increases in $[Ca^{2+}]_i$, and then, AA activates PPARα, which enhances Ca^{2+}-regulated exocytosis in antral mucous cells. A novel autocrine mechanism mediated via PPARα maintains Ca^{2+}-regulated exocytosis of the antral mucous cells of guinea pig.

3549-Pos

Dopamine Production in the Pancreatic β-Cells: a Possible Autocrine Regulatory Mechanism for Insulin Secretion

Alessandro Ustione, David W. Piston.
Vanderbilt University, Nashville, TN, USA.

Glucose homeostasis is maintained by small clusters of hormone secreting cells in the pancreas: the pancreatic islets. Insulin secreting β-cells make for 90% of each islet and secrete insulin in a tightly regulated manner.

Scattered observations in the literature report that β-cells express the required machinery to synthesize and secrete dopamine. Other lines of evidence show that dopamine inhibits glucose stimulated insulin secretion (GSIS) in vitro, and the effect is mediated by the D2 isoform of the dopamine receptor. Yet, there is no evidence of dopaminergic neurons innervating pancreatic islets, and therefore, the biological relevance of such sensitivity is not clear.

We test the hypothesis that pancreatic islets produce dopamine from circulating precursor L-dopa and that the resulting dopamine is released as an autocrine inhibitory signal to regulate insulin secretion. We use microfluidic devices to maintain isolated intact islets viable during imaging experiments: we monitor islet metabolic activity by imaging of NAD(P)H autofluorescence with two photon excitation and we measure intracellular $[Ca^{2+}]_i$ oscillations by confocal microscopy. Our data from wild type and transgenic mice lacking D2 dopamine receptor support the hypothesis that dopamine is an autocrine regulator of GSIS. The results show that metabolic activity is not affected by dopamine. On the contrary, $[Ca^{2+}]_i$ oscillation frequency is reduced by both dopamine and L-dopa, suggesting that D2 receptor activation affects GSIS downstream of glucose metabolism.

This finding provides a new target for drug development in the treatment of diabetes and could help understanding the reported increased risk of developing type 2 diabetes by patients treated with antipsychotic drugs.

3550-Pos

Cholesterol Stabilizes the Fusion Pore of Rat Chromaffin Granules before Its Rapid Dilation

Frederick W. Tse, Nan Wang, Christina Kwan, Xiandi Gong,
Elena Posse de Chaves, Amy Tse.
University of Alberta, Edmonton, AB, Canada.

Changes in cellular cholesterol level affect transmitter release but the role of cholesterol in the fusion machinery is not well understood. Using carbon fiber amperometry, we examined whether changes in cellular cholesterol level has any direct effect on the release of catecholamines from individual chromaffin granules. To avoid any possible effect of cholesterol perturbation on ion channels, exocytosis was stimulated directly via whole-cell dialysis of a Ca^{2+}-buffered solution. Cellular cholesterol level was either reduced by ~30% (via a cholesterol synthesis inhibitor and extracellular application of a cholesterol extractor) or increased by ~3 fold (via loading of cholesterol). Changes in cellular cholesterol level did not affect the rate of exocytosis, the quantal size or the kinetic parameters of the main amperometric spikes (which reflect the rapid release during and after the rapid dilation of the fusion pore). In contrast, cholesterol perturbation affected the amperometric foot signals (which reflect the catecholamine leakage via a semi-stable fusion pore). Reduction of cellular cholesterol destabilized the fusion pore while it was flickering (resulting in a decrease in the proportion of ''stand-alone foot'' signal) and before the onset of rapid dilation (resulting in a shortening of the foot signal). Elevation in cellular cholesterol level had opposite effects, suggesting that cholesterol elevation increased the stability of the semi-stable fusion pores. Acute extraction of cholesterol from the cytosolic side of the plasma membrane (via whole-cell dialysis of a cholesterol extractor) also shortened the foot signal and reduced the proportion of ''stand-alone-foot'' signals. However, acute extracellular application of cholesterol or its extractor did not affect the amperometric signals. We suggest that cholesterol on the cytosolic leaflet of the vesicular membranes constrained the fusion pores of chromaffin granules before the onset of rapid dilation.

3551-Pos

Integration of Electrical Stimulation together with Electrochemical Measurement of Quantal Exocytosis on Microchips

Jaya Ghosh, Kevin Gillis.
University of Missouri, Columbia, MO, USA.

