calcium transients will be important in physiological and pathophysiological processes.

Exocytosis & Endocytosis

503-Pos Board B382
Bioanalytical Analysis of Bis(monoacylglycero)phosphate (BMP) Model Lipid Membranes
Janetricks N. Chebukati, Gail E. Fanucci.
University of Florida, Gainesville, FL, USA.
Bis(monoacylglycero)phosphate (BMP) is an unusually shaped, negatively charged phospholipid found in elevated concentrations in the late endosomes. The unusual structure and stereochemistry of BMP are thought to play important roles in the endosome, including structural integrity, endosome maturation, and lipid/protein sorting and trafficking. We have utilized dynamic light scattering, fluorescence spectroscopy and transmission electron microscopy to characterize the morphology and size of BMP hydrated dispersions and extruded vesicles. We find that the morphology of hydrated BMP dispersions varies with pH, forming highly structured, clustered dispersions of 500 nm in size at neutral pH 7.4. However, at acidic pH 4.5, spontaneous hydrolysis of BMP occurs, altering the vesicle morphology to spherically shaped dispersions. BMP vesicles are also significantly smaller in diameter than palmitoyloleoylglycerophosphocholine (POPC) vesicles. In a stability assay using dynamic light scattering measurements to compare and monitor 30 nm extruded vesicles of BMP, POPC, and POPG over a 5 week period, we find that BMP vesicles do not fuse to form larger structures. BMP also forms lamellar vesicles evidenced by the fluorescence leakage assay studies. These results shed light on the possibility that the biosynthesis of BMP and the increasing acidity during the maturation process of late endosomes play an important role in the formation of intraendosomal vesicular bodies.

504-Pos Board B383
Pacap Acts As A Transmitter At The Sympatho-adrenal Synapse Under The Acute Stress Response
Barbara A. Kuri, Shyue-An Chan, Corey Smith.
Case Western Reserve University, Cleveland, OH, USA.
Chromaffin cells of the adrenal medulla release catecholamine as well as neuro- and vaso-active signaling peptide transmitters into the circulation under the control of the sympathetic nervous system. Exocytosis from chromaffin cells is evoked through cholinergic stimulation from the innervating splanchnic nerve. However, with sustained stimulation, cholinergic stimulation desensitizes rapidly. Yet chromaffin cells continue to release transmitter under the acute sympathetic stress response, indicating a secondary stimulation path. We investigated activity-dependent sympatho-adrenal signaling through direct nerve stimulation in a tissue slice preparation. Chromaffin excitation was determined by current clamp recordings, fura-based Ca^{2+} measurements and amperometric catecholamine detection. We provide data supporting a second transmitter involved in chromaffin cell excitation under conditions that mimic elevated sympathetic input. Pituitary Adenylate Cyclase Activating Peptide (PACAP) is packaged in the terminals of the innervating splanchnic nerve and is a potent secretagogue in catecholamine release from chromaffin cells. We demonstrate that PACAP elicits catecholamine release through cellular mechanisms separate from that evoked by cholinergic stimulation. PACAP stimulation causes cell depolarization to facilitate calcium influx through low voltage-activated T-type calcium channels resulting in catecholamine release. Furthermore, we show that the PACAP-evoked excitation is preferentially activated under elevated stimulation. Thus, PACAP-dependent sympatho-adrenal signaling under conditions that mimic elevated splanchnic firing is emerging as important regulator of catecholamine release under the acute stress response.

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Jamming Dynamics Of Stretch-induced Surfactant Secretion By Alveolar Epithelial Cells
Arnab Majumdar, Stephen P. Arold, Erzsebet Bartolak-Suki, Harikrishnan Parameswaran, Bela Suki.
Boston University, Boston, MA, USA.
Secretion of molecules by cells is a fundamental process of life that maintains the cellular micro-environment. In the lung, secretion of surfactant by alveolar epithelial type II cells is vital for the reduction of interfacial surface tension, thus preventing lung collapse. We find evidence of complex secretory dynamics of these cells in culture when exposed to cyclic mechanical stretch which is the primary stimulus for surfactant secretion. We find that (a) during and immediately following stretch, cells secreted less surfactant than unstretched cells and (b) cells stretched for 15 minutes secreted significantly more surfactant than unstretched cells after 45 min of rest. The subsequent increase in secretion suggests that stretch indeed induces an enhancement of surfactant secretion, but the delay implies that the rate of secretion is in fact decreased. To explain these dynamic features, we develop a model based on the hypothesis that stretching leads to jamming of surfactant traffic, escaping the cell through a limited number of channels. We solve the model analytically and show that its dynamics are consistent with experiments. The proposed mechanism of jamming highlights the importance of dynamics in cellular secretory response to applied stretch and could also be relevant to the dynamics of stimulated secretion from other cells in vivo.

