3544-Pos
A Role for Dysferlin and Agatoxin Sensitive Calcium Channels in the Calcium-Trigged Secretion of ATP Following Plasma Membrane Wounding
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Mutations in dysferlin cause Limb-Girdle and Miyoshi Muscular Dystrophy. Dysferlin is structurally related to Otoferlin, a protein involved in the calcium triggered release of neurotransmitters in cochlear hair cells. Dysferlin is a calcium-binding protein with a single membrane-spanning domain thought to be required for membrane wound repair. The specific function that dysferlin performs in wound healing is unknown. Here we report the activation of an intercellular signaling pathway in sea urchin embryos by membrane wounding that evokes calcium spikes in neighboring cells. This pathway was mimicked by ATP application, and inhibited by apyrase, cadmium, and omega-agatoxin IVa. Microinjection of dysferlin antisense morpholinos blocked this pathway; control morpholinos did not. Co-injection of mRNA encoding human dysferlin with the inhibitory morpholino rescued signaling activity. Our results indicate that membrane wounding triggers membrane depolarization, calcium influx through an agatoxin sensitive voltage-gated calcium channel, and ultimately the secretion of ATP. Moreover, dysferlin appears to mediate one of the essential steps in this signaling cascade.

3545-Pos
Evoked Catecholamine Release from Sympathetic Nerve Terminals in Cardiac Slices
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Neurotransmitter release from presynaptic cells can be recorded by postsynaptic potentials/currents in central nerve system. However, little is known about how to record nerve release in cardiovascular system in fresh tissue or in vivo. We have developed two novel methods for recording catecholamine release induced by nerve action potentials in cardiac slices and in vivo, respectively. Heart beating is regulated by peripheral (vagal and sympathetic) nerves, which innervate and release neurotransmitters in the heart. We are interested in how nerves regulate heart function. In the present study, we demonstrate first recordings from fresh cardiac slices. We have developed a novel method (termed cSEC) to record catecholamine release from rat and mouse sinus atrial nodes (SAN). Using micro carbon fiber electrode (CFE), catecholamine release from sympathetic nerve terminals were recorded as amperometric current (4°C) following depolarization made by field electric stimulation, high KCl, ACh, or hypoxia. The spatial mapping of evoked catecholamine signals is determined in SAN. Supported by NSFC and ‘973’ program.

3546-Pos
A Permissive Role for Protein Kinase a in Support of Epac Agonist-Stimulated Human Inslet Secretion
George G. Holz1, Oleg G. Chepurny1, Colin A. Leech1, Igor Dzhura1, Grant G. Kelley1, Michael W. Roe1, Elvira Dzhura1, Xiangquan Li1, J. Lu1,2, Y. Xiu1,2, O. Me-cAMP-AM (10 micromolar) was associated with evoked exocytosis activated by 1°C/3°C temperature. Thus, hypotonic shock-induced ATP secretion from A549 cells occurs mainly via Ca2+ dependent exocytosis. Supported by CIHR and CCFR (RG), Emerging Technologies Fund Grant Texas (Z.G.), NIH-HL090786 (JB).

3547-Pos
Tirf Microscopy Study of Exocytotic ATP Release from A549 Epithelial Cells
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Extracellular nucleotides, via interaction with cell surface purinergic receptors, regulate multiple physiological processes in the lungs, including airway mucociliary clearance and surfactant secretion. Release of ATP from nonexcitable cells can be provoked by mechanical perturbations and cell-swelling, but the underlying mechanisms remain incompletely understood. We have shown previously that cell-swelling induced ATP secretion from A549 cells tightly correlates with intracellular Ca2+ elevations and sought to establish whether Ca2+-dependent exocytosis is involved. In this study, 50% hypotonic shock-induced ATP release from A549 cells was examined by total internal reflection fluorescence (TIRF) microscope in an attempt to directly visualize ATP-loaded vesicle movement, recruitment and fusion with the plasma membrane. Cells were loaded with quinacrine, a fluorescent marker of ATR intracellular storage sites, and time-lapse imaging was performed using through the objective TIRF system. The time-course of fluorescence intensity changes of individual quinacrine-stained vesicles was evaluated during 1-2 min following hypotonic stimulation. Approximately 20%-30% of vesicles visible by TIRF at the cell base showed a quasi-instantaneous disappearance during the first minute post-stimulation, as expected for vesicle fusion and dispersal of their content. This was accompanied by recruitment of ~10% new vesicles into the evanescent field followed by their exocytosis. The hypotonic stimulus significantly (~5-fold) increased rate of exocytotic events compared to rate of spontaneous events in unstimulated cells. Exocytotic release mechanism is also consistent with ATP efflux measurements using luciferin-luciferase luminescence assay. Agents known to disrupt exocytotic process (brefeldin, monensin), or cytoskeleton (nocodazole, cytochalasin) reduced ATP release significantly (by up to 80%), while the release was completely blocked by N-ethylmaleimide (1mM), and low (10°C) temperature. Thus, hypotonic shock-induced ATP secretion from A549 cells occurs mainly via Ca2+-dependent exocytosis. Supported by CIHR and CCFR (RG), Emerging Technologies Fund Grant Texas (Z.G.), NIH-HL090786 (JB).

