Norepinephrine (NE) is a well-known physiological inhibitor of insulin secretion in pancreatic β cells. We investigated modulation of exocytosis and endocytosis by NE in INS 832/13 cells using whole-cell capacitance measurements. Exocytosis was stimulated by depolarizing pulses from −70mV to +10mV of variable duration and was followed by compensatory endocytosis. Inhibition of Ca^{2+}-evoked exocytosis by NE was overcome by increasing Ca^{2+} influx, either by increasing the depolarizing pulse duration (up to 500ms) or by increasing the extracellular Ca^{2+} concentration up to 10mM. When stimulated by a short train of 500ms pulses in the presence of NE (5μM), robust exocytosis was observed but endocytosis was markedly inhibited. The NE inhibition of endocytosis was abolished by the α_2-adrenergic receptor antagonist yohimbine (10μM) and was not affected by PTX-treatment (150ng/ml), demonstrating that NE inhibition of endocytosis is mediated via the α_2-adrenergic receptor and not via G_i and/or G_o proteins. When a synthetic peptide that mimicked the last 13 c-terminal amino acids of the $G_{z\alpha}$ subunit was dialyzed into the cells via the whole-cell patch pipette, NE inhibition of endocytosis was fully blocked, suggesting that G_z may be mediating the inhibition. Single vesicle recordings by cell attached capacitance measurements indicate that inhibition of endocytosis by NE is due to a decreased number of endocytic events without a significant change in endocytic vesicle size. Further analysis of fission pore kinetics revealed that NE selectively inhibited the rapid fission events. Our findings establish a novel action for NE and suggest the possibility that NE may modulate endocytosis in the central nervous system and elsewhere.

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Secretory Phospholipase A2 Type III Enhances α-secretase-dependent Amyloid Precursor Protein Processing by its Effect on Membrane Fluidity and Endocytosis

Xiaoguang Yang[1], Wenwen Sheng[1], Mark Haidekker[2], Grace Sun[1], James Lee[1].
[1]University of Missouri, Columbia, MO, USA, [2]University of Georgia, Athens, GA, USA.

Owing to the non-amyloidogenic pathway of amyloid precursor protein (APP) processing to produce neuroprotective and neurotrophic α-secretase-cleaved soluble APP (sAPPα), and preclude the amyloidogenic pathway which produces neurotoxic amyloid-β peptide (Aβ), increasing α-secretase activity and sAPPα levels have been suggested as pharmacological approaches for treatment of Alzheimer's disease (AD). In this study, we demonstrated that cytokines including TNFα, IL-1β and IFNγ stimulated immortalized astrocytes (DITNC cells) to secrete secretory phospholipase A2 type III (sPLA2-III) into culture medium. When this conditioned medium was applied to differentiated human neuroblastoma (SH-SY5Y cells), it enhanced sAPPα secretion from cells. To further demonstrate the effect of sPLA2-III on sAPPα secretion, SH-SY5Y cells were exposed to sPLA2-III from bee venom, which is homologous to mammalian sPLA2-IIIs, and hydrolyzed products of sPLA2 including arachidonic acid (AA), lysophosphatidylcholine (LPC) and palmitic acid (PA). We found that either sPLA2-III or AA, but not LPC and PA, increased membrane fluidity, increased the localization of APP at the cell surface without altering the total APP expression in cells, and enhanced sAPPα secretion in SH-SY5Y cells. In addition, neither sPLA2-III nor AA altered the expressions of α-secretases including ADAM 9, 10, and 17. APP has the motif which can target to clathrin-coated pits. We demonstrated that monodansylcadaverine (MDC), clathrin-mediated endocytosis inhibitor, can increase sAPPα secretion from SH-SY5Y cells by reducing APP internalization. Based on these results, sPLA2-III-enhanced sAPPα secretion is suggested to be a consequence in part, due to increased membrane fluidity, and reduced APP internalization through clathrin-mediated endocytosis. Further study will focus on the effect of sPLA2-III on clathrin-mediated endocytosis.

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Quantification of Noise Sources for Amperometric Measurement of Quantal Exocytosis Using Ultramicroelectrodes

Jia Yao[1,2], Kevin Gillis[1,2].
[1]University of Missouri, Columbia, MO, USA, [2]Dalton Cardiovascular Research Center, Columbia, MO, USA.