506-Pos Board B385
Action Potential Code And Cocaine Modulates Dopamine Release In Mice Striatum *in vivo*
P.L. Zuo[1], X.J. Kang[1], J. Fan[1], Q. Lei[1], S.R. Wang[1], W. Yao[1], T. Luo[1], Y.F. Xiong[1], H.Q. Dou[1], X.Y. Liu[1], C.H. Wang[1], S. Guo[1], C.X. Zhang[1], H.H. Gu[2], **Zhuan Zhou[1]**.
[1]Peking University, Beijing, China, [2]Ohio State University, Columbus, OH, USA.
Dopamine is a neurotransmitter crucial for movement, mood, drug addiction and many neural degeneration diseases including Parkinson's disease. Micro electrochemical carbon fiber microelectrode (CFE) can record dopamine release from brain in vivo. Stimulation action potentials (APs) induced secretion of dopamine in mouse brain striatum *in vivo*. The stimulus pattern is defined as AP code [N, m, f, d] (N = total stimulating number, m = burst-number, f = frequency, d = inter-burst interval) (Duan et al, JNS, 2003). In wide type mice (WT), with fixed AP number N, the evoked dopamine release was strongly modulated by code parameters m, f and d. In contrast to N and f, which regulate dopamine release by $[Ca^{2+}]_i$ accumulation, m and/or d may modulate secretion by recycling vesicle pool. To test this hypothesis, we used a knockin mice (KI) with the dopamine transporter (DAT) insensitive to cocaine (Chen et al, PNAS, 2006). In KI vs. WT mice, both amplitude and kinetics of dopamine release was drastically changed following given stimulation AP code. The effect of AP burst number ([144, m, 80Hz, 0.5s],m = 1 vs. 16), or "m-effect", on dopamine release is increased by $>$ 400% in KI vs. WT mice. As expected, cocaine increased AP-induced dopamine release for blocking DAT in WT but not KI mice. Surprisingly, the presynaptic vesicle recycling is also altered by cocaine in WT vs. KI mice, as revealed by reduced "m-effect" in KI mice. We propose that cocaine affects not only DAT, but also presynaptic dopamine vesicle pool in striatum in mice.
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Monitoring Exocytosis And Endocytosis At Neuronal Cells Using A Quartz Crystal Microbalance Technique With Simultaneous Amperometric Detection
Ann-Sofie Cans.
Chalmers University of Technology, Goteborg, Sweden.
A small population of neuronal-like cells was cultured on the surface of a quartz crystal disc. When stimulating the cells to exocytosis, the mass loss that occurs from vesicle neurotransmittor release and the mass re-gain by endocytosis was monitored using a quartz crystal microbalance in both the direct mode measuring mass changes and with dissipation (QCM-D) to measure changes in structure in the cell, all in real time. To specifically distinguish the onset of the later endocytosis from the events of exocytosis, the QCM-D instrument has been coupled to simultaneous electrochemical detection to directly measure release events. The one side of the quartz crystal electrode was held at an overpotential and used as an amperometric detector to monitor the oxidation of vesiclar neurotransmitters released from cell from exocytosis. These data allow deconvolution of the opposing events and to determine the amount of endocytosis that occurs immediately following exocytosis.

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Probing Exocytosis In Blood Platelets
Shencheng Ge, Nathan J. Wittenberg, Emily Woo, Christy L. Haynes.
University of Minnesota, Minneapolis, MN, USA.
Exocytosis, a fundamental process for information exchange among cells including neurons, has been extensively studied based on its critical role in many physiological processes. The recent application of techniques such as microelectrochemistry has enabled measurement of individual secretion events, facilitating a mechanistic understanding of the secretion process and chemical messenger storage. In the work presented herein, microelectrochemistry methods are used to study the exocytosis process in blood platelets for the first time. Exocytosis is utilized by platelets as a signaling pathway to accomplish their role in primary hemostasis, the arrest of bleeding. Because platelet exocytosis is similar in many ways to exocytosis in neurons, platelets have been historically treated as an easily obtainable neuronal model. Our work gives the first experimental evidence of quantal secretion from platelets, resulting from exocytosis of one type of specialized granule from platelets, dense-body granules,

