2601-Pos

Cystic Fibrosis Transmembrane Conductance Regulator in Mouse Pancreatic Beta-Cells

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Cystic fibrosis (CF) is a monogenic autosomal recessive disease caused by mutation in the cystic fibrosis gene that encodes the cystic fibrosis transmembrane conductance regulator (CFTR), which is an ion-channel that conducts negatively charged chloride ions. Cystic fibrosis related diabetes (CFRD) is the leading complication of CF and exocrine pancreatic dysfunction affects ~85% of patients. Recently, it was discovered that rat α-cells and β-cells in the islets of Langerhans express both CFTR mRNA and protein. The aim of this study was to investigate the presence of active CFTR channels in pancreatic β-cells and if these influence insulin secretion. For this purpose we have used the patch-clamp technique and capacitance measurements on single mouse β-cells and insulin secretion measurements using RIA. First we measured the presence of CFTR in β-cells using the patch-clamp technique. A membrane conductance of 0.05 ± 0.01 nS/pF (n=10) and 1.05 ± 0.22 nS/pF at negative and positive potentials, respectively, was activated by the cAMP-increasing agent forskolin. The conductance was significantly reduced ($P<0.001$) and the current almost totally inhibited in the presence of 10 μM of the CFTR-antagonist, CFTRinh-172. Glucose-stimulated and cAMP-amplified insulin secretion measured on islets was not reduced using this concentration although there was a tendency towards reduction. Moreover, exocytosis elicited by a train of ten membrane depolarisations and measured as an increase in membrane capacitance on single β-cells was significantly reduced by 70 ± 10% ($P<0.01$, n=9) in the presence of 10 μM CFTRinh-172. We conclude that active CFTR is present in mouse pancreatic β-cells and that it has a crucial role in exocytosis of secretory granules in the β-cells.

2602-Pos

Potassium Accumulation Dominates Short-Term Depression of Neurohypophysial Excitability

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Excitability of the axons and nerve terminals in the mammalian neurohypophysis strongly depends on the temporal pattern and intensity of stimulation. Within a given stimulus train, excitability tends to facilitate initially and begins to depress significantly during persistent stimulation. In this work we present both experimental evidence and numerical simulations indicating that depression of neurohypophysial excitability is dominated by activity-dependent potassium accumulation.

Using high speed optical recordings and fast potentiometric dyes (di-4-ANEPPDHQ), we monitored the tissue-averaged changes in excitability of the intact neurohypophysis during trains of action potentials. We examined the effects of three interventions, each supporting the potassium accumulation model. First, we increased and decreased the potassium concentration of the bathing solution. At low $[K^+]$, the depression was diminished, and at higher $[K^+]$, the depression was more prominent. By bathing the preparation in a hypertonic saline solution, we examined the effect of increased interstitial space on the modulation. With a greater volume into which the potassium dilutes, depression was noticeably reduced. Finally, we applied ouabain to inhibit the Na^+ / K^+ pumps. The reduced ability to clear accumulating potassium resulted in increased depression.

Potassium accumulation also resulted in changes to the waveform of the optically recorded action potential. Aside from the net loss of AP amplitude within a train of stimuli, the AP after-hyperpolarization vanishes and the action potential broadens. Numerical simulations of the effects of K^+ accumulation on action potential waveforms, using established properties for neurohypophysial Na^+ and K^+ ion channels, reproduced the experimentally observed behavior.

2603-Pos

Locking up the Guardian: Loopholes in the Lung Defense Program

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The human respiratory tract is continuously exposed to bacteria, dust and other airborne particle. Under normal conditions mucociliary transport (MCT) efficiently remove inhaled airborne particles from the airway. An additional airway defense mechanism is the presence of a family of antimicrobial peptides called cathelicidins. These peptides are known to be secreted by a variety of cells including neutrophils, acinae, and goblet cells. Cathelicidin LL37/hCAP18 has been shown to be expressed in the airway epithelium, however its storage and release have not been investigated (Proc. Natl. Acad. Sci. USA, 1998, 95:9541-9546).

While held inside the granule secretory products (SP) are caged in condensed polyanionic matrixes, including chromogranin, heparine, mucin, secretogranins, etc. Upon exocytosis the secretory matrix swells allowing SP to freely diffuse to the extracellular space (Biophys. J. 1991, 59: 1022-1027). In goblet cells SP are stored in a mucin matrix. Postexocytic swelling, driven by Na^+/Ca^{2+} ion exchange results in the formation of the mucus gel and the release SP stored in the mucin granule (Ann Rev. Physiol. 1990, 52: 157-176).

Airway infection, a hallmark among prevalent respiratory inflammatory diseases, including COPD and Cystic Fibrosis (CF), is consistently associated to defective hydration of mucus. Here we test the hypothesis that cathelicidin LL37/hCAP18 is stored in goblet cell granules and that defective mucus hydration hinders release of LL37/hCAP18 from the mucus gel. Preliminary results show that retarded swelling kinetics and decreased equilibrium hydration of mucus by decreasing extracellular $[Na^+]/[Ca^{2+}]$ results in failure to uncage LL37/hCAP18 from the mucin network. Supported by NSF grant # 0120579 to PV.

2604-Pos

Massive Endocytosis Activated by Perturbing the Outer Plasmalemmal Monolayer

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The roles played by lipids in endocytic processes are the subject of much ongoing debate. Using electrophysiological, optical, and ultrastructural methods, we describe massive endocytosis (MEND) of >50% of the plasmalemma in response to perturbing the outer plasmalemma monolayer of fibroblasts and cardiac myocytes by multiple means. Extracellular application of a bacterial sphingomyelinase causes MEND within seconds, and similar responses occur with the nonionic detergents, Triton X-100 and NP-40, proapoptotic drugs (e.g. edelfosine and tamoxifen), and an amphipathic phospholipase inhibitor, U73122. At the concentrations employed, the effective agents do not cause membrane permeability changes, and they are inactive from the cytoplasmic side. Ca transients that do not cause MEND decrease markedly the threshold concentrations of amphipaths that cause MEND, perhaps by generating a lipid catalyst of MEND. Noise analysis of NP-40 records suggests that the average vesicle size is initially small (<100nm). However, internalized vesicles evidently fuse rapidly, as horseradish peroxidase is found within seconds in large vacuoles and multi-lamellar bodies. These MEND responses do not require cytoplasmic ATP, Ca, or dynamins, and they can be repeated multiple times with reversal taking place over several minutes in the presence of ATP. For nonionic detergents, ongoing MEND stops within 2 to 4 seconds when detergent is removed. For dodecylsulfate and dodecylglucoside, MEND occurs only after detergent removal. These results suggest that endocytosis can be driven primarily by lipidic forces, possibly by lipid and protein partitioning into domains that pinch off to the cytoplasm as a result of line tension to their surround.