inhibited by H-89 (an inhibitor of protein kinase A (PKA)). The concentration-response studies demonstrated that procaterol and FK shift the concentration-response curves of CBA to a low concentration compared with those of CBF, but IBMX does not. Thus, procaterol increases CBA and CBF via cAMP accumulation. However, cAMP compartmentalization in the microdomains for regulating CBA and CBF activity may be different. Latex microbead movements, which were driven by ciliary beating in small airways, were measured using a lung slice. A low concentration of procaterol (10 pM), which increased CBA but not CBF, increased microbead transport in small airways. In conclusion, increases in the waveform of cilium, particularly CBA increase, are of particular importance in increasing the rate of mucociliary transport in small airways of the lung.

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Examination of the Effects of Cholesterol on Cholecystokinin Receptor Function using Model Cell Lines

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The Family A G protein-coupled type 1 cholecystokinin receptor (CCK1R) has been shown to be particularly sensitive to membrane cholesterol, with elevated levels induced by cholesterol-loaded methyl-β-cyclodextrin interfering with receptor-G protein coupling and hormone-induced signaling (JBC, 2005). This is important, because CCK is a physiologic satiety factor, and a defect in CCK-induced activation of this receptor can result in obesity with associated hyperlipidemia. These problems can then worsen the CCK signaling defect and contribute to a cycle of further clinical problems. Of note, the structurally closely-related CCK2R is not sensitive to membrane cholesterol when manipulated in the same manner (unpublished data). Here, we have attempted to study the function of these receptors in 25RA cells derived by T.Y. Chang from CHO cells (JBC 1980). These cells have elevated membrane cholesterol secondary to a gain-of-function defect in the SCAP gene (sterol regulatory element binding protein (SREBP) cleavage-activating protein). We established and characterized 25RA cell lines stably expressing CCK1R and CCK2R. Increased membrane cholesterol was visualized with a quantitative filipin-binding morphologic assay. In this cholesterol-rich environment, CCK radioligand binding and CCK-stimulated intracellular calcium assays were performed, with results compared to those in parental CHO cells. Like the CHO cells with acute physical enhancement of cholesterol, these genetically modified cells demonstrated defective CCK-induced signaling, despite normal CCK radioligand binding. We also analyzed CCK receptor trafficking in the environment of 25RA cells. We found that both CCK1R and CCK2R internalize in response to agonist occupation and to recycle to the plasma membrane normally despite the increase in membrane cholesterol.. (Supported by NIH grants DK32878 and DK78385, and a Kinney Career Development Award).

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T-Cell Signaling Microcluster Transport Toward the Synaptic Center is Influenced by Membrane Ligand Mobility

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The engagement of the T cell antigen receptor (TCR) by antigens induces dynamic molecular reorganization and leads to the recruitment to the plasma membrane of many critical effectors, including zeta-chain associated protein kinase of 70kDa (ZAP70) and adaptor SH2 domain containing leukocyte phosphoprotein of 76kDa (SLP76). Live-cell tracking experiments have revealed the dynamic segregation of these signaling molecules and the formation of immunological synaptic (IS) at the cell-cell interface. However, the correlation between microcluster movement and membrane ligand mobility has remained unclear. Studies so far have focused on either extremely mobile ligands on single-component bilayer surfaces or completely immobile stimuli on glass coverslips. We prepared a supported lipid bilayer system composed of a binary mixture of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) that allows us to vary ligand mobility by adjusting the mixing ratio of phospholipids. Biotinylated anti-human CD3 antibodies are adsorbed via neutravidin to supported lipid bilayers containing polyethylene glycol biotinylated lipids. Human Jurkat T cells are thus activated by our stimulatory bilayer and we track microclusters via fluorescence imaging. We found that ZAP70 microclusters exhibit faster tracking velocity and longer trajectory length in response to increasingly mobile ligands. In contrast, SLP76 microclusters are less responsive to varied ligand mobility. Our measurements of the radial microcluster velocity suggests both ZAP70 and SLP76 microclusters exhibit acceleration toward the center of the cell. Our measurements may help understand the physiological relevance of T-cell synapse dynamics.

