receptor aggregates that represent the functional signaling complex. Here, using a combination of receptor mutagenesis, experiments in living cells (including FRET), and experiments with purified peptides we report receptor oligomerization driven through both covalent and non-covalent interactions in membrane-proximal regions of the receptor. These results represent the first findings that pre-ligand receptor oligomerization and signal propagation into the cell may be driven in part through receptor interactions within and near the membrane.

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A Similar Motif Shared by HIV-1 gp41 and TCRA Trans-Membrane Domains Exploited by the HIV Virus to Modulate T-Cell Proliferation

Yechiel Shai, Tomer Cohen, Shmuel Jaffe Cohen, Niv Antonovsky, Irun R. Cohen.

The transmembrane domains (TMDs) of the T-cell receptor (TCR) play an important role in the assembly of the receptor complex. Previous studies have shown that the fusion peptide (FP) of HIV-1 inhibits T-cell activation and by that suppress the immune response against the virus. FP gains this activity by specifically binding to the TMD of TCRα and interfering with the assembly of the TCR complex We utilized *in-silico* testing of a TMD sequence library derived from virus protein sequences, and pin-pointed a nine amino-acid motif shared by a group of different viruses; this motif resembles the transmembrane domain of the α-subunit of the T-cell receptor (TCRα). The highest similarity was found within SIV and HIV glycoprotein 41 TMD (gp41 TMD). Previous studies have shown that stable interactions between TCRα and CD3 are localized to this nine amino acid motif within TCRα, and a peptide derived from it interfered and intervened in the TCR function when added exogenously. By combining biophysical and biochemical approaches we found that the gp41 TMD peptide co-localizes with CD3 within the TCR complex and inhibits T-cell proliferation *in vitro*. We also found that the inhibitory mechanism of gp41 TMD differs from that of FP. Disassociated from HIV, the gp41 TMD molecule provides a novel mechanism for down regulating undesirable responses and might be be used as an immunotherapeutic tool.

2275-Pos Board B261

Cooperativity in the Binding of Agonists to Reconstituted Tetramers of the M_2 Muscarinic Receptor

Dar'ya S. Redka, Heiko Heerklotz, James W. Wells.

G protein-coupled receptors display a dispersion of affinities in the binding of agonists, the breadth of which is a measure of efficacy. The implied heterogeneity commonly is thought to be induced by the G protein in an otherwise homogeneous population of receptors. In some early studies, however, purified M_2 muscarinic receptor also revealed heterogeneity under some conditions. We have shown that M_2 receptors extracted from *Sf*9 cells can be purified as monomers devoid of G protein and reconstituted almost exclusively as tetramers in phospholipid vesicles (POPC/POPS/cholesterol), as indicated by cross-linking. Monomers in solution appeared homogeneous, as indicated by Hill coefficients near 1 for the inhibitory effect of agonists on binding of the antagonist *N*-[^{3}H]methylscopolamine; in contrast, reconstituted tetramers appeared heterogeneous ($n_H < 1$) in a manner that correlated with efficacy as reported for GTPase activity ($p = 0.013$). It follows that efficacy is intrinsic to the receptor in its tetrameric state.

To examine whether heterogeneity arises from pre-existing asymmetry within the tetramer or is induced through cooperativity between ligands, the inhibitory effect of two agonists (oxotremorine-M, arecoline) and two antagonists (*N*-methylscopolamine, atropine) was measured at two concentrations of *N*-[^{3}H] methylscopolamine. The data were analyzed simultaneously in terms of four dissimilar and non-interacting sites (*i.e.*, asymmetry) and four interacting sites (*i.e.*, cooperativity). The radioligand was assumed to label only two of the four sites, because the capacity for *N*-[^{3}H]methylscopolamine doubled upon dissolution of reconstituted receptors in digitonin; one-half of the reconstituted sites therefore were inaccessible to the radioligand. Whereas the data could not be described in terms intrinsic asymmetry alone, the fit improved markedly upon the introduction of cooperative interactions ($p < 0.00001$). Efficacy therefore appears to arise at least in part from cooperativity within the tetramer.

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Mechanistic Insights into Reversal of Pulmonary Hypertension by Estrogen Therapy

Soban Umar, Humann Matori, Andrea Iorga, Michelle Afkhami, Jingyuan Li, Federica Maltese, Mansoureh Eghbali.

