allows us to monitor channel formation of alamethicin peptides and a-HL. From the microwave tranmission signature we can correlate the peptides' insertion and reveal radiation rectification. The results offer a new tool for high-frequency spectroscopy on single channels.

2232-Pos Ion Exclusion by Sub-2 nm Carbon Nanotubes: a Simplified Model for Ion Channels

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Board B347

Narrow and relatively hydrophobic pore regions are often encountered in biological channels, including aquaporins, proton, and ion channels. Moreover, charged residues are frequently found in the selectivity filter of membrane ion channels. Understanding the mechanism of ion transport and ion selectivilty in biological pores is very difficult due to their inherent complexity.

Carbon nanotubes are considered simplified models of membrane channels because of their hydrophobicity, narrow diameter, and fast flow of water, comparable to membrane aquaporins. Thus, to avoid the complexity of biochannels while retaining their basic features, we investigate ion transport through carbon nanotube model pores. The nanofluidic platform used in this study consists of membranes made of aligned double-walled carbon nanotubes with sub-2 nm diameter. Negatively charged groups are introduced at the opening of the carbon nanotubes by oxygen plasma treatment. Pressure-driven filtration experiments coupled with capillary electrophoresis analysis of permeate and feed are used to understand the mechanism of ion transport for both large and small ions. Ion exclusion and selectivity is investigated as a function of solution ionic strength, pH, and ion valence. Observed trends show similarities with biological ion channels.

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Ion Channels, Other

2233-Pos Identification of the Pore-Lining Residues of the BM2 Ion Channel of Influenza B Virus

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Board B348

The influenza virus BM2 proton-selective ion channel is essential for virus replication. The BM2 channel conducts proton into the interior of the virus particle, which results in viral matrix protein-RNPs dissociation. The BM2 protein is similar to the A/M2 protein of influenza A virus in that it is a single span proton channel and it contains a $H_{19}XXXW_{23}$ motif. Unlike the A/M2 protein, the BM2 protein is not inhibited by the antiviral drug amantadine. The identification of the pore-lining residues of the BM2 proton channel is essential to understand the mechanism of ion transport and to reveal the drug action target. In this study we used cysteine scanning mutagenesis in combination with the substituted cysteine accessibility measurement to ascertain the pore-lining residues of this ion channel. The specific activity (relative to wild-type), reversal voltage, and susceptibility to modification by MTSEA and NEM of the mutant proteins were measured in oocytes. We found that substitutions of cysteine at positions of Ser9, Ser12, Phe13, Ser16, His19, and Trp23 ion channels were most disruptive for ion channel function and/or most susceptible to MTSEA and NEM modification. Based on experimental data, a first BM2 transmembrane domain model is proposed. The presence of polar residues in the pore is a probable explanation for the amatadine-insensitivity of the BM2 protein and suggests that related, but more polar, compounds might serve as useful inhibitors of the protein.

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2234-Pos Channel Properties Of The Translocator Hmw1b Of The Two Partner Secretion System Of *Hemophilus Influenzae*

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Board B349

H. influenzae is a pathogenic Gram-negative bacterium that colonizes the nasopharynx. A major virulence factor is the adhesin HMW1 which provides attachment of the bacterium to the host epithelium. The secretion of HMW1 requires the outer membrane translocator HMW1B. HMW1B belongs to the Omp85-Tps superfamily, which also includes proteins involved in the membrane biogenesis of mitochondria and chloroplasts. It is believed that HMW1B contains an N-terminal surface-exposed domain, followed by a large periplasmic domain possibly involved in HMW1 adhesin recognition and a C-terminal beta-barrel domain which may act as a pore for the translocation of the adhesin. Here, the whole HMW1B and the C-terminal domain CTD (HMW1B 234-545) were purified, and the pore-forming nature of the proteins was assessed by electrophysiology using planar lipid bilayers. CTD was a somewhat more unstable protein and required prior reconstitution into liposomes before insertion into planar membranes. Both proteins show similar channel activity. The proteins exhibit opening transitions to two distinct levels of conductance of 343 and 1417 pS in 1 M KCl. Transitions of small conductance tend to be frequent and extremely flickery. The large conductance level is accessed less frequently and

displays a high level of open channel noise. The selectivity of the channel derived from the large conductance indicates a cation-selective channel (PK/PCl = 11.2). When measured at pH 5.2, the conductance increases by 1.5 fold and the selectivity decreases to a PK/PCl of 4.7. These results suggest the involvement of acidic residues in the pore. Although HMW1B purifies as a tetramer, it remains uncertain at this time whether the two conductance levels represent conductance states within a single monomer, or the activity of individual monomers within the larger oligomeric unit.

