3544-Pos

A Role for Dysferlin and Agatoxin Sensitive Calcium Channels in the Calcium-Triggered 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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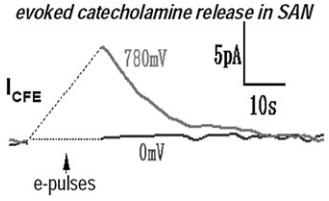
Neurotransmitter release from presynaptic cells can be recorded by postsynaptic potentials/currents in central nerve system. However, little is known about how to record nerve release in cardiovascular system in fresh tissue or in vivo. We have developed two novel methods for recording catecholamine release induced by nerve action potentials in cardiac slices and in vivo, respectively.

Heart beating is regulated by peripheral (vegas 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 ($\mathbf{I_{CFE}}$) following depolarization made by field electric stimulation, high KCl, ACh, or hypoxia. The spatial mapping of evoked catecholamine signals is determined in SAN.



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3546-Pos

A Permissive Role for Protein Kinase a in Support of Epac Agonist-Stimulated Human Islet Insulin Secretion

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Potential insulin secretagogue properties of an acetoxymethyl ester of a cAMP analog (8-pCPT-2′-*O*-Me-cAMP-AM) that activates the guanine nucleotide exchange factors Epac1 and Epac2 were assessed using isolated human islets of Langerhans. QPCR demonstrated that the predominant variant of Epac expressed in human islets was Epac2, although Epac1 was clearly detectable. Under conditions of islet perifusion, 8-pCPT-2′-*O*-Me-cAMP-AM (10 micromolar) potentiated 10 mM glucose-stimulated insulin secretion (GSIS), while failing to influence insulin secretion measured in the presence of 3 mM glucose. The secretagogue action of 8-pCPT-2′-*O*-Me-cAMP-AM was associated with K-ATP channel inhibition, depolarization and an increase of $[Ca^{2+}]_i$ measured in single beta cells or whole islets. As expected for an Epac-selective cAMP analog, 8-pCPT-2′-*O*-Me-cAMP-AM (10 micromolar) failed to stimulate phosphorylation of PKA substrates CREB and Kemptide in human islets. Furthermore, 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 viral transduction. Surprisingly, treatment of human islets 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 Ca^{2+}-dependent exocytosis.

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 Ca^{2+} elevations and sought to establish whether Ca^{2+}-dependent exocytosis is involved. In this study, 50% hypotonic shock-induced ATP release from A549 cells was examined by total internal reflection fluorescence (TIRF) microscopy 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 ATP 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 hypoosmotic 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, cytocholasin) reduced ATP release significantly (by up to 80%), while the release was completely blocked by N-ethylmaleimide (1 mM), and low (10oC) temperature. Thus, hypotonic shock-induced ATP secretion from A549 cells occurs mainly via Ca^{2+}-dependent exocytosis. Supported by CIHR and CCFF (RG), Emerging Technologies Fund Grant Texas (Z.G.), NIH-HL090786 (JB).

3548-Pos

Arachidonic Acid/ppara Enhancement of Ca2+-Regulated Exocytosis in Antral Mucous Cells of Guinea Pig

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Indomethacin (IDM, 10 μM) enhanced the Ca^{2+}-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 Ca^{2+}-regulated exocytosis. AA (2 μM) enhanced Ca^{2+}-regulated exocytosis in antral mucous cells similarly to IDM, moreover, an analogue of AA, $AACOCF_3$ (Arachidonyltrifluoromethyl ketone, a PLA_2 blocker) also enhanced it. These indicate that the Ca^{2+}-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-induced enhancement of Ca^{2+}-regulated exocytosis, because AA is a natural ligand for PPARα. MK-886 (40 μM) abolished the enhancement of Ca^{2+}-regulated exocytosis induced by AA, IDM and $AACOCF_3$. Moreover, WY14643 (a PPARα agonist) enhanced the Ca^{2+}-regulated exocytosis, similarly to AA. MK-886 decreased the frequency of the Ca^{2+}-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