stable cell lines derived from incorporation of a single short hairpin RNA expressed varying levels of syt I knockdown. This variability offers the advantages of studying the functional effects of graded levels of syt I protein expression in regulated release of transmitter. We measured Ca^{2+}-stimulated release of two transmitters, neuropeptide Y (NPY) and norepinephrine (NE). NPY was measured by an enzyme-immunoassay after depolarizing with 50 mM K^+ solution. Stable cell lines that expressed 50-60% of control levels of syt I exhibited NPY release that were similar to control cells. NPY release was reduced to about 18% of control cell release when expression of syt I was reduced to ~20%, and NPY release was abolished when syt I expression was abolished. Stimulated NE release from single vesicles was measured by carbon-fiber amperometry. Unlike NPY release, NE release ranged from 50-100% compared to control release, but was not abolished even for the cells that did not express syt I. These results show that syt I is required for NPY release, but NE release is only partially dependent on syt I expression.

2212-Pos Board B198

Visualization of Spatially Controlled Glucokinase Activation in Living Pancreatic Beta Cells using an Optimized FRET-Based Biosensor

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Glucokinase (GCK) activity in pancreatic beta cells limits the rate of glucose metabolism and controls the glucose threshold for insulin secretion. Regulation of GCK activity by cell surface receptors can occur through activation of nitric oxide synthase (NOS) on insulin secretory granules, and subsequent S-nitrosation of GCK. It is thought that interaction between NOS and GCK is essential to the S-nitrosation reaction. Nonetheless, activation of an existing Förster resonance energy transfer (FRET)-based sensor suggests that the activation occurs diffusively. To examine whether GCK activation has a spatial regulatory component, we developed an improved GCK biosensor using cyan fluorescent protein derived from mCerulean3. Circularly permuted mCerulean3 proteins were constructed with the intent of optimizing rotational positioning of the FRET pair. Several cpmCer3 variants were constructed. The brightest variant, cpmCer174, retains a very high quantum yield (> 0.8) similar to mCerulean3, and produced the highest contrast sensor when incorporated into the FRET-GCK biosensor. Expression in pancreatic beta cells revealed evidence supporting tight spatial control over GCK activation at the insulin secretory granules. These results have numerous implications for the nature of post-translational regulation of glucose sensing in pancreatic beta cells.

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Spatial Organization of Microtubules Specifies Sites of Glucose Transporter-4 Vesicle Fusion with the Plasma Membrane

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In adipocytes, vesicles containing glucose transporter-4 (GLUT4 vesicles) redistribute from intracellular stores to the cell periphery in response to insulin. Vesicles then fuse with the plasma membrane, facilitating glucose transport into the cell. While the cytoskeleton is known to be important in GLUT4 vesicle trafficking, the details of microtubule involvement in this important process are unclear. We therefore examined the spatial organization and dynamics of microtubules in relation to GLUT4 vesicle trafficking in living 3T3-L1 adipocytes using total internal reflection fluorescence (TIRF) microscopy. We found that insulin stimulated an increase in density of microtubules within the TIRF-illuminated region of the cell. The time-course of the density increase correlated with that of the increase in intensity of GLUT4-GFP in this same region. In addition, portions of the microtubules are highly curved and are pulled closer to the cell cortex, as confirmed by Parallax (Sun et al. Nano Lett. 2009) microscopy. Occasionally microtubules are translated parallel to the plasma membrane. We detected fusion events and determined their spatial relationship to microtubules using a pH-sensitive GFP variant (pHluorin) fused to insulin-regulated aminopeptidase (IRAP), a protein that co-traffics with GLUT4. Quantitative analysis revealed that fusions preferentially occur in proximity to the microtubules. We conclude that microtubules may be important in providing spatial information for fusion events, although they are not required for GLUT4 vesicle fusion.