We are developing microfabricated devices consisting of arrays of electrochemical electrodes in order to increase the throughput of single-cell measurements of quantal exocytosis from neuroendocrine cells and to develop technology that allows simultaneous electrochemical detection and fluorescence imaging of single fusion events. One component of this effort is to develop on-chip methods for stimulating exocytosis from select cell population on the chip. Here we describe our efforts to use the same electrode to electrically stimulate the adjacent cell and subsequently measure exocytosis using amperometry. Voltage pulses were applied to planar electrodes while recording the membrane potential of an adjacent cell using a patch clamp pipette in current-clamp mode. We found that the threshold for eliciting action potentials is typically between 2.0 and 3.0 V for cells that are well adhered to Au electrodes (2.23 + 0.49 V, 0.2 ms pulse, n=20 cells). Trains of stimuli, however, often lead to electroporation of the cell membrane, therefore we turned our attention to designing stimuli to promote efficient cell electropermeabilization to trigger exocytosis upon Ca^{2+} influx from Ca^{2+}-buffered bath solutions. The amplifier was modified to allow it to transiently pass large currents to enable electroporation, yet record pA amperometric currents with low noise. We found that trains of voltage pulses of 5-8 V of 0.2-0.5 ms duration can reliably elicit Ca^{2+}-dependent exocytosis lasting for tens of seconds, presumably by eliciting electroporation of the cell membrane. Preliminary experiments with transparent electrodes and the fluorescent Ca^{2+} indicator fura-4F demonstrate a rise in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) upon electrical stimulation. Experiments are in progress to determine if this method allows one to clamp $[Ca^{2+}]_i$ to the Ca^{2+} level of the buffered bath solution.

Supported by NIH grant NS048826.

3552-Pos

Quantitative Modeling of Synaptic Release at the Photoreceptor Synapse

Gabriel Duncan[1], Katalin Rabl[2], Ian Gemp[1], Wallace B. Thoreson[2],
Ruth Heidelberger[1].
[1]University of Texas Medical School at Houston, Houston, TX, USA,
[2]University of Nebraska Medical Center, Omaha, NE, USA.

Exocytosis from the rod photoreceptor is stimulated by submicromolar calcium and exhibits an unusually shallow dependence on presynaptic calcium. This weak cooperativity may contribute to the linear relationship between calcium influx and release at photoreceptor synapses and contrasts with release at other ribbon and conventional synapses, which exhibit a fourth or fifth-order calcium dependence. To provide a quantitative description of the photoreceptor calcium sensor for exocytosis, we tested a family of conventional and allosteric computational models describing the final calcium-binding steps leading to exocytosis. Simulations were fit to two measures of release, evoked by flash-photolysis of caged calcium: exocytotic capacitance changes from individual rods and post-synaptic currents of second-order neurons. The best simulations supported the occupancy of only two calcium binding sites on the rod sensor rather than the typical four or five. For most models, the on-rates for calcium binding and maximal fusion rate were comparable to those of other neurons. However, the off-rates for calcium unbinding were unexpectedly slow. In addition to contributing to the high-affinity of the photoreceptor calcium sensor, slow calcium unbinding may support the fusion of vesicles located at a distance from calcium channels, perhaps located higher up the synaptic ribbon or away from the ribbon. In addition, partial sensor occupancy due to slow unbinding may further contribute to the linearization at this first synapse in vision.

3553-Pos

Inertia of Synaptic Vesicle Exocytosis

Oliver Welzel, Teja W. Groemer, Jasmin Jung, Armin M. Stroebel,
Katrin Ebert, Johannes Kornhuber, Andreas W. Henkel.
Department of Psychotherapy and Psychiatry, Erlangen, Germany.

Reliable synaptic vesicle exocytosis in primary hippocampal neurons depends on the number and availability of release-competent vesicles, their recharging with neurotransmitters and the kinetics of exo- endocytosis. We have analyzed the correlations between several exocytotic kinetic parameters by measuring FM-styryl dyes (FM 1-43, FM 4-64 and FM 5-95) discharge from electrically stimulated synapses: initial fluorescence, relative fluorescence loss, half-decay time and number of neighbors of each synapse. All terminals were maximally loaded and subsequently destained by three different stimulations, using 1200 action potentials (APs) at frequency of 40 Hz for loading and 600 APs at 30 Hz, 20 Hz and 10 Hz for destaining, respectively. Nerve terminals that contain more dye and thus more vesicles released styryl dyes slower compared to synapses that contain fewer vesicles. Furthermore, vesicle-rich synapses exhibited a lesser relative fluorescence loss than those with fewer vesicles. Interestingly, synapses with more neighbors matched these with high initial fluorescence . Computer model simulations revealed that the results of the exocytosis parameter measurements were not compromised by statistical and system-specific appraisal artifacts. The results of this study show that exocytosis is qualitatively

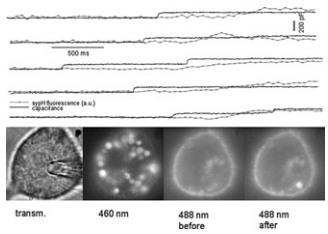
and quantitatively different in vesicle-rich and vesicle-poor synapses and depends on the spatial localisation of the synapse and their number of neighbors, respectively. This variation could be the basis for specific information-processing circuits in the hippocampus.

