vesicles labelled with FM 1-43. These data suggest that VMS astrocytes respond to a decrease in pH by releasing ATP via vesicular exocytosis.

507-Pos

The Role of Cell Adhesion Molecule 1 (CADM1) in Nerve-Mast Cell Communication

Tadahide Furuno[1], Akihiko Ito[2], Naohide Hirashima[3], Mamoru Nakanishi[1].
[1]Aichi Gakuin University, Nagoya, Japan, [2]The University of Tokyo, Tokyo, Japan, [3]Nagoya City University, Nagoya, Japan.

It has long been demonstrated that the nervous and immune systems are not disparate entities. The nerve-mast cell relationship served as prototypic association, and unequivocal evidence has been considerably presented for the consistent anatomical association and the functional interaction between nerves and mast cells. We found that nerve-mast cell communication can occur bidirectionally in the absence of intermediary cells using in vitro coculture approach and calcium imaging analysis. We have studied the molecular mechanism in nerve-mast cell communication and showed that substance P was an important mediator from superior cervical ganglia (SCG) to mast cells and induced the degranulation to mast cells attached with SCG neurites. In addition, ATP released from antigen-stimulated mast cells was found to activate SCG neurites attached with mast cells. To investigate the adhesion molecules involved in the nerve-mast cell communication, we here focused an adhesion molecule of immunoglobulin superfamily, CADM1, which is expressed on bone marrow-derived mast cells from wild type mice. When mast cells with or without CADM1 were cocultured with SCG and dorsal root ganglia (DRG) neurons, the number of CADM1-expressing mast cells attached to neurites was much higher than CADM1-deficient cells. The transfection with CADM1 to CADM1-deficient mast cells recovered the attachment to neurites. The responding rate of mast cells with CADM1 attached to neurites following specific activation of neurons by scorpion venom was higher than ones without CADM1. Ectopic expression of CADM1 increased this proportion. CADM1 was also found to be locally concentrated at points of contact between neurites and mast cells. These results suggested that CADM1 on mast cells not only functions as simple glue in nerve-mast cell interaction but also promotes development of a microenvironment to communicate efficiently each other.

508-Pos

Tunneling Membrane Nanotubes Generate Local Calcium Signals and May Actively Propagate Calcium Signals Between Cells

Ian F. Smith[1], Jianwei Shuai[2], Ian Parker[1].
[1]Neurobiology and Behavior, Irvine, CA, USA, [2]Department of Physics, Xiamen University, Xiamen, China.

Cells have long been known to employ gap junctions and synapses to communicate with their neighbors. A new mechanism has recently been proposed following the discovery of tunneling membrane nanotubes (TNTs) between cells [1]. TNTs are dynamic membrane protrusions with lengths up to several tens of microns and diameters of 50-800nm, which permit the exchange of membrane components and cytoplasmic molecules between neighboring cells. Ca^{2+} diffusion along TNTs has been proposed as a means of intercellular communication [2], yet our modeling simulations show that passive diffusion alone is insufficient to account for efficient transmission of Ca^{2+} between cells. Instead, we observe local spontaneous and inositol trisphosphate (IP_3)-evoked mediated Ca^{2+} signals within the length of TNTs formed between cultured SHSY-5Y neuroblastoma cells. Moreover, immunostaining demonstrates the presence of both ER and IP_3 receptors along the TNT. We propose that IP_3Rs are involved in actively propagating intercellular Ca^{2+} signals along TNTs, acting as amplification sites to overcome limitations of passive diffusion in a chemical analog of electrical transmission of action potentials along axons.

Supported by grants NIH GM 40871 and GM65830.

1. Rustom, A. et al. (2004) Science. 303, 1007-1010
2. Watkins, S. C. and Salter, R. D. (2005) Immunity. 23, 309-318.

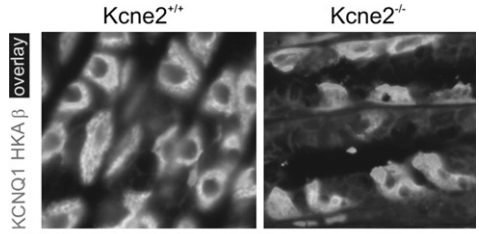
Epithelial Channels & Physiology

509-Pos

Effects of Kcne Subunit Deletion on Polarized Trafficking of the KCNQ1 Potassium Channel *in Vivo*

Geoffrey W. Abbott[1], Kerry Purtell[1], Elizabeth C. King[1], Gianina Panaghie[1], Daniel J. Lerner[2], Torsten K. Roepke[3].
[1]Cornell University, Weill Medical College, New York, NY, USA, [2]Tyrx Inc, Monmouth Junction, NY, USA, [3]Clinic for Cardiology and Angiology, Charite University-Medicine, Berlin, Germany.

