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All processes in living cells are built from random molecular events, but the law of large numbers usually guarantees predictability. Intracellular Ca^{2+} oscillations mediated by inositol trisphosphate receptor channels (IP_3Rs) are widespread and important. Their apparent regularity and the large numbers of molecules involved have led to a perception that Ca^{2+} oscillations are deterministic and predictable. But we show that for four different cell types, Ca^{2+} oscillations are stochastic, consistent with theoretical predictions claiming that they are a sequence of random spikes. The existence of a minimal interspike interval allows for regular oscillations despite such fluctuations. Random molecular fluctuations usually destroy coherence and blur spatiotemporal structures, but Ca^{2+}-induced Ca^{2+} release deploys random fluctuations constructively to generate Ca^{2+} signals that spread throughout the cell. Ca^{2}+ signals use array enhanced coherence resonance to orchestrate noise into regular oscillations.

2131-Pos IP3 Induced Ca2+ Release In Endothelial Cells Of Single Coronary Arteries Of An Intact Beating Heart

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The Ca^{2+} dynamics of endothelial cells has been usually addressed in cell lines or primary cultures. Under these circumstances however, the interaction between the endothelial tissue and the smooth muscle of a coronary artery is completely lost. Here, the Ca^{2+} dynamics of endothelial cells were studied for the first time at the single coronary artery level on an intact beating heart. Ca^{2+} dynamics were measured in a mouse heart mounted on horizontal Langendorff, on an upright confocal mycroscope (Zeiss 510 slm, Germany). The hearts were perfused with a Ca^{2+} indicator (either Fluo-4 or Rhod-2). Additionally, the heart was perfused with acetomethyl esther form of a caged IP_3 or nitrophenyl EGTA. These caged compounds were rapidly uncaged by applying 1 ms UV pulses generated by a frequency tripled Nd-Yag DSPP. The UV pulses were locally delivered through a multimode optical fiber micropositioned on the epicardial site of the left ventricular wall. Substantial changes in basal [Ca^{2+}] were obtained when the caged IP_3 was photo-hydrolyzed. These changes were not dependent on the extracellular Ca^{2+} concentration and were blocked by inhibiting the SERCA pump with thapsigargin. The mechanical response of a single artery was also evaluated. Our results demonstrate that this technique can be a unique tool to evaluate the endothelial function of single coronary arteries under normal or physiopatological conditions

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Epithelial Channels & Physiology

2132-Pos The Selectivity Mechanism of Aquaporins and Aquaglyceroporins

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Aquaporins (AQPs) constitute a family of pore proteins that facilitate the efficient flux of water across biological membranes. Related aquaglyceroporins additionally transport small organic solutes such as glycerol or urea. Within the last years substantial progress has been made in understanding the permeation mechanism through AQPs, however questions regarding their selectivity for different solutes remain challenging. Moreover, the role of aqua(glycero)porins in gas transport across membranes is still a matter of debate. Using molecular dynamics simulations, we studied the selectivity of Aquaporin-1 and the bacterial glycerol facilitator GlpF for a wide range of physiologically relevant solutes. We present potentials of mean force (PMFs) for solute permeation through the AQP channels and compare them to PMFs for the alternative route across the lipid membrane. In addition, the effects of point mutations on the channel characteristics have been studied. The results help to rationalize permeation experiments and allow to identify the molecular mechanisms underlying the selectivity of aquaporins and aquaglyceroporins.

2133-Pos Store-operated Ca^{2+} Channels (SOC) In Pancreatic Duct Epithelial Cells (PDEC)

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Ca^{2+} influx through store-operated Ca^{2+} channels (SOC) is activated by depletion of intracellular Ca^{2+} stores following agonist-mediated Ca^{2+} release. We previously established that Ca^{2+} entry *via* SOC contributes to exocytosis in pancreatic duct epithelial cells (PDEC) (Kim et al., 2007). We now characterize electrophysiological properties, modulation, and expression of the SOC on PDEC using Ca^{2+} imaging and patch-clamp techniques. The agonists ATP, UTP, acetylcholine, and epinephrine stimulated a Ca^{2+} influx dependent on extracellular Ca^{2+}. Inclusion of 100 μM IP_3 or 5 μM thapsigargin in the internal pipette solution elicited whole-cell currents, mediated by the SOC, of ~ 1 pA/pF (−100 mV) with 20 mM Ca^{2+} in the external medium. In a divalent cation-free medium, the SOC-mediated current (measured as a Na^{+} current stimulated by thapsigargin and inhibited by 10 μM $LaCl_3$) increased 6–7 fold. Ca^{2+} influx through SOC was completely blocked by 10 μM La^{3+} or 100 μM 2-aminoethoxydiphenyl borate (2-APB) but only partially by 50 μM SK&F 96365. Influx was also reduced by 100 μM W-7, an inhibitor of calmodulin, suggesting that Ca^{2+}-activated calmodulin modulated SOC. In polarized PDEC, thapsigargin-induced Ca^{2+}

influx only occurred though the basolateral membrane, localizing the SOC to this membrane. RT-PCR, using mRNA from

PDEC, established the expression of TRPC1 and TRPV6, members of the transient receptor potential (TRP) channel family, as possible molecular candidates for SOC. Thus, in PDEC, different stimuli activate the basolateral SOC that will mediate Ca^{2+} influx to increase cytoplasmic Ca^{2+}, subsequently modulating salt secretion *via* ion transport mechanisms and mucin secretion *via* exocytosis.