3554-Pos

Simultaneous Optical-Electrical Measurement of the Delay between formation of the Fusion Pore and Proton Equilibration in Exocytosis of Single Vesicles

Daniel Steinbrenner, Nadine Harmel, Nikolaj Klöcker, Jan C. Behrends.
University of Freiburg, Freiburg, Germany.

Optical detetction of neutralization of pH in granules or vesicles is often used to define exocytotic events. However, combined measurents of ensemble capacitance and pH-dependent vesicular fluorescence changes have suggested that the movement of protons only becomes possible after fusion pore expansion with a mean delay of $>$ 300 ms (1). To enhance the temporal resolution of such measurements, we have combined capacitance recordings of single vesicle fusion in RBL cells transfected with synapthpHluorin as a reporter of vesicular pH. To monitor cell capacitance steps due to exocytosis of single granules in whole cell patch-clamp mode, we used the piecewise linear technique. Internal solution contained 10 μM free Ca2+ and 300 μM GTPγS. Before establishing whole cell recordings, punctate fluorescence signals could be detected with excitation at 460 nm, while during perfusion with internal solution and excitation at 480 nm, punctate fluorescence signals gradually appeared at corresponding sites. Fluorescence increases clearly lagged capacitance steps by several 100 ms- seconds, supporting the idea that pH equilibration through the fusion pore is delayed.



(1) Barg et al.: Neuron, 33, 287-299, 2002.

Intracellular Channels

3555-Pos

Functional Properties of SR Cl^- and K^+ Channels during Postnatal Development of Cardiac Muscle

Maura Porta[1], Kristin Harris[1], Eric Winkel[1], Josefina Ramos-Franco[2], Rafael Mejia-Alvarez[1].
[1]Midwestern University, Downers Grove, IL, USA, [2]Rush University Medical Center, Chicago, IL, USA.

In adult (AD) heart, the sarcoplasmic reticulum (SR) contains Cl^- & K^+ channels presumably involved in controlling RyR-mediated SR Ca^{2+} release. These channels provide a countercurrent mechanism that attenuates the drop in Ca^{2+} driving force across the SR membrane, thereby preventing early termination of Ca^{2+} release. We showed that in newborn (NB), Ca^{2+} sparks occur with similar frequency than in AD but have shorter duration and smaller amplitude, implying an early termination of Ca^{2+} release. Although the functional properties of SR Cl^- & K^+ channels have been thoroughly described in AD, little is known about their presence and their role in NB. Consequently, we first tested the hypothesis that the early termination of Ca^{2+} release in NB coincides with absence/low density of SR Cl^- & K^+ channels at this stage. To this end, the heavy microsomal fraction was obtained from 5-days-old NB and AD rat hearts and SR Cl^- & K^+ channels were reconstituted into artificial planar lipid bilayers. Our results indicate that Cl^- & K^+ channels can be reconstituted from NB heavy SR microsomes with a similar success rate (number of SR channel incorporations / total number of bilayers) than in AD (~0.2 for Cl^- channels & ~0.1 for K^+ channels). Thus, an alternative mechanism would imply that in NB, smaller counterion fluxes result from different functional properties of SR Cl^- & K^+ channels. This assumption was tested by measuring their unitary conductance, open probability, and voltage dependence. The results in NB channels revealed no significant differences in any of these parameters in comparison to AD. Thus, we concluded that SR Cl^- & K^+ channels do not contribute to the developmental changes of Ca^{2+} release in NB cardiomyocytes. Supported by AHA-0655656Z to RMA.

3556-Pos

Role of TRIC-A Channel in Circulatory Function

Daiju Yamazaki[1], Satomi Kita[2], Shinji Komazaki[3], Aya Mishima[1], Miyuki Nishi[1], Takahiro Iwamoto[2], Hiroshi Takeshima[1].
[1]Grad. Sch. of Pharmaceutical Sci., Kyoto Univ., Kyoto, Japan, [2]Faculty of Med., Fukuoka Univ., Fukuoka, Japan, [3]Saitama Med. Univ., Saitama, Japan.