The KCNQ1 potassium channel alpha subunit generates essential K^+ currents in human heart and in a range of polarized secretory epithelia. The polarity of KCNQ1 trafficking varies between different epithelia, but neither the importance nor the mechanism for this polarity are well understood. KCNQ1 co-localizes apically with the KCNE2 beta subunit in gastric parietal cells but basolaterally with KCNE3 in colonic crypts. Both KCNE2 and KCNE3 convert KCNQ1 to a constitutively active channel. Here, genetic deletion of *Kcne2* in mice resulted in 5-fold upregulation of Kcne3, formation of Kcnq1-Kcne3 complexes, and basolateral Kcnq1 targeting in parietal cells, and gastritis cystica profunda stemming from achlorhydria and earlier hyperplasia. In contrast, *Kcne2*$^{-/-}$*Kcne3*$^{-/-}$ mice exhibited apical parietal cell Kcnq1 localization. Thus, in parietal cells, apical Kcnq1 localization is required for gastric acid secretion, and the apical localization *per se* does not require Kcne2. Kcne3, if present, actively targets Kcnq1 basolaterally, ultimately causing a preneoplastic condition which in humans could predispose to gastric cancer.



Apical (left) versus basolateral (right) localization of KCNQ1 (red) in gastric parietal cells of *Kcne2*$^{+/+}$ (left) versus *Kcne2*$^{-/-}$ (right) mice. Green: H^+/K^+-ATPase β subunit (apical marker).

510-Pos

Regulation of Delta-Enac Ion Channels by the Neuronal-Specific Sgk1.1 Kinase

Diana Wesch[1], Pablo Miranda[1], Diego Alvarez de la Rosa[2,3], **Teresa Giraldez**[1].
[1]Unidad de Investigacion HUNSC, Tenerife, Spain, [2]Physiology Dept, ULL School of Medicine, Tenerife, Spain, [3]Instituto de Tecnologias Biomedicas (ITB), Tenerife, Spain.

The epithelial sodium channel (ENaC) is a voltage-independent ion channel that plays a fundamental role in kidney transepithelial sodium transport and extracellular volume homeostasis. Previous work from our group and others have identified a novel ENaC subunit that is prominently expressed in neurons but not in kidney epithelia. The physiological role of delta-ENaC channels in neurons is unknown, but it could be involved in the regulation of membrane resting potential and hence of neuronal excitability. Kidney ENaC activity is increased by the serum and glucocorticoid-induced kinase 1 (SGK1). Recently, a new neuronal-specific isoform of SGK1, named SGK1.1, has been identified. We have tested whether SGK1.1 regulates delta-ENaC activity. Co-injection of SGK1.1 and delta-ENaC channels in *Xenopus* oocytes increased sodium current by two-fold. SGK1.1 increased delta-ENaC plasma membrane expression by 1.6-fold. *In situ* hybridization experiments confirmed the co-expression of delta ENaC and SGK1.1 in pyramidal neurons of the human cerebral cortex, indicating that this regulation could be physiologically relevant. In summary, we have identified a new regulator of delta-ENaC ion channels that could play a role in the control of neuronal resting potential and excitability.

511-Pos

A Multidomain Model For Electrodiffusion and Water Flow

Yoichiro Mori[1], Robert S. Eisenberg[2].
[1]University of Minnesota, Minneapolis, MN, USA, [2]Rush Medical Center, Chicago, IL, USA.

Fluid flow and its coupling to electrodiffusion is involved in many physiological systems from the kidney to the lens of the eye, where it has been studied in some detail (Journal of Membrane Biology (2007) 216:1-16). We formulate a mathematical model that describes electrodiffusion and water flow in three dimensions with resolution and scale appropriate for analysis of tissues. The mathematical model presented can be seen as a coarse-grained version of a model used in (PNAS(2008) 105:6463-6468) to model cellular and subcellular electrodiffusion. We shall discuss the relationship of the general model to other macroscopic models in electrophysiology, and show preliminary computations and applications.

Calcium Signaling Pathways

512-Pos

Orai3 and the Selective Activation of the Arc Channel by Arachidonic Acid

Trevor Shuttleworth[1], Olivier Mignen[2], Jill Thompson[1].
[1]University Of Rochester Medical Center, Rochester, NY, USA, [2]Université de Bretagne Occidentale, Brest, France.

