Our results indicate that along with complexes between GPCRs that are coupled to the same class of G-protein, heterocomplexes are also possible, enabling simultaneous signaling of distinct signaling pathways in response to a single agonist.

**3483-Pos Board B530**

**Cellular Association and N-glycosylation of Glucagon and Related Receptors**

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Increasing evidence suggests that oligomerization and N- glycosylation influence cell surface expression of G-Protein Coupled Receptors (GPCRs). However, the importance or occurrence of these post-translational events remain controversial or unknown for many GPCRs. For the GPCRs in the glucagon family, which play important roles in regulating cardiac activity, insulin secretion and glucose metabolism, an understanding of the post-translational mechanisms that regulate their association and cell surface expression is particularly limited. Therefore, we initiated studies on glucagon (Gluc), glucagon-like peptide 1 (GLP-1), and glucose-dependent insulinotropic polypeptide (GIP) receptors to determine if they self-associate preferentially and if N-glycosylation plays a role in their function or cell surface expression. These three families of GPCRs are similar in primary sequence, suggesting they may both homo- and heterodimerize, and contain between 2 and 4 N-glycosylation sequons in predicted extracellular regions. Using transient expression in Chinese Hamster Ovary cells and Bioluminescence Resonance Energy Transfer (BRET), we show high levels of GFP emissions of GIP, GLP-1 and Gluc receptors with themselves, but lower levels when they are co-expressed. Mutation of putative N-glycosylation Asn residues to Gln produces shifts in mobility consistent with the removal of N-glycans, and raises perinuclear levels of intracellular receptors as determined by immunocytochemistry and structured illumination microscopy. Furthermore, preliminary data from cAMP assays suggest that the Gluc receptor is not functional after glucagon stimulation when all putative N- glycosylation sites are mutated. These data suggest that each of these three related families of GPCRs associate preferentially with themselves, and that they are N-glycosylated in CHO cells, which augments their cell surface targeting.

**3484-Pos Board B531**

**Strong cAMP Response to a GPCR Agonist Challenge Despite Apparent Inactivation**

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A large variety of neurotransmitters, hormones, paracrine agents, and odorants exert their effects through G protein coupled receptors (GPCRs). These receptors activate intracellular G proteins that in turn modulate the activity of different effector proteins. Based largely on the study of the light-activated receptor rhodopsin and the beta-adrenergic receptor, the vast majority of GPCRs are assumed to undergo a process of inactivation or silencing involving receptor phosphorylation and capping by one of the arrestins. The advent of improved cAMP sensors in living cells and enhanced molecular information about phosphodiesterases has led to an increased appreciation of the role that these enzymes play in the shaping and termination of cAMP signals. We report here a high-resolution measurement of vasoactive intestinal peptide (VIP)-triggered cAMP signals near the surface membrane in pituitary GH4C1 cells, using modified cyclic nucleotide-gated ion channels. The signals are transient and the falling phase is due solely to an increase in phosphodiesterase activity. There is no detectable receptor inactivation on this timescale. Surprisingly, this system is highly responsive to subsequent increases in VIP levels. We show that such responsiveness can arise in a system in which receptors remain active and phosphodiesterase activity is just slightly higher than adenylyl cyclase activity, but not in a system that exhibits classical receptor inactivation. The upregulation of phosphodiesterase activity represents a type of inactivation or desensitization that causes a decline in the response to the initial agonist dose and limits the intracellular spread of cAMP. However, this mechanism has the unique feature of allowing the cell to respond to subsequent agonist challenges.

