role as a link in transmembrane signal transduction. Recently, Hulko et al. determined the solution NMR structure of the HAMP domain (PDB ID:2ASW) of an archaeal protein AF1503, a putative transmembrane receptor [Hulko, M., et al. (2006) *Cell 126*, 929-940]. Based on the NMR structure, we have modeled the relative orientation of transmembrane domains TM1 and TM2 using replica exchange molecular dynamics simulations (REXMD). We then performed MD simulations of the HAMP and TM complex in explicit lipid bilayers to investigate possible thermal motions as well as signal transduction mechanisms. In addition, as Hulko et al. suggested a signal transduction mechanism related to the canonical packing by rotation of the helices, we have investigated the helix rotation propensity through MD simulations. In this work, we will present the simulation results of the HAMP and TM complex as well as the HAMP domain alone.

3478-Pos Board B525

Redistribution Of The Human Mu-opioid Receptor (hMor) In HEK 293 Cell Membranes In Response To Agonists

Tanya E. S. Dahms[1], Dominique Massotte[2], Tianming Sun[1].
[1]University of Regina, Regina, SK, Canada, [2]CNRS/INSERM/ULP, Illkirch cedex, France.

Opioid receptors play a role in a whole battery of physiological processes, including affective behaviour, neuroendocrine physiology and pain perception. The cell membrane, which is the physical environment of the receptor, is known to consist of domains with distinct lipid/protein composition and physical characteristics. Previous work (Moffet *et al.* (2000) *J. Biol. Chem.* 275: 2191-2198.) has shown that the G-protein signalling partners of hMOR localize to the ''so called'' detergent resistant membrane (DRM). Our previous studies using ligand-affinity atomic force microscopy (AFM) of hMOR-expressing Sf9 cell membranes indicated that hMOR localizes to mixed lipid domains, presumably corresponding to detergent soluble membranes (DSM). These results would indicate a physical separation of the receptor and its signalling partner prior to activation. However, the relationship between microdomains characterized using biochemical techniques (DSM/DRM) and membrane rafts defined by biophysical techniques remains unclear. Here we track active hMOR localization and lipid composition in detergent and detergent-free separated membranes. For each fraction, hMOR activity was assessed using a modified binding assay, and Western blot analysis was used to determine the relative amount of lipid raft marker, flotillin-1, and G-proteins. We show a redistribution of the receptor in response to its agonists, implying that hMOR distribution at the cell membrane helps to regulate it function.

3479-Pos Board B526

Evidence for precoupling of inactive M3 muscarinic receptors and G_q Protein

Kou Qin, Kuravi K. Sudha, Nevin A. Lambert.
Medical College of Georgia, 30912, GA, USA.

Two models have been proposed to explain how G protein-coupled receptors (GPCRs) interact with heterotrimeric G proteins to transduce physiological signals. One model suggests that GPCRs and G proteins collide with each other randomly after receptor activation and that binding is transient. An alternative model suggests that GPCRs and G proteins are bound to each other (precoupled) before receptor activation. We have studied interactions between GPCRs and G proteins using fluorescence recovery after photobleaching (FRAP) and avidin-mediated crosslinking in HEK 293 cells. We have previously shown that immobile CFP-labeled α_{2A}-adrenoreceptors (C-α2ARs) do not decrease the mobility of the G proteins that they activate, consistent with a collision-coupling model. Here we show that immobile CFP-labeled M3 muscarinic receptors (C-M3Rs) decrease the lateral mobility of citrine-labeled $G\alpha_q$. C-M3Rs failed to decrease the mobility of venus-labeled $G\alpha_{oA}$. Conversely, the C-M4Rs (which activate $G_{i/o}$ proteins) failed to decrease the mobility of G_q-citrine. Slowing of $G\alpha_q$-citrine by immobile C-M3R was unaffected by an agonist (carbachol) or an inverse agonist (atropine), and thus did not depend on activation of the receptor. Slowing of $G\alpha_q$-citrine by immobile C-M3Rs was enhanced by carbachol when nucleotides were depleted, as predicted by the ternary complex model of G protein coupling. These results suggest that inactive M3Rs precouple with G_q proteins, and that different coupling models apply to different GPCR-G protein pairs.
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3480-Pos Board B527

Active α2A-receptors induce GTPγS release from activated Go proteins and subsequently sequestrate G proteins

Leif G. Hommers, Christian Dees, Moritz Bünemann.
University of Wuerzburg, Wuerzburg, Germany.