The store-operated CRAC channels and the store-independent, arachidonic acid-activated ARC channels represent the founding members of a new family of biophysically similar, highly Ca^{2+}-selective, Ca^{2+} entry channels - the ''Orai channels''. Both of these channels are dependent on STIM1 for their activation, but they differ in the pool of STIM1 responsible. Thus, whereas STIM1 in the ER regulates the CRAC channels on store-depletion, ARC channels are exclusively regulated by the pool of STIM1 that constitutively resides in the PM.
Recent studies have shown that the functional CRAC channel pore is formed by a tetrameric arrangement of Orai1 units. In contrast, a heteropentameric assembly of three Orai1 subunits and two Orai3 subunits forms the functional ARC channel pore (Mignen *et al*. J. Physiol. 587: 4181). Importantly, this inclusion of Orai3 subunits in the channel structure has been shown to play a specific, and unique, role in determining the selectivity of the ARC channels for activation by arachidonic acid. Using an approach based on the generation and expression of various concatenated constructs, we examined the basis for this Orai3-dependent effect on selectivity for arachidonic acid. These studies revealed that, whilst heteropentamers containing only one Orai3 subunit are sensitive to arachidonic acid, *specific selectivity* for activation by this fatty acid is only achieved on inclusion of the second Orai3 subunit in the pentamer. Further studies identified the cytosolic N-terminal domain as the region of the Orai3 molecule that is specifically responsible for this switch in selectivity. Substitution of just this domain into an otherwise complete Orai1 subunit within a concatenated 31111 pentamer is sufficient to change the resulting channel from one that is predominantly store-operated, to one that is essentially exclusively activated by arachidonic acid.

513-Pos

Stim-Dependent and Independent Effects of 2-APB on Orai3 Crac Channels

Megumi Yamashita, Agila Somasundaram, Murali Prakriya.
Northwestern University, Chicago, IL, USA.

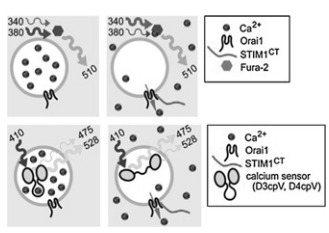
The compound 2-aminoethyldiphenyl borate (2-APB) has received widespread attention for its ability to modulate store-operated CRAC channels. 2-APB elicits complex effects in native and ectopic CRAC channels arising from the over-expression of Orai1 (the pore subunit), causing a several-fold enhancement of ICRAC at low concentrations (20 μM). However, recent studies indicate that 2-APB produces strikingly different effects in the Orai3 variant. Here, high 2-APB concentrations activate (rather than inhibit) Orai3 channels. Moreover, the 2-APB activated Orai3 currents differ from store-operated Orai3 (and Orai1) currents in manifesting altered ion selectivity. The multiplicity of 2-APB effects in the different Orai isoforms has confounded efforts to understand its mode of action. Here, we find that 2-APB (50 μM) induces Orai3 current in two kinetically distinct phases: an initial increase in current with no change in ion selectivity is followed by secondary activation of Orai3 channels with altered ion selectivity. Lower concentrations of 2-APB (< 10 μM) potentiated Orai3 currents with no change in ion selectivity, resembling effects seen in Orai1. In contrast to the activation of Orai3 channels by high concentrations of 2-APB, the potentiation by low concentrations of 2-APB was entirely dependent on STIM1. High concentrations of 2-APB also eliminated fast Ca2+-dependent inactivation of Orai3 currents. Collectively, our results indicate that as seen with Orai1 and native CRAC channels, 2-APB causes dual effects on Orai3 channels: low concentrations potentiate Orai3 currents with no change in ion selectivity, whereas high concentrations activate Orai3 currents while also altering ion selectivity and removing fast inactivation. Our results suggest that the complex effects of 2-APB on Orai1 and Orai3 channels share common mechanisms.

514-Pos

Minimal Requirement for Store-Operated Calcium Entry: STIM1 Gates ORAI1 Channels in Vitro

Yubin Zhou[1], Paul Meraner[1], Hyoung T. Kwon[1], Danya Machnes[1], Masatsugu Oh-hora[1], Jochen Zimmer[2], Yun Huang[1], Antonio Stura[1], Anjana Rao[1], Patrick G. Hogan[1].
[1]Immune Diseasea Institute, Harvard Medical School, Boston, MA, USA, [2]HHMI and Department of Cell Biology, Harvard Medical School, Boston, MA, USA.

Store-operated Ca2+ entry through the plasma membrane CRAC channel in mammalian T cells and mast cells depends on the sensor protein STIM1 and the channel subunit ORAI1. In order to dissect the essential steps in STIM-ORAI signaling in vitro, we have expressed ORAI1 in a sec6-4 strain of the yeast Saccharomyces cerevisiae, which allows isolation of sealed membrane vesicles carrying ORAI1 from the Golgi compartment to the plasma membrane. S cerevisiae itself has no significant reservoir of Ca2+ in the ER, does not possess orthologues of the ER Ca2+-ATPase or IP3 receptor, and has no STIM or ORAI homologues. We show by in vitro Ca2+ flux assays that bacterially-expressed recombinant STIM1 opens wildtype ORAI1 channels, but not channels assembled from the ORAI1 pore mutant E106Q or the ORAI1 immunodeficiency mutant R91W. These experiments demonstrate that the STIM1-ORAI1 interaction is sufficient to gate recombinant human ORAI1 channels in the absence of other proteins of the human ORAI1 channel complex, and set the stage for further biochemical and biophysical dissection of ORAI1 channel gating. (*Y.Z. and P.M. contributed equally to this work.)



