3683-Pos

Voltage Gated Trapping of fcAMP in HCN2 Channels

Jana Kusch[1], Susanne Thon[1], Christoph Biskup[1], Eckhard Schulz[2], Thomas Zimmer[1], Klaus Benndorf[1].
[1]Friedrich-Schiller-University, Jena, Germany, [2]Fachhochschule Schmalkalden, Schmalkalden, Germany.

HCN channels are nonselective tetrameric cation channels that are activated by hyperpolarizing voltages and modulated by the ligand cAMP. They generate spontaneous rhythmic activity in the heart and brain. Ligand binding to the intracellular cyclic nucleotide-binding site accelerates the activation kinetics, shifts the steady-state activation to more positive voltages and increases the open probability. We expressed homotetrameric HCN2 channels in *Xenopus* oocytes. Using fast solution exchange by a piezoelectric system we observed that removal of cAMP from the patch induces significant current deactivation if the channels are weakly activated by voltage but only minor deactivation if the channels are strongly activated by voltage. To visualize ligand unbinding directly, simultaneous measurement of activation and ligand binding was performed in excised inside-out macropatches by means of patch-clamp fluorometry with confocal resolution, using a fluorescent cAMP derivate (fcAMP) that activates the channels closely similar to cAMP. As a result, fast removal of saturating fcAMP (7.5 μM) from maximally activated HCN2 channels (−130 mV) caused a fluorescence decay with a fast and a slow component. Assuming four bound ligands per channel at a saturating ligand concentration and at saturating hyperpolarization, the amplitudes of the two exponentials of ~50% each suggest that two of the for bound ligands unbind fast, whereas the remaining two are trapped. This trapping can be shown to depend on channel activation. Comparing the time courses of deactivation and unbinding following a fast removal of the ligand, the results make it likely that only two of the four binding sites have to be occupied to cause maximal ligand induced modulation, resembling the situation in related CNGA2 channels, where two of the four ligands are sufficient to open the channel maximally.

Ion Channels, Other II

3684-Pos

Descent of the Prion Protein Gene Family from the Extracellular Domain of an Ancestral Zip Metal Ion Transporter

Holger Wille[1], Sepehr Ehsani[2], Joel C. Watts[1], David Westaway[3], Gerold Schmitt-Ulms[2].
[1]University of California, San Francisco, San Francisco, CA, USA, [2]University of Toronto, Toronto, ON, Canada, [3]University of Alberta, Edmonton, AB, Canada.

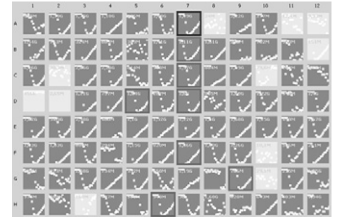
Since its discovery over twenty years ago, both the phylogenetic origin and the cellular function of the prion protein (PrP) have remained enigmatic. The subsequent discovery of the PrP paralog Doppel (Dpl) has strengthened the argument that the copper-binding ability of PrP and Dpl may be part of their cellular function.
Chemical crosslinking provides insights into a possible function of PrP through the characterization of its molecular neighborhood on the cell surface. Quantitative interactome data demonstrated the spatial proximity of two putative zinc ion transporters of the ZIP family, ZIP6 (Slc39a6) and ZIP10 (Slc39a10), to mammalian prion proteins in vivo. A subsequent bioinformatic analysis revealed the unexpected presence of a PrP-like amino acid sequence within the N-terminal, extracellular domain of a distinct sub-branch of the ZIP protein family, which includes ZIP5, ZIP6 and ZIP10. Structural threading and orthologous sequence alignment analyses argue that the prion protein gene family is phylogenetically derived from a ZIP-like ancestral molecule. The level of sequence homology and the presence of prion protein genes in most chordate species place the split from the ZIP-like ancestor gene at the base of the chordate lineage. This relationship explains structural and functional features found within mammalian prion proteins as elements of an ancient involvement in the transmembrane transport of divalent cations, presumably zinc and/or copper. The phylogenetic and spatial connection to ZIP proteins is expected to open new and thus unexplored avenues of research to elucidate the biology of the prion protein in health and disease.

3685-Pos

96 Parallel Gigaseal Patch Clamp Recordings

Andrea Bruggemann, Cecilia Farre, Timo Stengel, Claudia Haarmann, Christian Patzig, Christian Grad, Corinna Voll, Bertholt Heintzel, Stephanie Simmel, Juergen Steindl, Niels Fertig, Michael George.
Nanion Technologies, Munich, Germany.