Severe pulmonary hypertension(PH) leads to right-ventricular failure(RVF) and death. Estrogen(E2) pretreatment has been shown to attenuate development of PH. Here, we tested the hypothesis that E2 may also reverse advanced PH and investigated its underlying mechanisms. Male rats were treated with monocrotaline(MCT,60 mg/kg) to induce PH(n=36). At day-21 when PH had established, one group was euthanized(PH, n=8), and 5 other groups for 10 days received either i) E2 (0.017 mg/day, n=12) ii) E2 plus angiogenesis inhibitor TNP-470 (1.2 mg/day, n=3) iii) ERb-agonist DPN(0.34 mg/day, n=3) iv) ERa-agonist PPT(0.34 mg/day, n=3) or v) left untreated(RVF, n=7). Saline-treated rats served as controls(CTRL, n=6). Echocardiography, cardiac catheterization, RT-PCR, Western-blot and immunocytochemistry were performed. MCT-group developed severe PH at day21 as RV-pressure (RVP) increased from 31 ± 2 in CTRL to 69 ± 2 mmHg. E2 reversed PH(RVP=38 ± 2mmHg, $P<0.05$) completely resulting in full recovery. Interestingly, E2 failed to rescue PH when applied together with TNP. VGEF protein in RV and lungs was reduced significantly ~5fold in PH and E2 increased VEGF significantly ~10 fold. Reduced RV capillary-density in PH was restored fully by E2. E2 reduced cardiopulmonary inflammation as IL-6 transcript upregulation in PH(~22 fold in lung, ~7 folds in RV) was partially reversed by E2. Lungs showed 4-fold increase in ED1-inflammatory cells in PH and E2 reversed this increase. Lung ERb-transcripts, but not ERa, were significantly reduced 6-fold in PH and were restored by E2. ERb protein in lung and RV was also significantly reduced ~2 fold in PH and E2 restored ERb. ERb agonist DPN was as effective as E2 to rescue PH (RVP=34 ± 1 mmHg), whereas PPT was not as effective. In conclusion, E2 rescues PH by stimulating cardiopulmonary neoangiogenesis and suppressing inflammation mainly via ERb.

2277-Pos Board B263

Expression and Spectroscopic Characterization of Melanopsin and Squid Rhodopsin

Nazhat Shirzad-Wasei, **Erica Saint Clair**, John I. Ogren, Christie Stanzel, Javier Navarro, Willem J. deGrip, Kenneth J. Rothschild.

Melanopsin, found in intrinsically photosensitive retinal ganglion cells, drives circadian rhythm and pupillary response. A variety of studies indicate that melanopsin shares many properties with invertebrate rhodopsin such as found in the eyes of squid including activation by an initial photon absorption and regeneration to the original state with a second red-shifted photon. We report the expression and initial spectroscopic characterization of recombinant expressed melanopsin. Mouse melanopsin, carrying a C-terminal deca-his-tag, was expressed in Sf9 insect cells, using recombinant baculovrius. Good expression levels of intact melanopsin protein were achieved (2-4 mg/l). To regenerate the active photopigment 11-cis retinal was added. However, solubilization efficiency was quite low for most detergents. Best results were obtained with dodecylphosphocholine (DPC). Efficient single-step purification was achieved with affinity chromatography over immobilized Ni-2+ ions. So far only a small percentage of the purified melanopsin was photoactive, showing a maximum absorbance at 480-500 nm. Preliminary low-temperature FTIR difference and resonance Raman spectroscopic measurements are reported for melanopsin and squid rhodopsin in order to compare structural features and conformational changes upon light activation including retinal isomerization and protein backbone changes. Supported by a grants from the NIH-NEI to KJR and from NWO-CW to WJdG.

Voltage-Gated Na Channels

2278-Pos Board B264

FGF13 is a Regulator of the Cardiac Voltage-Gated Sodium Channel Nav1.5

Chuan Wang, Jessica A. Hennessey, Robert D. Kirkton, Chaojian Wang, Victoria Bryson, Paul B. Rosenberg, Nenad Bursac, Geoffrey S. Pitt.

The four members of the intracellular Fibroblast Growth Factor (iFGF) subfamily, FGF11-14, regulate voltage-gated sodium (Nav) channels. A missense mutation in FGF14 causes spinocerebellar ataxia 27, thought to be due to Nav channel dysfunction. iFGFs are expressed in the heart, but whether they regulate cardiac Nav channels is not known. Using quantitative real-time RT-PCR, we identified that FGF13 isoforms are the dominant iFGFs in adult mouse heart. Using whole cell patch-clamp configuration, we determined a functional link between FGF13 and Nav1.5. We found that the FGF13 isoforms, FGF13S, FGF13U, and FGF13VY differentially modulate Nav1.5 current density when transiently co-expressed in HEK293 cells. Steady-state activation was not altered. In contrast, steady-state availability was significantly shifted towards the depolarizing direction by each of the FGF13 isoforms. Most strikingly, FGF13S induced a dramatic slowing of recovery from inactivation. Co-immunoprecipitation showed that FGF13 interacted with Nav1.5 in cardiomyocytes. Using a pull down assay, we found that FGF13 directly interacted with C-terminus of Nav1.5. Immunostaining showed that FGF13 co-localized with Nav1.5 on sarcolemma. Some FGF13 is present in subcellular regions devoid of Nav1.5, suggesting other roles than sodium channel modulation. FGF13 knockdown by adenoviral infection with shRNA in adult mice cardiomyocytes affected sodium current density and steady-state availability. FGF13 knockdown in a neonatal rat cardiomyocyte monolayer reduced cardiac impulse conduction and the velocity of the action