calcium transients will be important in physiological and pathophysiological processes.

Exocytosis & Endocytosis

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Bioanalytical Analysis of Bis(monoacylglycero)phosphate (BMP) Model Lipid Membranes
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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.

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Pacap Acts As A Transmitter At The Sympatho-adrenal Synapse Under The Acute Stress Response
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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
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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.

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Action Potential Code And Cocaine Modulates Dopamine Release In Mice Striatum *in vivo*
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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
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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
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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,