2134-Pos siRNA Directed at KCNQ1 K^+ Channels Inhibits Epithelial Anion Secretion

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Board B249

Basolateral membrane K^+ channels in secretory epithelial tissues play a critical role in anion secretion by establishing membrane potential as an electrical driving force. To identify these K^+ channels we examined activation of membrane current by the neurotransmitter VIP in a human liver duct cell line, Mz-ChA1, using whole-cell voltage-clamp. VIP (300 nM) rapidly activated K^+ current, which was blocked by bath Ba^{2+} (5 mM). KCNQ1 is a voltage-gated K^+ channel expressed in the heart, but it can also be associated with the ancillary subunit KCNE3, rendering it voltage-insensitive. We demonstrated the presence of KCNQ1 and KCNE3 messages in Mz-ChA1 cells using RT-PCR, and showed using immunofluorescence that KCNQ1 is expressed in rat liver duct cells. Mz-ChA1 cells do not form high resistance monolayers. To demonstrate the role of KCNQ1 in anion secretion, we measured short circuit current (Isc) across T84 human colonic secretory epithelial cell monolayers. VIP (300 nM) activated a large Isc across T84 monolayers, which was rapidly blocked by the addition of Ba^{2+} (5 mM) to the serosal bath. Transfection (confirmed by confocal microscopy) of these monolayers with short interfering RNA (siRNA) designed to degrade KCNQ1 mRNA significantly reduced the VIP-stimulated Isc. We conclude that VIP activates KCNQ1 and KCNE3 in secretory epithelial tissues.

2135-Pos Cyclically Stretching Polarized Bronchial Epithelial Cultures at an Air-Liquid Interface

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Board B250

Mechanical strain on cells plays a vital role in cell signaling and gene expression. Polarized human bronchoepithelial cell cultures have emerged as a fruitful model system for studying airway surface liquid volume regulation and muco-ciliary transport. These cultures require a membrane porous on the nanoscale to develop as well-differentiated, pseudo-stratified, polarized cell cultures with significant ciliation. This study presents a novel membrane which enables the application of cyclic strain on epithelial cells while maintaining the air-liquid interface. This is achieved by electrospinning a nanoporous, nonwoven mesh and coupling it to a silastic membrane. Applying vacuum to the silastic membrane applies an equibiaxial strain to the porous membrane and any attached cells. This allows for detailed study of the mechanical dependencies of epithelial cultures at an air-liquid interface. Furthermore it creates the opportunity to examine the relationship between aspects of mucociliary clearance and mechanical strain.

2136-Pos A Three-dimensional Model of Epithelial Sodium Channel Based on the Homology with Acid-Sensing Ion Channel

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Board B251

Epithelial sodium channels (ENaCs) are highly selective ion channels that allow an amiloride sensitive transport of sodium across polarized epithelial cells. The three-dimensional structure of ENaC is unknown. ENaC is a heteromultimeric channel, composed of three homologous subunits. The subunit stoichiometry of ENaC was controversial; ENaC was thought to be a tetramer or a nonamer. Both ENaC and acid-sensing ion channel (ASIC) are members of the ENaC/DEG family and share a ~70% amino acid sequence identity. This study presents a model of ENaC based on sequence homology with the ASIC1 channel, whose crystal structure in the closed state was recently solved. ASIC1 channel is a trimer and it is very likely that ENaC channels also are trimers. The pore of ENaC was modelled in a closed conformation, without any ions on the permeation pathway. The model was further refined using molecular dynamics methods. The proposed model is consistent with the functional data of wild-type and mutant ENaC channels. The extracellular 'loop' of ENaC model appears as a compact structure that comprises alpha-helices and beta-sheets. The extracellular region is stabilized by the seven disulphide bonds from each subunit, involving cysteine residues conserved in ENaC/DEG family. Surprisingly, according to this model, the selectivity filter, previously thought to precede TM2, lies in the cytoplasmatic half of the lipid bilayer and has a helical conformation. One of the putative amiloride binding sites, thought to lie in the extracellular 'loop', in the present model is positioned in the TM2 helix, in the middle of the lipid bilayer. The other putative amiloride binding site from the extracellular 'loop' was not included in the present model because it is positioned in an area without homology in ASIC sequence.