Over the last several years ion channels have received more and more interest as potential drug targets, because of their known involvement in chronic and acute disease. Patch clamp is the gold standard for obtaining highly relevant information about ion channels and their effectors but it is a notoriously laborious technique. To meet the ever increasing demand for higher throughput in ion channel screening and safety testing we have developed a highly parallel patch clamp platform, the SyncroPatch 96. The platform supports giga-seal recordings, continuous recording during compound application and addition of multiple compounds at each of the 96 cells recorded from at a time.
The figure shows 96 individually recorded IV's of the sodium channel hNav1.5 expressed in HEK 293 cells.



Here we present high quality data of voltage gated and ligand gated channels expressed in various cell lines. Whole cell access was achieved using either pore forming agents such as Nystatin, or by short applications of suction pulses.
The SyncroPatch 96 offers high throughput without compromising data quality.

3686-Pos

Acid Extrusion from Human Spermatozoa is Mediated by Flagellar Hv1 Proton Channel

Polina V. Lishko, Andriy Fedorenko, Inna L. Botchkina, Yuriy Kirichok.
UCSF, San Francisco, CA, USA.

Human spermatozoa are quiescent in the male reproductive system and must undergo activation once introduced into the female reproductive tract. After ejaculation, and during their transit through the female reproductive tract, sperm motility is initiated and then hyperactivated to allow the spermatozoa to penetrate through the viscous oviductal mucus and the egg's protective vestments. These processes are known to require alkalinization of sperm cytoplasm, but the mechanism responsible for transmembrane proton extrusion has remained unknown due to the inability to measure membrane conductances in human sperm. Here, by successfully patch clamping human spermatozoa, we show that proton channel Hv1 is their dominant proton conductance. Hv1 is confined to the principal piece of the sperm flagellum, where it is expressed at an unusually high density. Robust flagellar Hv1-dependent proton conductance is activated by membrane depolarization, an alkaline extracellular environment, and removal of extracellular zinc, a potent Hv1 blocker.
Surprisingly, endocannabinoid anandamide was also found to modulate Hv1 activity in the sperm cells as well as in the heterologously expression system. Hv1 allows only outward transport of protons and is therefore dedicated to inducing intracellular alkalinization and activating spermatozoa. In contrast to the large Hv1 current in human sperm cells, the amplitude of the outward current recorded under similar conditions from mouse spermatozoa was 30 times smaller; therefore, mouse sperms seem to have a different mechanism for acid extrusion.
In human sperm, Hv1-induced intracellular alkalinisation should activate pH-dependent Ca2+ channel CatSper and control intracellular Ca2+ concentration. Finally, since Hv1 lies upstream in the signalling cascades leading to sperm activation, hyperactivation, and capacitation, Hv1 is an attractive target for the control of male fertility.

3687-Pos

Ion Channel Reconstitution on a Pore-Suspending Membrane on Microstructured Glass Chip: Towards Artificial Gap Junction Formation Between Bilayers and Cells

Mohamed Kreir[1], Christoph Methfessel[2], Michael Habig[2], Mathias Winterhalter[3], Niels Fertig[1].
[1]Nanion Technologies GmbH, Munich, Germany, [2]Bayer Technology Services, Leverkusen, Germany, [3]Jacobs University Bremen, Bremen, Germany.

Chip based automated patch clamping is an attractive biophysical tool for studying ion channel proteins. Solvent-free planar lipid bilayers can be formed in an automated fashion by positioning and subsequent bursting of giant unilamellar lipid vesicles containing membrane proteins on micron-sized apertures in a borosilicate glass substrate. The use of proteoliposomes for bilayer formation on such chips allows for the direct recording of single channel activity without need for commonly difficult reconstitution of membrane protein after bilayer formation. This approach is specifically attractive for investigations of membrane proteins not accessible to patch clamp analysis, like e.g. proteins from organelles or proteins from bacteria.
Here, the biophysical and pharmacological characterization of different membrane proteins was performed. A wide variety of ion channels have been studied with this technique, for example potassium channels (KcsA, Kv1.2), sodium channels (NachBac) as well as other ligand-dependent (IP3 receptor) or mechanosensitive channels (MscL, TRP channels). Also, screening for influx