3548-Pos
Arachidonic Acid/parra Enhancement of Ca2+-Regulated Exocytosis in Antral Mucous Cells of Guinea Pig
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Indomethacin (IDM, 10 μM) enhanced the Ca2+-regulated exocytosis stimulated by 1 μM ACh in guinea-pig antral mucous cells, but not aspirin (ASA, 10 μM). The differences in pharmacological actions between IDM and ASA suggest that IDM accumulates arachidonic acid (AA), which enhances Ca2+-regulated exocytosis. AA (2 μM) enhanced Ca2+-regulated exocytosis in antral mucous cells similarly to IDM, moreover, an analogue of AA, AACOCF3 (Arachidonitrifluoromethyl ketone, a PLA2 blocker) also enhanced it. These indicate that the Ca2+-regulated exocytosis is directly enhanced by AA, not by the products of the AA cascade, such as PGs, LXs and LTs. We examined the effects of MK886 (an inhibitor of peroxisome proliferation activation receptor α, PPARα) on the AA-enhanced induced of Ca2+-regulated exocytosis. AA is a natural ligand for PPARα. MK886 (40 μM) abolished the enhancement of Ca2+-regulated exocytosis induced by AA, IDM and AA+COFC3. Moreover, MY14643 (a PPARα agonist) enhanced the Ca2+-regulated exocytosis, similarly to AA. MK-886 decreased the frequency of the Ca2+-regulated exocytosis activated by 1 μM ACh or thapsigargin by 25-30 %. Western blotting and immunohistochemical examinations demonstrated that PPARα exists in antral mucous cells. Thus, ACh stimulates AA accumulation via

more, 8-pCPT-2'-O-Me-cAMP-AM (10 micromolar) had no significant ability to activate AKAR3, a PKA-regulated biosensor expressed in human islet cells by viva transcription. Surprisingly, co-administration of 8-pCPT-2'-O-Me-cAMP-AM with an inhibitor of PKA activity (H-89, 10 micromolar), or treatment with a camp antagonist that blocks PKA activation (Rp-8-CPT-cAMPS; 200 micromolar), reduced GSIS measured in the absence of 8-pCPT-2'-O-Me-cAMP-AM. Furthermore, the action of 8-pCPT-2'-O-Me-cAMP-AM to potentiate GSIS was nearly abolished by H-89 and Rp-8-CPT-cAMPS. Thus, there exists a permissive role for PKA in support of glucose-stimulated and Epac-regulated human islet insulin secretion. We propose that this permissive action of PKA may be operative at the insulin secretory granule recruitment, priming, and/or post-priming steps of Ca2+-dependent exocytosis.
increases in \([\text{Ca}^{2+}]_i\), and then, AA activates PPARα, which enhances \(\text{Ca}^{2+}\)-regulated exocytosis in antral mucous cells. A novel autocrine mechanism mediated via PPARα maintains \(\text{Ca}^{2+}\)-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**

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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 morphometry and β-cell activity by imaging NADPH and autofluorescence with two photon excitation and we measure intracellular \([\text{Ca}^{2+}]_i\), 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, \([\text{Ca}^{2+}]_i\), 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 Dilatation**

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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 \(\text{Ca}^{2+}\)+ solution by intracellular \([\text{Ca}^{2+}]_i\) 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, \([\text{Ca}^{2+}]_i\), 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.

**Quantitative Modeling of Synaptic Release at the Photoreceptor Synapse**

**3552-Pos**

**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 morphometry and β-cell activity by imaging NADPH and autofluorescence with two photon excitation and we measure intracellular \([\text{Ca}^{2+}]_i\), 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, \([\text{Ca}^{2+}]_i\), 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.

**3553-Pos**

**Inertia of Synaptic Vesicle Exocytosis**

**3551-Pos**

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

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We are developing microfabricated devices consisting of arrays of electrochemical electrodes in order to increase the throughput of single-cell measure-