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The Prostaglandin G-Protein Coupled Receptor EP4 Activates Both the Stimulatory G_s and the Inhibitory G_i Signaling Pathways

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Understanding the molecular mechanisms that shape an effective immune response is a fundamental question in biology. Antigen-presenting dendritic cells (DCs) are key regulators of the immune system: in peripheral tissues, they recognize foreign antigens, subsequently migrate towards the lymph nodes to activate naïve T cells and initiate an immune response.

On DCs, Prostaglandin E2 (PGE2) is a key modulator of cell migration and cytokine production, exerting its action through the co-expressed non-redundant G-protein coupled receptor (GPCR) subtypes EP2 and EP4, both expressed at the plasma membrane. Both receptors couple to the stimulating G-protein G_s and activate adenylyl cyclase and production of cyclic AMP. We prove with FLIM that PGE2-stimulated EP4 also signals via the inhibitory G_i, thereby inhibiting adenylyl cyclase, suggesting crosstalk between the two signaling pathways, which results in fine-tuning the cell's response to PGE2. Furthermore, it suggest that cross-talk between EP2 and EP4 might occur. Currently we are investigating with TIRF-based single-particle tracking and FRAP how cross-talk is regulated at the molecular level and influenced by the DC plasma membrane nanoenvironment. Our approach is to integrate molecular, immunological and biophysical disciplines to unravel the nanoscale membrane organization, dynamics and cross-talk of the PGE2 receptors on DCs. Our results could potentially provide an important conceptual advance in our understanding of PGE2 signaling and possibly in the GPCR field.

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ATP Stimulates Caveolar Endocytosis of $P2X_7R$ in MC3T3-E1 Osteoblasts

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Purinergic signaling plays a critical role in mechanotransduction in bone. The nucleotides released by the bone cells in response to mechanical stimulation initiate a cascade of signaling events in osteoblasts. Purinergic signaling plays a critical role in mechanotransduction in bone The nucleotides released by the bone cells in response to mechanical stimulation initiate a cascade of signaling events in osteoblasts. $P2X_7$ activation is required for PGE2 release, COX2 synthesis and bone formation, however current research lacks to identify the detailed signaling events and mechanism leading to this activation. We determined the localization of $P2X_7$ receptors on the plasma membrane and it's co-loclaization using the family of Image Correlation Spectroscopy combined with molecular biological methods. Additionally, we used detergent free isolation of lipid rafts to prove that $P2X_7$ receptors are present in caveolae and that release of ATP leads to caveolae endocytosis. To visualize the caveolar endocytosis in response to ATP treatment, MC3T3-E1 cells were transfected with caveolin-1 GFP and visualized under Zeiss 5 Live confocal microscope. We used AF-555 albumin as a selective marker for caveolar endocytosis. The cells treated with 250μM ATP for 40 minutes showed increased AF-555 albumin uptake and its co-localization with caveolin-1 GFP demonstrates endocytosis via caveolae. These data suggest ATP stimulation translocates caveolin-1 from the membrane lipid rafts into the cytosol. Presence of $P2X_7$ receptors in the same fraction as caveolin-1 demonstrates its existence in caveolae of MC3T3-E1 osteoblasts and ATP stimulation induces caveolar endocytosis of $P2X_7$ receptor. The caveolae is a major signaling hub in mammalian cells, the presence $P2X_7$ receptor and its endocytosis in response to ATP stimulation could be a vital process in mechanotransduction in osteoblasts.

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Role of EGFR Juxtamembrane Domain in EGFR Activation

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The intracellular juxtamembrane domain of several receptor tyrosine kinases (RTKs) has been proposed to play an autoinhibitory role. The epidermal growth factor receptor (EGFR) has been widely used as a model in studies of the RTK activation mechanism. Extensive efforts have been devoted to studying the functions of EGFR's different domains; however, the role of the juxtamembrane domain in EGFR phosphorylation and activation is yet to be clarified. In this work, we replaced the 30 amino acids (674-704) of the EGF juxtamembrane domain with ten repeats of Glycine-Glycine-Serine (GGS). Preliminary results from western blots showed decreased phosphorylation of Tyr1148 in the EGFR-GGS chimeric receptor, which suggests that the juxtamembrane domain of EGFR plays an activating role rather than an inhibitory one. Currently we are using ligand titration and Foster resonance energy transfer (FRET) experiments to further explore the difference between wild type EGFR and the EGFR-GGS chimeric receptor. Results from this work will yield further insights into the function of the juxtamembrane domain in the EGFR activation process.