CAPTURING 7TMR SIGNALING PLURIDIMENSIONALITY AND LIGAND BIAS IN LIVE CELLS BY AN IMPEDANCE-BASED BIOSENSOR

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Seven transmembrane helical receptors (7TMRs; G protein-coupled receptors) are embedded into the cell membrane of almost any cell and are essential for orchestrating the function of cells in order to maintain homeostasis of the organism and its adaptation to environmental changes. In addition, modulation of 7TMR-function by drugs is a mainstay for the prevention and treatment of disease. Binding of the endogenous agonist to its 7TMR often leads to activation of more than one type of adaptor protein which is referred to as “promiscuous” or “balanced” signaling. In contrast, artificial ligands may selectively address only a subset of signaling pathways. As biased ligands might be superior to balanced agonists with regard to their therapeutic potential they are promising candidates in drug discovery. Here, we apply a label-free impedance-based biosensor (CellKey® 384 System, Molecular Devices) as powerful approach to deconvolute signaling pluridimensionality of 7TMRs in live cells. For this purpose, FlpIn™ CHO cells -engineered to stably express the human muscarinic M_2 receptor- are seeded into microtiter plates equipped with electrodes at the bottom of each well. Alternating voltage resulting in a flow of extracellular currents is supplied to the cell monolayer and impedance is measured. Activation of 7TMRs leads to changes in cell shape and cellular adhesion as well as cell-cell interactions, resulting in a shift of impedance most remarkably in a G protein-characteristic fashion. Using the acetylcholine M_2 receptor as a paradigm for small ligand 7TMRs we show that classic muscarinic agonists (i.e. acetylcholine and iperoxo) activate more than one class of G proteins in untreated CHO-hM_2 cells, i.e. G_i and G_s proteins. This promiscuous signaling is reflected by bell-shaped concentration-effect curves displaying stimulatory and inhibitory impedance-responses at low and high agonist concentrations, respectively (e.g. iperoxo (mean ± s.e.m.): $pEC_{50,\ stim.}$ = 9.61 ± 0.13, $pEC_{50,\ inhib.}$ = 8.20 ± 0.26, n = 4). Applying pertussis toxin (PTX, 100 ng/ml, 16-24 h) and cholera toxin (CTX, 100 ng/ml, 8 h) to chemically knock-out G_i and G_s proteins, respectively, we assign the stimulatory phase to G_i activation and the inhibitory phase to G_s activation. Beyond that, the method identifies a set of dualsteric ligands (Antony et al., 2009) with pronounced G_i bias by means of monophasic concentration-effect curves and the lack of impedance change in PTX-pretreated cells. Thus, assaying cellular impedance in live cells may be a promising screening platform to identify pluridimensional 7TMR signaling and ligand bias and should therefore be of considerable interest to drug discovery.

References:

Antony, J. *et al*. (**2009**) Dualsteric GPCR targeting: a novel route to binding and signaling pathway selectivity. *FASEB J*. 23: 442–450.

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