515-Pos

Proteomics Analysis of the Drosophila CRAC Channel Complex in the Resting and Active State

Aubin Penna[1], Robyn Kaake[1], Olga Safrina[1], Luette Forrest[1], Andriy V. Yeromin[1], Peter Kaiser[2], Lan Huang[1], Michael D. Cahalan[1].
[1]Department of Physiology & Biophysics, UCI, Irvine, CA, USA, [2]Department Biological Chemistry, UCI, Irvine, CA, USA.

Recent genome-wide RNAi screens have revealed Stim and Orai as critical components of the Ca^{2+} release-activated Ca^{2+} (CRAC) channel. Upon release of Ca^{2+} from the ER, Stim senses Ca^{2+}depletion, aggregates, relocalizes to ER-plasma membrane (PM) junctions, and interacts with Orai pore-forming subunits in the PM to open the CRAC channel. This signaling cascade is spatially confined, regulated by specific protein-protein interactions between Stim and Orai, and may require additional binding proteins such as regulatory subunits, trafficking proteins, or kinases. We developed an extensive and sensitive proteomics approach to screen for binding partners of Stim and Orai in resting and store-depleted conditions. Histidine-Biotine (HB)-tagged *Drosophila* Stim or Orai proteins were stably expressed in *Drosophila* S2 cells; the HB tag module consisting of a hexahistidine tag (H), a bacterially-derived *in vivo* biotinylation signal peptide (B), and a TEV protease cleavage site (T). HBTH-Orai and Stim-HTBH complexes were purified from resting or Ca^{2+} store-depleted S2 cells lines following two complementary approaches: native purification by high-affinity streptavidin binding and TEV cleavage elution; or, alternatively, *in vivo* chemical cross-linking to freeze both stable and transient interactions in intact cells prior to lysis, followed by tandem-affinity purification (TAP) of the cross-linked protein complexes under fully denaturing conditions. After endoproteolytic digestion and two-dimensional LC, the Stim/Orai interacting proteins were identified by tandem mass spectrometry (MS). By these methods, proteins involved in scaffolding, cytoskleton dynamics, trafficking, chaperone function, and signaling were identified. In addition to the subunit composition and interacting partners, we also characterized Stim/Orai posttranslational modifications. This work represents the first comprehensive characterization of CRAC channel complex by affinity purification and tandem mass spectrometry and will provide a detailed proteomic profiling of the dynamic protein interaction network in the CRAC channel pathway.

516-Pos

Phosphorylation of STIM1 Underlies Suppression of Store-Operated Calcium Entry During Mitosis

Jeremy T. Smyth, John G. Petranka, Rebecca R. Boyles, Wayne I. DeHaven, Miwako Fukushima, Katina L. Johnson, Jason G. Williams, James W. Putney.
National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA.

When endoplasmic reticulum (ER) Ca^{2+} stores are depleted, Ca^{2+} influx via plasma membrane (PM) Ca^{2+} channels is activated by store-operated Ca^{2+} entry (SOCE). SOCE involves Orai1 Ca^{2+} influx channels and STIM1 ER Ca^{2+} sensors. ER Ca^{2+} depletion induces rearrangement of STIM1 from a diffuse localization throughout the ER membrane into punctate structures near the PM, where it activates Orai1 channels. Interestingly, SOCE is strongly suppressed during mitosis, the only known physiological situation in which SOCE is negatively regulated; however, the mechanisms that underlie SOCE suppression during mitosis are unknown. We found that both endogenous STIM1 and expressed eYFP-tagged STIM1 (eYFP-STIM1) immunoprecipitated from mitotic but not interphase HeLa and HEK293 cells were recognized by the phosphospecific MPM-2 antibody, suggesting mitosis-specific phosphorylation of STIM1. We also found that rearrangement of eYFP-STIM1 into near-PM puncta in response to ER Ca^{2+} depletion was suppressed during mitosis. We therefore hypothesized that STIM1 phosphorylation underlies prevention of STIM1 puncta formation and suppression of SOCE during mitosis. MPM-2 recognizes phospho-serine or threonine followed by proline, and human STIM1 contains 10 occurrences of S/T-P, all downstream of amino acid 482. eYFP-STIM1 truncated at amino acid 482 (482STOP) was not recognized