Recently, analysis of agonist-induced G protein activation and receptor/G protein interaction has become possible in single living cells by means of FRET. Using these methods, we aimed to investigate whether GTPγS-bound Go proteins interact with active α2A-receptors and whether bound-GTPγS can be released from Go proteins upon this interaction. In order to conduct these experiments, we had to gain control of the intracellular nucleotide composition by permeabilizing the cell membrane of transiently transfected HEK293 cells via short application of saponine.
Agonist-induced receptor/G protein interaction, monitored by means of FRET between tagged α2A-receptors and tagged Gγ, was significantly stronger in the presence of low concentration of GDP, GTP and GTPγS compared to corresponding 1000-fold higher concentrations of nucleotide. Superfusion of cells with GTPγS in the presence of agonist resulted in attenuation of FRET between G protein subunits corresponding to maximal activation of G proteins. When omitting GTPγS, the FRET signal recovered with kinetics accelerating with increasing concentration of agonist. This result suggested deactivation of G proteins due to receptor-induced dissociation of GTPγS from preactivated G proteins. As a second experiment, non-labeled GTPγS could displace radioactively labeled GTPγ35S in an agonist dependent manner. In the presence of saturating concentration of agonist, increased FRET between G protein subunits suggested a reduced activation of G proteins compared to non-saturating concentration of agonist. This effect was found for both intact cells and permeabilized cells in the presence of low concentration, but not in the presence of high concentration of GTPγS. These results suggested (I) interaction of active G proteins with active receptors (II) receptor-induced dissociation of GTP/ GTPγS from G proteins and (III) sequestration of inactive nucleotide-free G proteins at the active receptors.

3481-Pos Board B528

Rapid Binding of G$\beta\gamma$ Dimers to the c-terminus of GRK3

Bettye Hollins, Sudhakiranmayi Kuravi, Gregory J. Digby, **Nevin A. Lambert**.
Medical College of Georgia, Augusta, GA, USA.

One proposed function of macromolecular signaling complexes is acceleration of signal onset, as complexes would obviate the need for collision of randomly-diffusing molecules. Here we study the kinetics of heterotrimeric G protein signaling between molecules that are unlikely to be part of a complex. Binding of the GRK3 c-terminus (GRK3ct) to G$\beta\gamma$ dimers was detected using confocal microscopy and fluorescence or bioluminescence resonance energy transfer (FRET or BRET). Activation of pertussis toxin-insensitive G_{oA} heterotrimers led to translocation of GRK3ct-venus from the cytosol to the plasma membrane with a monoexponential time constant of 431 $\pm$ 35 ms (n=9) at room temperature. FRET between a membrane-associated GRK3ct-cerulean (masGRK3ct-C) and G$\beta\gamma$-venus (G$\beta\gamma$-V) increased with a time constant of 213 $\pm$ 32 ms (n=10) at 26.5 °C, and 67 $\pm$ 13 ms (n=13) at 37 °C. Fluorescence recovery after photobleaching suggested that masGRK3ct-V and heterotrimers were not part of a complex prior to activation. Agonist-induced BRET between masGRK3ct-Rluc8 and G$\beta\gamma$-V decreased as the relative expression of Gα increased. This is consistent with buffering of free G$\beta\gamma$ by excess inactive Gα subunits, suggesting that masGRK3ct-Rluc8 bound to free G$\beta\gamma$-V dimers rather than to rearranged heterotrimers. These results suggest that G protein signals that occur on a timescale of ~100 ms are not necessarily mediated by preassembled signaling complexes. *Supported by grants GM078319 from the NIH and MCB0620024 from the NSF.*

3482-Pos Board B529

Functional Evidence for Gi-Gq crosstalk through G protein-coupled Receptor Heterocomplexes

Miguel Fribourg-Casajuana[1], Javier González-Maeso[1], Stuart C. Sealfon[1], Diomedes E. Logothetis[2].
[1]Mount Sinai School of Medicine, New York City, NY, USA, [2]Virginia Commonwealth University, Richmond, VA, USA.

We have studied signaling through a complex of two distinct G protein-coupled receptor (GPCR) types: a serotonin (Gq-coupled) and a metabotropic glutamate (Gi-coupled). We used two-electrode voltage clamp in Xenopus oocytes expressing the receptors and used endogenous calcium-activated chloride or heterologously expressed Kir channels to monitor the effects of GPCR signaling. Previous biochemical studies had shown Gi signaling by stimulating the serotonin receptor in the serotonin-glutamate receptor complex (González-Maeso et al., Nature 2008 452:93-97). Our work here focused on studying whether Gq signaling could also take place when stimulating the glutamate receptor.
Upon glutamate receptor activation, oocytes expressing both receptors elicited calcium-activated chloride currents, not observed in oocytes injected with the glutamate receptor alone. Furthermore, these currents could be blocked by a specific regulator of Gq-protein signaling (RGS2) and were accompanied by phosphatidylinositol (3,4)-bisphosphate (PIP2) hydrolysis in the membrane, results consistent with Gq-signaling. Finally, receptor chimeric studies further confirmed that the glutamate-serotonin complex needs to be formed in order to allow Gi-Gq cross activation.