and beta2-AR: beta1-AR exhibited a transient mode of interaction whereas in contrast the vast majority of beta2-AR formed stable higher order complexes.

3497-Pos Board B544

The Effect of Detergent on Human Mu-opioid Receptor (hMOR) Localization as a Function of Pretreatment with Agonist and Antagonist

Tianming Sun, Tanya E.S. Dahms.
University of Regina, Regina, SK, Canada.

Like many other seven transmembrane G-protein coupled receptors (GPCRs), the human mu-opioid receptor (hMOR) interacts with multiple members of the pertussis toxin-sensitive Gi and Go protein families, to regulate adenylyl cyclase, Ca2+ and K+ channels. Notably, opioid agonists represent the most powerful analgesic drugs for the clinical management of pain, through binding opioid receptors. However, not all agonists exert the same level of effect.

Plasma membranes are organized into specialized micro-domains differing in composition, biological function and physical properties. In recent years, detergent resistant membranes (DRM) are thought to serve as molecular sorting platforms to concentrate signalling molecules (e.g. opioid receptors) based on membrane fractionation and cholesterol depletion experiments. It remains unclear whether membrane organization with detergent has an effect on hMOR localization.

Here we track active hMOR and lipid composition in isopycnic membrane fractions in the presence and absence of CHAPS detergent. hMOR activity was assessed using a modified binding assay. The relative amount of lipid raft marker (flotillin-1), actin and G-proteins were assessed by Western blot analysis. The data show the effects of detergent on receptor distribution. Relocation of the hMOR receptor in the membrane indicates an additional level regulation at the cell membrane.

3498-Pos Board B545

The Effect of Agonist Activation and Homodimerisation on the Membrane Diffusion of the Human Histamine H_1 Receptor

Rachel H. Rose, Stephen J. Briddon, Stephen J. Hill.
University of Nottingham, Nottingham, United Kingdom.

There is considerable evidence to support the existence of dimers of G protein-coupled receptors, including the histamine H_1 receptor (H_1R), although their functional significance remains unclear. We have used bimolecular fluorescence complementation (BiFC) in combination with fluorescence correlation spectroscopy (FCS) to selectively monitor histamine-mediated changes in the diffusion characteristics of the dimeric H_1R (using BiFC), as well as those of the total H_1R population (using labelling with YFP).

cDNAs encoding YFP or the C-terminal and N-terminal YFP fragments were cloned into pcDNA3.1 to produce fusions to the C-terminus of the H_1R. CHO-K1 cells were transiently transfected with the relevant cDNAs and FCS measurements were performed on the upper cell membrane and analysed as previously described (Briddon, *et al.* (2004) PNAS, 101, 4673-4678).

Translational diffusion of the H_1R in the cell membrane, measured as the average diffusion time through the FCS detection volume, was significantly faster for oligomeric H_1R (14.1 ± 1.1ms) than the total receptor population (17.3 ± 1.1ms). Following stimulation with 0.1mM histamine, there was a significant increase in both the diffusion time (17.3 ± 1.1ms vs. 21.6 ± 1.0ms) and particle number (1.02 ± 0.08 vs. 1.28 ± 0.07) of the total receptor population after 10 minutes. This returned to control values after 20 and 40 minutes. For the dimeric receptor population, however, there was no significant change in either translational receptor diffusion or particle number following histamine exposure.

Since FCS only detects the diffusion of mobile particles, the increase in particle number for H_1YFP following 10 minutes agonist stimulation may reflect mobilisation of previously immobile receptors. The increased diffusion time could indicate association with larger protein complexes involved in receptor signalling, desensitisation or internalisation. The absence of such changes for dimeric receptors suggests fundamental functional differences between monomeric and oligomeric receptor populations.

3499-Pos Board B546

Monitoring The Activation Of Rhodopsin By The Transient Fluorescence Of Fluorescently Labeled Helix 8

Daniel Hoersch, **Harald Otto**, Ingrid Wallat, Maarten P. Heyn.
Freie Universitaet Berlin, Berlin, Germany.

The transient changes of the fluorescence of bovine rhodopsin in ROS membranes selectively labeled with Alexa594 at cysteine 316 in helix 8 were followed in time from 1 μs to 10 s after flash excitation of the photoreceptor. A large light-induced transient fluorescence increase was observed with time constants in the ms- range at pH6. Using transient absorption spectroscopy the kinetics of this structural change at the cytoplasmic surface was compared to the formation of the signaling state MII (360 nm) and to the kinetics of proton uptake as measured with the pH indicator dye bromocresol purple (605 nm). The fluorescence kinetics lags behind the deprotonation of the Schiff base. The proton uptake is even further delayed. These observations show that in ROS membranes (at pH 6), the sequence of events is: Schiff base deprotonation, structural change, proton uptake. From the temperature dependence of the kinetics we conclude that the Schiff base deprotonation and the transient fluorescence have comparable activation energies, whereas that of proton uptake is much smaller.