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2235-Pos Determination of the Active Oligomeric State of Influenza B Virus BM2 Channel

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Board B350

Influenza A virus and Influenza B virus both contain the M2 integral membrane protein (A/M2 and BM2 respectively) that functions as a pH-sensitive proton channel and is essential for virus replication. Although A/M2 and BM2 proteins have similar structural and functional properties, the only homology between their sequences is found in the membrane-spinning region, which is critical for the ion-channel activity. The mechanism of action of M2 channels became a subject of scientific and medical interest, since A/M2 channel was shown to be a target for the action of the antiviral drug amantadine. Unfortunately, there are no known inhibitors of the BM2 channel activity. Thus, knowledge of the structural and functional properties of BM2 channels is essential for the development of potent antiviral drugs and vaccines. The characterization of the oligomeric state of BM2 channels is an essential first step in the understanding of channel function. In the current work we determined the stoichiometry of BM2 proton channel by utilizing three different approaches. (1) We demonstrated that BM2 monomers can be chemically cross-linked to form dimers, trimers and tetramers. (2) We studied electrophysiological and biochemical properties of mixed oligomers consisting of wild type and mutated BM2 subunits, and related these data to predicted binomial distribution models. (3) We used FRET in combination with biochemical measurements to estimate the relations between BM2 channel subunits expressed on plasma membrane. Our experimental data are consistent with the tetrameric structure of BM2 channel. Finally, we demonstrated that BM2 trans-membrane domain is responsible for the channel oligomerization. Determination of the BM2 channel oligomeric state provides an essential platform for further dissection of BM2 channel properties and antiviral drug design.

2236-Pos Post-natal Developmental Changes In Ion Channels In The Sinoatrial Node

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Board B351

There are important post-natal changes in the sinoatrial node (SAN): as compared to the neonate, in the adult, the intrinsic heart rate is slower and the action potential is longer. These changes may be due to differences in ion channel expression between the neonate and adult. We have investigated post-natal developmental changes in the SAN using neonatal (2–7 days of age; n=26) and adult male (~6 months of age; n=15) New Zealand white rabbits using extracellular potential recording and quantitative PCR (qPCR). Our results showed that the intrinsic heart rate was significantly slower in the adult. mRNA for HCN4 (the major isoform responsible for the pacemaker current, I_f) in the SAN declined significantly to 31 % during development and this may contribute to the slowing of pacemaking. Paradoxically, the slowing of pacemaker activity by 2 mM Cs^+ (blocker of I_f) was significantly greater ($P<0.001$) in the adult, but this may be an indirect consequence of the longer cycle length in the adult (expected to result in greater activation of HCN4). qPCR revealed a significant decline in the SAN during development of other transcripts: $Na_V1.5$ (responsible for I_{Na}) to 28 %, $Ca_V1.3$ (in part responsible for $I_{Ca,L}$) to 42 % and NCX1 (responsible for inward I_{NaCa}) to 39 %. These declines could also contribute to the slowing of pacemaking during development. There was a significant decline during development in mRNA for delayed rectifier K^+ channel subunits ($K_V1.5$ responsible for $I_{K,ur}$, K_VLQT1 and minK responsible for $I_{K,s}$, and ERG responsible for $I_{K,r}$) to 63 % and this could explain the prolongation of the action potential. In conclusion, there are significant post-natal developmental changes in ion channel expression in the SAN that can explain the changes in heart rate and action potential duration.

2237-Pos Modulation Of Cell-to-cell Dye Transfer By Transjunctional Voltage In Heterotypic Gap Junction Channels Exhibiting Gating Asymmetry

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Board B352

We demonstrate that transjunctional voltage (V_j) can modulate cell-to-cell metabolic communication in heterotypic gap junction (GJ) channels exhibiting strong voltage gating asymmetry. Studies were performed in heterologous cell pairs of HeLa cells expressing Cx43 and Cx45 that form Cx43/Cx45 heterotypic gap junction channels. We demonstrate that permeability of Cx43/Cx45 channels for Alexa fluor-350 and LY can be strongly modulated by relatively small changes in the difference of the resting potentials of communicating cells, ΔV_{jh}. Day diffusion declines or accelerates substantially by making cell-expressing Cx45 relatively more negative or positive, respectively, to the one expressing Cx43. Our data show that similar changes of ΔV_{jh} modulate metabolic communication more efficiently than electrical cell-cell coupling. Voltage steps (~+100 mV in amplitude and 5–50 ms in duration) repeated with high frequency reduced metabolic communication to zero when arose in the cell expressing Cx43 and enhanced it when arose in the cell expressing Cx45. We demonstrate that this type of regulation of metabolic

communication is potential to occur in other types of heterotypic gap junction channels that contain on one side Cx45 hemichannel and may arise between cells of the conduction system of the heart, between endothelial and smooth muscle cells in the vessels, and between neurons and astrocytes in the nervous system.