by employing fast-scan cyclic voltammetry and amperometry at carbon-fiber microelectrodes. While cyclic voltammograms confirm that the released molecules are serotonin, amperometry provides not only the first evidence of quantal secretion of serotonin from platelets, but also a real-time record of the secretion events. Individual platelets secrete an average of ~15 serotonin-containing granules, each with a serotonin concentration of ~0.6M and a secretion time course of ~7 ms. By combining experimental data with simulation results, we conclude that serotonin, along with other small molecules, is stored in the dense-body granules as a protein-free macromolecular complex rather than in a free solution state. This finding is in stark contrast to storage mechanisms proposed for other cell types where a proteinacious matrix is usually present. This work also examines the effects of variations in physiologically relevant extracellular conditions such as osmolarity, pH, temperature and cholesterol concentrationl, on serotonin secretion.

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Ca^{2+} Syntillas Inhibit Spontaneous Exocytosis In Mouse Adrenal Chromaffin Cells

Jason J. Lefkowitz, Kevin E. Fogarty, John V. Walsh, Jr., Valerie De Crescenzo.
University of Massachusetts Medical School, Worcester, MA, USA.

A central concept in the physiology of neurosecretion is that a rise in cytosolic [Ca^{2+}], resulting from Ca^{2+} influx, triggers exocytosis. But how does Ca^{2+} efflux from intracellular stores affect exocytosis? Here we examine the effect on exocytosis of a focal rise in cytosolic [Ca^{2+}] due to release from internal stores in the form of Ca^{2+} syntillas.

Ca^{2+} syntillas are spontaneous, focal cytosolic transients mediated by ryanodine receptors (RyRs), first found in hypothalamic magnocellular neuronal terminals. (*Scintilla*, Latin for spark, found in a nerve terminal, normally a synaptic structure.) Ca^{2+} syntillas are also found in mouse adrenal chromaffin cells, where they do *not* cause exocytosis because they appear to arise in a microdomain different from the one where the final exocytotic steps occur [1].

We report here that suppressing syntillas in mouse chromaffin cells leads to an increase in spontaneous exocytosis measured amperometrically. Two independent lines of experimentation each lead to this conclusion. In one case release from internal stores was blocked by ryanodine; in another, stores were emptied using thapsigargin plus caffeine. Additionally, the effects of ryanodine were not altered in the presence of reserpine and therefore could not be explained by an inhibitory effect on the vesicular monoamine transporter (VMAT). The effect of syntillas on spontaneous exocytosis can be accounted for by a simple two state model.

We conclude that Ca^{2+} syntillas act to *inhibit* spontaneous exocytosis.

1. ZhuGe, R. et al. Syntillas release Ca2+ at a site different from the microdomain where exocytosis occurs in mouse chromaffin cells. Biophys J 90, 2027–37 (2006).

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F-actin Re-organization Through MARCKS and Myosin II Activity Regulates Quantal Exocytosis

Bryan Doreian, Robert Mecklemburg, Corey Smith.
Case Western Reserve University, Cleveland, OH, USA.

Chromaffin cells of the adrenal medulla are innervated by the sympathetic nervous system and play a crucial role in determining the appropriate physiological response to stressors. Under diverse physiological conditions, differential release of catecholamines and vasoactive peptide transmitters helps determine the appropriate metabolic status and physiological response. As both classes of transmitters are contained within the same dense core secretory granule, their differential release must be regulated after granule fusion with the cell membrane. At basal firing rates, catecholamines are selectively released though 'kiss-and-run' fusion events characterized by a restricted exocytic fusion pore. Under acute stress, increased sympathetic input elevates cytosolic calcium, driving dilation of the fusion pore and expulsion of both catecholamines and the proteinacious core through the 'full-collapse' exocytic mode. Thus, activity-dependent differential transmitter release is regulated by fusion pore dilation. Previously, we showed that F-actin and myosin II play an important role in regulating the transition from 'kiss-and-run' exocytosis to 'full-collapse' exocytosis. Here, we employ electrochemical, electrophysiological and fluorescence based approaches to further investigate the molecular mechanisms responsible for the transition in secretion mode. We show that under light stimulation, myristoylated-alanine-rich¬-c-kinase-substrate (MARCKS) and myosin II remained inactive and cortical F-actin stabilizes 'kiss-and-run' fusion events. Increased cell stimulation resulted in activation of myosin II and MARCKS. The result was disruption of the actin cortex and collapse of the 'kiss-and-run' fusion event. These data demonstrate a role for activity-evoked cytoskeletal re-arrangement through the action of myosin II and MARCKS and define their roles as regulators of the sympathetic stress response.

