

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 proteinaceous matrix is usually present. This work also examines the effects of variations in physiologically relevant extracellular conditions such as osmolarity, pH, temperature and cholesterol concentration, on serotonin secretion.

This study is supported by the Searle Scholars Program.

509-Pos Board B388

Ca²⁺ 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 [Ca²⁺], resulting from Ca²⁺ influx, triggers exocytosis. But how does Ca²⁺ efflux from intracellular stores affect exocytosis? Here we examine the effect on exocytosis of a focal rise in cytosolic [Ca²⁺] due to release from internal stores in the form of Ca²⁺ syntillas.

Ca²⁺ 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.) Ca²⁺ 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 occur¹.

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 Ca²⁺ 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).

510-Pos Board B389

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

Bryan Doreian, Robert Mecklenburg, 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 through '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 proteinaceous 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.

511-Pos Board B390

Fusion Pore Regulation of Peptidergic Vesicles

Jernej Jorgačevski¹, Matjaž Stenovec^{1,2}, Marko Kreft^{1,2}, Aleksandar Bajič³, Boštjan Rituper¹, Nina Vardjan^{1,2}, Stanko Stojilkovic³, Robert Zorec^{1,2}.

¹Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia, ²Celica Biomedical Center, Ljubljana, Slovenia,

³Section 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.

512-Pos Board B391

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 motoneuron 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 motoneuron 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.

513-Pos Board B392

Norepinephrine Inhibits Endocytosis In Insulin-secreting Cells

Ying Zhao, Qinghua Fang, Susanne G. Straub, Lindau Manfred, Geoffrey W.G. Sharp.

Cornell University, Ithaca, NY, USA.