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Mechanistic Insights into Exocytosis Dysfunction After Noble Metal Nanoparticle Exposure

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As nanoparticle use in consumer products and medical applications continues to increase drastically, the field of nanotoxicology has developed to examine the impacts of these nanoparticles in biological systems. Previous work,[1, 2] with primary culture chromaffin and mast cells exposed to Au and Ag nanoparticles, has shown a general trend for nanoparticle effects on cellular exocytosis. That work showed that 24 hours of exposure, to nanoparticles at concentrations from 0.1 to 10 nM for nanoparticles, results in fewer molecules being secreted over a longer period of time. This work investigates the underlying causes of these changes in exocytosis in nanoparticle-exposed cells. Several possible mechanisms of dysfunction could be related to these changes, including altered membrane properties, reactive oxygen species (ROS) generation, or changes in calcium signaling. To examine these potential mechanisms of exocytosis dysfunction, carbon-fiber microelectrode amperometry (membrane properties) and fluorescence methods (vesicle matrix-nanoparticle interactions, calcium signaling, and ROS generation) were employed. Preliminary results indicate there were no significant changes in calcium signaling following nanoparticle exposure, nor were there changes in the pre-spike foot occurrence, which would indicate an alteration in the membrane properties post-exposure. However, preliminary results suggest that there were changes in the rate of ROS generation, which could impair exocytosis machinery in addition to having other downstream effects.

1. Love, S. A.; Haynes, C. L., Assessment of functional changes in nanoparticle-exposed neuroendocrine cells with amperometry: exploring the generalizability of nanoparticle-vesicle matrix interactions. Analytical and Bioanalytical Chemistry 2010, 398 (2), 677-88.
2. Marquis, B.; Maurer-Jones, M.; Braun, K.; Haynes, C., Amperometric assessment of functional changes in nanoparticle-exposed immune cells: varying Au nanoparticle exposure time and concentration. Analyst 2009, 134 (11), 2293-300.

2215-Pos Board B201

The Role of Ceramide in the Clearence of Apoptotic Cells

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Cell fusion is a critical physiological process involving the specific interaction of lipid molecules that reside on the plasma membrane of all cells. The clearance of apoptotic cells (efferocytosis) requires the recognition and subsequent fusion of alveolar macrophages with those membranes of apoptotic cells that display a phosphatidylserine (PS) on the outer membrane, allowing for the physiological clearance of dead and/or dying cells. Recognition of this PS group by macrophages has critical importance as inefficient clearance of apoptotic cells may lead to localized inflammation that may exacerbate the pathogenesis of disease, such as that observed in emphysema. However, the biochemical mechanisms underlying cell fusion and efferocytosis remain unclear. Because ceramide-induced changes in biological membrane composition may have profound effects on cellular functions, we studied the biological effect of ceramide on plasma membrane fusion using model membranes. To do this we examined the inter- and intra-membrane forces of model membranes containing different ratios of PS:PC (phosphatidylcholine) in the presence of ceramides using SAXS and solid-state deuterium NMR. The addition of ceramides to the model membranes reduced the equilibrium spacing between membranes and increased the order of lipid chains. In a complementary cell culture model of engulfment using alveolar macrophages and model membranes, the incubation of either macrophages or model membranes with ceramides reduced the engulfment efficiency significantly. These data demonstrate that ceramide plays a critical role in the processes that lead to the recognition of PS and the subsequent fusion between plasma membranes. These studies suggest that modulating ceramide levels may lead to more efficient efferocytosis and thereby may attenuate lung tissue inflammation in emphysematous lungs.

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Weibel-Palade Body Membrane Identity and Intra-Organelle Protein Mobilities Following Transient Plasma Membrane Fusion

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Weibel-Palade bodies (WPBs) are large rod-shaped regulated secretory organelles unique to endothelial cells (ECs) that contain proteins important in blood clotting and inflammation. During cell stimulation the majority of WPBs fusing with the plasma membrane undergo complete exocytosis, however, in a small fraction of the WPBs the fusion event was transient, resulting in a marked morphological change from rod to spherical shape with the retention of cargo proteins. Using confocal microscopy and FRAP techniques we examined the impact of transient fusion on the