cerulean) in the presence of non-fluorescent μOR. Moreover, we could pick up FRET between the μOR and Gβγ in the presence of non-fluorescent GRK2. Comparison of the on- and off-kinetics of the μOR-GRK2 interaction with that of the μOR-Gβγ interaction in the presence of GRK2 revealed similar time constants both for the on- and off-kinetics (GRK2: k_{on} 0.16 s^{-1}; k_{off} 0.087 s^{-1}; Gβγ in the presence of GRK2: k_{on} 0.23 s^{-1}, k_{off} 0.069 s^{-1}).

Conclusion: The results suggest that, in the absence of GRK2, the orientation of the two fluorophores on the μOR and Gβγ may be unfavorable to produce an appreciable FRET signal. In the presence of GRK2, however, Gβγ changes its position relative to the μOR in a way that allows the interaction of the GRK2-Gβγ complex with the μOR to be detected by FRET. Similarly, we measured FRET between Gβγ and the α_{2A}-adrenergic receptor or the M_3 muscarinic acetylcholine receptor in the absence and presence of GRK2 and compared the kinetics with the kinetics of GRK2 binding and unbinding to these receptors. In both cases, we found that GRK2 and Gβγ in the presence of GRK2 associate and dissociate from these receptors with comparable kinetics. Our results suggest that ligand efficacy for μORs is already apparent on the level of receptor-GRK interaction.