We are developing transparent multi-electrochemical electrode arrays on microchips in order to automate measurement of quantal transmitter exocytosis from individual neuroendocrine cells. Features of interest in amperometric recordings of quantal exocytosis can be <1 pA in amplitude, therefore low current noise is essential. Consequently we are seeking to understand the relationship between current noise and working electrode area, series resistance, bandwidth, and choice of fabrication materials. We have measured the current power spectral density (PSD) from electrode arrays with working areas that vary in size using a low-noise amplifier. Arrays are shielded from interfering signals. The capacitance of the working Indium-Tin-Oxide electrode varies linearly with area with a specific capacitance of 36 fF/μm^2. In the absence of an analyte, current noise is thermal in origin because the PSD is well described by the Nyquist relationship: PSD = 4kT times the Real part of the electrode Admittance. We find the PSD amplitude scales ~linearly with working electrode area and with frequency from ~30 Hz to at least 3 kHz. The dependence of the PSD on electrode area is similar for carbon-fiber electrodes and our patterned chip electrodes, therefore the electrode material and fabrication method are not key determinants of electrode noise. The choice of material and thickness (>2 μm) for insulating the non-working areas of the electrodes also does not affect the PSD. We conclude that the standard deviation of current noise increases ~ linearly with recording bandwidth, and microchip electrodes can achieve the same noise performance as carbon-fiber microelectrodes.

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Calcium/synaptotagmin-mediated Compound Fusion Increases Quantal Size And Causes Post-tetanic Potentiation At Synapses

Liming He[1], Lei Xue[1], Jianhua Xu[1], Benjamin D. McNeil[1], Ernestina Melicoff[2], Roberto Adachi[2], Ling-Gang Wu[1].
[1]NIH, Bethesda, MD, USA, [2]Department of Pulmonary Medicine, University of Texas MD Anderson Cancer Center, Houston, TX, USA.

Exocytosis at synapses generally refers to fusion between vesicles and the plasma membrane. Although fusion between vesicles, known as compound fusion, occurs in non-neuronal secretory cells and has recently been proposed at ribbon-type synapses, it remains unclear whether it exists, how it is mediated, and what role it plays at the vast majority of synapses, where release occurs at conventional active zones. Here we addressed this issue in rats and mice at a large nerve terminal containing conventional active zones. High potassium application induced giant capacitance up-steps at the release face of nerve terminals, which were larger than the membrane capacitance of regular vesicles. These giant up-steps were not comprised of several smaller steps, nor were they bulk endocytic vesicles that had re-fused. High potassium application also induced giant vesicle-like structures in nerve terminals and giant miniature EPSCs (mEPSCs) that reflected release of a large amount of transmitter. The giant up-steps, giant vesicle-like structures, and giant mEPSCs were abolished by removing the extracellular calcium or by knocking out synaptotagmin II, the calcium sensor mediating fusion at calyces. These results suggest that calcium binding with synaptotagmin II mediates compound fusion and increases quantal size. Compound fusion significantly contributed to the generation of a widely observed synaptic plasticity, post-tetanic potentiation (PTP) of the EPSC, because 1) action potential trains that generated PTP also evoked giant up-steps and increased the mEPSC amplitude, 2) the time course and the degree of the mEPSC amplitude increase paralleled those of PTP, and 3) both the mEPSC amplitude increase and PTP were abolished by the calcium buffer EGTA or synaptotagmin II knockout. Our finding may be of wide application because intense nerve activity, PTP, and giant miniature currents occur in physiological conditions at many synapses.

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Evidence Of A Role For SNAP-25 As A v-SNARE In Vitro

Brandon E. Forbes, Nathan C. La Monica, Gary R. Edwards, Dixon J. Woodbury.
Brigham Young University, Provo, UT, USA.

In neurons, SNARE proteins form a complex that drives membrane fusion leading to neurosecretion. SNAP-25 is an integral part of the neuronal SNARE complex and is clipped by the extremely toxic protease Botulinum Neurotoxin type E (BoNT/E). SNAP-25 is thought to act in the plasma membrane by forming a 1:1 complex with Syntaxin 1A which forms the binding site for the vesicular SNARE, synaptobrevin. SNAP-25 is also found in the vesicle membrane where its physiological role, if any, has yet to be defined. We show that BoNT/E cuts SNAP-25 in rat brain synaptic vesicles (SVs) decreasing their fusion to model membranes (BLM) containing reconstituted syntaxin 1A. We hypothesize that SNAP-25's role *in vivo* may depend on the lipid composition of the plasma and vesicular membranes. SNAP-25 is the only SNARE protein with no membrane spanning domain however, it is anchored in the membrane through the palmitoylation of one or more of its cysteines residues. In order to examine how SNAP-25 functions under different lipid conditions we used our planar lipid bilayer-base fusion system. Fusion rates are determined for target membranes composed of PE:PC (7:3) and with DPPC above and below its Tm. In order to simulate a more native neuronal environment, cholesterol (up to 50mol%) is added to the membrane By understanding the dynamics of the SNARE protein complex in different lipid environment, we hope to understand more about how neurons utilize SNARE proteins to release neurotransmitter.