Measuring receptor motility and filopodia dynamics

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Signaling through G protein-coupled receptors (GPCRs) involves the orchestrated interactions of the receptor complex itself with elements of a dynamic actin cytoskeleton. Important components of the motile cell membrane include actin rich protrusions, termed filopodia, which can mediate interactions between the cell and its environment. However, the dynamics of GPCRs in a highly motile cell membrane are not well understood. Therefore, we have developed an experimental approach to measure membrane motility and GPCR motility in tandem, in live cells, during application of receptor agonists and modifiers of actin dynamics. We have utilised a Chinese Hamster Ovary (CHO) cell line expressing functional angiotensin II type 1a receptor (AT1AR) tagged with enhanced green fluorescent protein (eGFP) as a GPCR model system. Taking advantage of the imaging capabilities of our Leica SP5 laser scanning confocal microscope equipped with the unique combination of a high-speed resonant scanner and avalanche photo diode detectors, we are able to image receptor and membrane dynamics at very high speed and single photon sensitivity with minimal photodamage. Series of 50 images of live CHO-AT1AR-eGFP cells were captured at a rate of 20-25 frames per second, t=0, 10, and 20 min after addition of fluorescently labelled ATIAR agonist, angiotensin II-AlexaFluor647 (AngII-A647) (100nM). Effects of the actin polymerisation inhibitor, cytochalasin B (CB) (0.5μM, 2.5μM, 5μM), and the dynamin GTPase inhibitor dynasore (80μM) were also tested. Images were collated, processed and analysed primarily with ImageJ software. In the absence of agonist, high resolution imaging revealed a surprising amount of motility in the cell membrane and constitutive internalisation of the receptor was observed. In the presence of agonist, the high level of membrane motility remained in the presence of internalisation of ligand-bound receptors. In 4 of 5 cells, application of 0.5μM CB led to collapse and aggregation of filopodia 10 min after application. By 20 min post application, significant recovery of filopodial motion was observed. Immunocytochemistry labelling with antibodies for β-actin demonstrated a classic text-book distribution of actin including the appearance of stress fibres at the basolateral surface of control cells. Treatment with CB (0.5μM, 2.5μM, 5μM) revealed a reorganisation of the actin cytoskeleton, including the appearance of actin rich aggregations along the membrane and the disappearance of actin stress fibres. CB treated cells also showed a change to the overall cell morphology. In all 7 cells treated with 80μM dynasore, no obvious effect on filopodia motility was observed. This model system enables the measurement of filopodia movement in different states of receptor activation. Our results to date suggest that the analysis of agonist-receptor interactions, at least in cell lines, will be significantly confounded by the consequences of membrane motility. By coupling this model with advanced image analysis to extract and quantify different aspects of membrane and receptor motility (*e.g.* particle tracking, Raster Image Correlation Spectroscopy), we will enhance our understanding of GPCR signalling in a dynamic membrane environment.