TRIC (trimeric intracellular cation) channels in the sarco-/endoplasmic reticulum likely act as counter-ion channels that conduct monovalent cations in a synchronized manner with release of stored Ca^{2+}. TRIC channel subtypes display differential expression patterns as TRIC-A is predominantly expressed in excitable tissues, including brain and muscle, and TRIC-B is present throughout many tissues. TRIC-A knockout mice are viable and fertile, while TRIC-B knockout mice exhibit neonatal lethality due to respiratory failure (Yamazaki et al., Development 2009), and double-knockout mice lacking both subtypes show embryonic cardiac failure (Yazawa et al., Nature, 2007). To resolve the physiological role of TRIC-A, we are currently focusing on abnormal circulatory function in TRIC-A-knockout mice during young adulthood. These mutant mice showed significant hypertension and bradycardia. Autonomic blocking agents (co-application of atropine and metoprolol) greatly improved the bradycardic condition without affecting hypertension in the mutant mice. This observation suggests that a hyperactive baroreceptor reflex leads to development of the bradycardic condition in the mutant mice. Blockers for vasoactive humoral factors, such as angiotensin, endothelin and vasopressin, did not significantly improve hypertension in the mutant mice, suggesting normal blood-vasopressor levels. Importantly, isometric tension measurements indicated that contractility is markedly impaired in aortic ring preparations from the mutant mice, and that acetylcholine-induced relaxation is hypersensitive in mutant mesenteric artery. Our results suggest a vital role for TRIC-A channels in the physiological regulation of vessel tonus by vascular smooth muscle and endothelial cells. To further examine the pathogenesis of hypertension at the molecular level, we plan to examine TRIC-A expression and agonist-evoked Ca^{2+} transients in smooth muscle and endothelial cells from TRIC-A-knockout and wild-type mice.

3557-Pos

Tic110 a Channel-Forming Protein at the Inner Envelope of Chloroplasts Electrophysiology and Regulation

Tom A. Goetze[1], Richard Wagner[1], Monica Balsera[2], Bettina Bölter[3], Jürgen Soll[3].
[1]University of Osnabrueck, Osnabrueck, Germany, [2]Paul Scherrer Institut, Villigen, Switzerland, [3]Ludwig-Maximilians-Universität, Munich, Germany.

Tic110 has been proposed to be a channel-forming protein at the inner envelope of chloroplasts whose function is essential for the import of proteins synthesized in the cytosol. Sequence features and topology determination experiments presently summarized suggest that Tic110 consists of six transmembrane helices. Its topology has been mapped by limited proteolysis experiments in combination with mass spectrometric determinations and cysteine modification analysis. Two hydrophobic transmembrane helices located in the N terminus serve as a signal for the localization of the protein to the membrane as shown previously. The other amphipathic transmembrane helices are located in the region composed of residues 92-959 in the pea sequence. This results in two regions in the intermembrane space localized to form supercomplexes with the TOC machinery and to receive the transit peptide of preproteins. A large region also resides in the stroma for interaction with proteins such as molecular chaperones. In addition to characterizing the topology of Tic110, we show that Ca2+ has a dramatic effect on channel activity in vitro and that the protein has a redox-active disulfide with the potential to interact with stromal thioredoxin.

3558-Pos

Luminal Ca^{2+} is a Major Sensitiser of Two-Pore Channels to NAADP

Samantha Jane Pitt[1], Tim Funnell[2], Michael X. Zhu[3], Mano Sitsapesan[1], Elisa Venturi[1], John Parrington[2], Magarida Ruas[2], Antony Galione[2], Rebecca Sitsapesan[1].
[1]University of Bristol, Bristol, United Kingdom, [2]University of Oxford, Oxford, United Kingdom, [3]The Ohio State University, Columbus, OH, USA.

It has been suggested that two-pore domain channels (TPCs) are the NAADP receptors responsible for NAADP-mediated Ca^{2+}-release from lysosome-related stores yet there is evidence that NAADP could also regulate RyR channels. We have therefore compared the effects of NAADP on native RyR1, RyR2 and purified human TPC2, reconstituted into artificial membranes under identical experimental conditions. Similar to RyR channels, we find that TPC2 behaves as an ion-channel permeable to both monovalent (300 ± 14pS; symmetrical 210 mM K^+; SD; n=3) and divalent cations (15 ± 2pS; 10μM *cis*/50 mM *trans* Ca^{2+};SD; n=5) with no evidence for anion permeability (in a 210mM *trans*: 510mM *cis* KCl gradient, the reversal potential coincides with the calculated value for a channel ideally selective for cations (E_{rev}=-23mV)). Addition of *trans* NAADP had no effect on TPC2, but *cis* ap-