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Fusion Pore Regulation of Peptidergic Vesicles

Jernej Jorgačevski[1], Matjaž Stenovec[1,2], Marko Kreft[1,2], Aleksandar Bajić[3], Boštjan Rituper[1], Nina Vardjan[1,2], Stanko Stojilkovic[3], Robert Zorec[1,2].
[1]Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia, [2]Celica Biomedical Center, Ljubljana, Slovenia, [3]Section on Cellular Signaling, Developmental Neuroscience Program, NICHD, National Institutes of Health, Bethesda, Slovenia.

Neuroendocrine secretory vesicles discharge their cargo in response to a stimulus. This process begins with the formation of a fusion pore, an aqueous channel between a spherical vesicle and the nearly ''flat'' plasma membrane, through which cargo molecules diffuse into the cell exterior. After formation the fusion pore can expand, leading to the complete merger of the vesicle membrane and releasing all of the vesicle cargo (full fusion exocytosis). On the other hand, fusion pore can relatively swiftly close, allowing only partial release of vesicle cargo and retaining vesicle physical integrity (transient exocytosis).

We studied the release of the pituitary hormone prolactin by hypotonicity, because this hormone also contributes to osmoregulation. Perifused cells spontaneously released prolactin at room temperature, and hypotonicity evoked a transient increase in prolactin release, followed by a sustained depression, as monitored by radioimmunoassay. In single cells imaged by confocal microscopy, hypotonicity elicited discharge of the fluorescently-labelled atrial natriuretic peptide cargo from ~2% of vesicles/cell, while KCl-induced depolarization resulted in a response of ~10% of vesicles/cell, with different unloading/loading time-course of the two fluorescent probes. High resolution changes of membrane capacitance were recorded in both, unstimulated and stimulated conditions, reflecting single vesicle fusion/fissions with the plasma membrane. In stimulated cells, the probability of occurrence of full fusion events was low and unchanged, since over 95% of fusion events were transient. However, stimulation prolonged the average pore dwell-time (hypotonicity and KCl depolarization for 25%, respectively), increased the frequency of occurrence (hypotonicity for 35%, KCl depolarization tenfold) and the fusion pore conductance (hypotonicity and KCl depolarization for 50%, respectively). Hypotonicity only rarely elicited new fusion events in silent membrane patches.

The results indicate that transient exocytosis appears to be the dominant mode of exocytosis at spontaneous as well as at stimulated conditions.

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Synchronous Versus Asynchronous Contributions to Frequency-induced Synaptic Depletion in Zebrafish

Hua Wen, Paul Brehm.
Oregon Health and Sciences University, Portland, OR, USA.

Paired spinal motorneuron and target muscle recordings were used to examine transmitter depletion and subsequent recovery during high frequency stimulation. The skeletal muscle is sufficiently compact that the unitary synaptic events (~400pA average) were fully resolved through whole cell voltage clamp. Also, the evoked endplate current reflected the sum of <10 unitary events at physiological calcium concentrations. Stimulating at frequencies >20Hz led to drops in quantal content and eventual failure, with no associated change in unitary quantal size. The time required to deplete 80% of the transmitter corresponded to 25 sec at 20Hz, 10 sec at 50Hz and 5 sec at 100 Hz. Recovery occurred abruptly after a 40 sec rest and the rate of recovery was calcium-dependent. Analysis of the depletion profile during 100 Hz stimulation revealed two partially overlapping processes. Initially, all release was synchronous upon repolarization of the motorneuron action potential. With continued stimulation the release was delayed and asynchronous with respect to the action potential. The quantal size underlying both synchronous and asynchronous modes was unchanged during the depletion and following recovery. At 100 Hz the synchronous endplate current converted to asynchronous unitary openings in a manner that was qualitatively reciprocal. Moreover, traces composed principally of synchronous events had few asynchronous events and conversely traces with the largest numbers of asynchronous events lacked synchronous release. This reciprocity suggested that the two modes share common release sites. However, measurements of total synchronous and asynchronous events during the time of maximal overlap revealed a period of facilitation, suggesting that the synchronous and asynchronous modes were capable of additivity and might represent different release sites. Experiments are ongoing to distinguish between these alternative possibilities.

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Norepinephrine Inhibits Endocytosis In Insulin-secreting Cells

Ying Zhao, Qinghua Fang, Susanne G. Straub, Lindau Manfred, Geoffrey W.G. Sharp.
Cornell Uinversity, Ithaca, NY, USA.