3500-Pos Board B547

Structural And Dynamic Effects Of Cholesterol At Preferred Sites Of Interaction With Rhodopsin Identified From Microsecond Length Molecular Dynamics Simulations

George Khelashvili[1], Alan Grossfield[2], Scott E. Feller[3], Michael Pitman[4], Harel Weinstein[1].
[1]Weill Medical College, New York, NY, USA, [2]University of Rochester School of Medicine and Dentistry, Rochester, NY, USA, [3]Wabash College, Crawfordsville, IN, USA, [4]IBM T.J. Watson Research Center, Yorktown Heights, NY, USA.

A key unresolved question about GPCR function is the role of membrane components in receptor stability and activation. In particular, cholesterol is known to affect the function of membrane proteins, but the details of its effect on GPCRs are still elusive. Here, we describe how cholesterol modulates the behavior of TM1-TM2-TM7-helix 8(H8) functional network, that comprises the highly conserved NPxxY(x)5,6F motif, through specific interactions with the receptor. The inferences are based on the analysis of microsecond molecular dynamics (MD) simulations of Rhodopsin in an explicit membrane environment. We found that cholesterol primarily affects specific local perturbations of the TM domains such as the helical kink parameters in TM1, TM2 and TM7, and that these local distortions, in turn, relate to rigid-body motions of the TMs in the TM1-TM2-TM7-H8 bundle. The specificity of the effects stems from the non-uniform distribution of cholesterol around the protein. We find three regions that exhibit the highest cholesterol density throughout the trajectory. In one of these regions, cholesterol interacts with Pro7.38 in TM7 and with nearby residues in the extracellular (EC) loop 3, a location that resembles the high-density sterol area from the electron microscopy data (Ruprecht et al., 2004, EMBO J;23:3609-3620). A second cholesterol concentration region is in agreement with the recent X-ray crystallography data on beta2-adrenergic GPCR (Cherezov et al.,2007,Science,318:1258-1265), near residues Val1.58, Tyr2.41 and Ile4.43. In the third region, we find cholesterol interacting strongly with Tyr2.63 in TM2 and proximal residues Phe3.30, Leu3.27, Thr3.23 and Phe3.20 on the EC side of TM3. Through correlation analysis we connect local effects of cholesterol on structural perturbations with a regulatory role of cholesterol in signaling.

Membrane Receptors & Signal Transduction II

3501-Pos Board B548

NMR Structure of the "Finger" Loop of Rod Arrestin Induced by Meta-II Rhodopsin Binding

Sophie Feuerstein[1,2], Alexander Pulvermüller[3], Joachim Granzin[1], **Bernd W. Koenig**[1,2].
[1]Institute of Neuroscience and Biophysics 2, Research Centre Jülich, D-52425 Jülich, Germany, [2]Physical Biology Institute, Heinrich-Heine-University, D-40225 Düsseldorf, Germany, [3]Institute of Medical Physics and Biophysics, Charité University Hospital, D-10098 Berlin, Germany.

An unstructured ''finger'' loop connects β-strands V and VI in x-ray crystal structures of arrestin. Several recent studies identified this region as a possible major interaction site for specific binding of arrestin to photo-activated and/or phosphorylated meta-II rhodopsin. We studied binding of a peptide representing the amino acid sequence of the loop around residue 75 of visual arrestin to light-activated bovine rhodopsin. At low millimolar concentrations the peptide enhances formation of the photointermediate meta-II rhodopsin after receptor activation. The extra meta-II assay reveals competition of the peptide with transducin and arrestin for binding to activated (Rh*) and activated phosphorylated rhodopsin (P-Rh*), respectively. The high resolution structure of the meta-II rhodopsin-bound arrestin peptide was derived from the difference of two-dimensional liquid state NMR spectra recorded in the presence of either dark-adapted or light-activated rhodopsin-rich disc membranes. The peptide remains unstructured upon addition of dark-adapted rhodopsin. However, receptor activation causes dramatic changes in 2D NOESY spectra of the peptide. Specifically, the ligand is in rapid exchange between a free unstructured and a conformationally well defined meta-II-bound form. Efficient cross-relaxation of proton spins in the bound state causes strong NOESY cross-peaks reflecting the bound peptide structure. The specific pattern of binding-induced NOESY peaks indicates a helical conformation of the bound peptide. Restrained molecular dynamics calculations confirmed an α-helical peptide structure. A