**3485-Pos Board B532**

**Interaction Between Cationic Lipids and Endotoxin Receptors**

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It has been recently demonstrated that diC14-amidine, a cationic lipid, activate cytokine secretion (TNF-alpha, IL-12, IFN-gamma, IP-10) in dendritic cells through a Toll-like Receptor-4-dependent mechanism [Tanaka et al., 2008]. This receptor is involved in the recognition of the bacterial endotoxins (lipopolysaccharides, LPS) and activation of innate immune system. DiC14-amidine could activate this cascade by different mechanism [Lonez et al., 2008].Our first hypothesis is that diC14-amidine interacts with the co-receptor of TLR4, MD-2. DiC14-amidine has 2 acyl chains that mimic LPS acyl chains. Molecular dynamics simulations of the insertion of diC14-amidine in the MD-2 cavity revealed that two amidine molecules do occupy a volume identical to that of one tetra-acylated lipid A molecule, an antagonist of TLR4, in the cavity. A non-exclusive alternative could consist in a modification of the membrane environment of TLR4, upon insertion of cationic lipids in the membrane bilayer. The ability of diC14-amidine liposomes to fuse with cell membranes was demonstrated by confocal microscopy or (FRET) Fluorescence Resonance Energy Transfer measurements. Fusion (lipid mixing) with the cell membrane would be a way to insert cationic lipid in the lipid bilayer of the plasma membrane and to modify the lipid-protein interactions involved in the function of membrane proteins. Biophysical studies (AFM, X-ray scattering) revealed that amidine molecules adopts an interdigitated structure and does not require additional lipids (PE) to be fusogenic. This raises also the question of the role of interdigitated structures in the fusion (lipid mixing) mechanism.

References
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**3486-Pos Board B533**

**Dynamic Changes In The Monomer-Dimer Equilibrium Of The Intercellular Adhesion Molecule CEACAM1 Revealed By Live Cell TIRF-Based FRET Microscopy**

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The carcinoembryonic-antigen-related cell-adhesion molecule (CEACAM) family of proteins has been implicated in various intercellular-adhesion and intracellular signaling-mediated effects that govern the growth and differentiation of normal and cancerous cells. Recent studies also show that members of the CEACAM family play an important role in modulating the immune responses associated with infection, inflammation and cancer. At the epithelial cell surface, CEACAM1 (also known as BGP, C-CAM and CD66a) is believed to exist as a cis-homodimer. The dimeric state is thought to regulate the ability of the receptor to recruit signaling molecules, including SRC-family kinases and the tyrosine phosphatase SRC homology 2 (SH2)-domain-containing protein tyrosine phosphatase 1 (SHP1). To characterize the dynamics and monomer-dimer equilibrium of CEACAM1, we have applied a live-cell combinatorial microscopy imaging approach. Time-lapse total internal reflection fluorescence (TIRF) imaging of HELA cells overexpressing cyan fluorescent protein (CFP)- and yellow fluorescent protein (YFP)-CEACAM1 constructs revealed a heterogeneous distribution of bright, micron-sized jagged structures that rapidly changed shape and diffused across the cell surface. Homo- and hetero-Forster resonance energy transfer (FRET) imaging techniques (acceptor photobleaching, sensitized emission, and polarization anisotropy) were then used in combination with TIRF microscopy to determine that these jagged structures are likely aggregates of monomeric CEACAM1 proteins while the rest of the membrane contains lower concentrations of dimeric CEACAM1. This combinatorial microscopy strategy not only compliments traditional biochemical assays, but also provides new quantitative insights into spatial-temporal dynamics of this important membrane protein. We are exploiting these insights to further understand the various immune responses associated with the CEACAM family.

**3487-Pos Board B534**

**Mobile Haptens in Lipid Bilayers Cause Large-Scale Clustering of IgE Receptors**

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Rat basophilic leukemia (RBL) mast cells express IgE receptors on the membrane that, when aggregated, initiate biochemical events that lead to the exocytosis of inflammatory mediators (degranulation). Low concentrations of monovalent haptens in fluid supported lipid bilayers have been found to cause degranulation, but microscopically visible aggregation has not been previously observed. To investigate possible receptor aggregation at higher hapten concentration, RBL cells were loaded with a fluorescent anti-DNP IgE and then deposited onto fluid supported lipid bilayers containing DNP-lipids at up to 50 mole %. Total internal reflection fluorescence microscopy was used to image the cells; large clusters were observed at DNP concentrations $\geq$ 10 mol %. The characteristic cluster sizes and densities were analyzed with image