2238-Pos Aqp4 Expression In Striatal Glial Cultures Is Regulated By Dopamine: Implications On Proliferation Of Astrocytes

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Board B353

Astrocytes are found ubiquitously in the mature central nervous system (CNS) and play an important role in the regulation of brain function. Proliferation of glial cells takes place during ontogeny, where proliferation and differentiation of radial glia cells into neurons and astrocytes represent important mechanisms of neuronal genesis and differentiation. In the mature CNS, proliferation of astrocytes occurs following different stimuli such as trauma, inflammation and neurodegenerative diseases. Even though a series of studies on the regulation of *in vivo* and *in vitro* proliferation of astrocytes has been performed, the cellular mechanisms underlying proliferation remain poorly understood.

Proliferation involves -among other things- a change in cell volume, which normally comes along with water movement across the membrane. Aquaporins constitute the principal pathway of water through biological membranes, and in astrocytes AQP4 is the main aquaporin subtype expressed. Therefore we hypothesized, that AQP4 might play a role in the regulation of proliferation of astrocytes.

In this study, we investigated the effect of dopamine on the proliferation of striatal astrocytes *in vitro* and the expression of AQP4 and its possible involvement in proliferation.

Dopamine reduced proliferation in a cell density-dependent fashion. This effect was presumably mediated by AQP4 since we observed a dopamine-induced down-regulation of the AQP4-M23 isoform. The M1 isoform expression was not affected. The increase in proliferation by bFGF-treatment was abolished in cultures either by blocking AQP4 with 10 μM TEA or where AQP4 was knocked down by siRNA treatment. Our results indicate for the first time a role for AQP4 in regulation of proliferation and might initiate the development of therapeutical tools that modulate AQP expression and function for the treatment of diseases correlated with excessive proliferation of glial cells.

2239-Pos Biochemical and biophysical characterization of mitsugumin 23 Hiroshi Takeshima

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Board B354

Mitsugumin 23 (MG23) is a protein on the endo/sarcoplasmic reticulum (SR) and nuclear membranes and is abundantly expressed in muscle (FEBS Lett. *432*, 191, 1998). MG23 has predicted transmembrane segments, but its functional role is totally unknown. We therefore conducted biochemical and biophysical experiments on MG23. When muscle SR vesicles were subjected to proteinase treatment, the mature MG23 with an apparent molecular mass of ∼20 kDa generated a cleaved form of ∼16 kDa. The cleaved MG23 was recognized by an antibody to the N-terminal region, but not by an antibody to the C-terminal region, indicating that the N- and C-termini reside on the luminal and cytoplasmic sides, respectively. Further investigations using limited proteolysis suggest that MG23 has three membrane-spanning segments. Although affinity-purified MG23 using specific antibodies was detected as a single protein band on SDS-PAGE, chemical cross-linkers reproducibly generated high molecular weight proteins corresponding to homo-dimeric to hexameric products. MG23 purified from rabbit skeletal muscle could be reconstituted into artificial membranes under voltage-clamp conditions. Single-channel current fluctuations were observed and analysis of the data demonstrates that MG23 forms an ion channel that is permeable to K^+ but not to Cl^-. Single-channel conductance was ∼430 pS in symmetrical 260 mM K^+. Similar results were obtained with a purified N-terminal His-tagged MG23 protein expressed in yeast. Our results suggest that MG23 forms a homo-oligomeric structure and functions as a novel cation channel in intracellular membrane systems.

2240-Pos Fluorescence Biosensor yields pH And Chloride Concentration for in vivo Systems

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Board B355

Chloride ion is the most abundant physiological anion. It participates in many physiological functions including: control of mem-

brane excitability and stabilization of neuronal resting potential; charge balance during endosome acidification; fluid secretion, and regulation of cell volume. As a consequence dysfunctions in regulating membrane chloride permeability originate various diseases. For instance cystic fibrosis arises from a genetic alteration of a transmembrane conductance regulatory channel (CFTR).

At present processes regulating intracellular chloride ion concentrations are still widely unexplored mainly as a consequence of limiting methods to quantify chloride fluxes in living cells.

In the present work a novel genetically-encoded sensor with double ratiometric readout is presented. The sensor was obtained by fusion of a red fluorescent protein (DsRed-monomer), insensitive to chloride and pH, to a GFP variant containing a specific chloride-binding site (GFP-Chl) [1]. The latter mutant is obtained from EGFP by the single-point mutation T203Y. GFP-Chl binds the chloride ion following a fluorescence static quenching mechanism that allows to measure the intracellular pH in a chloride independent manner. Chloride concentration is subsequently determined from the Grynkiewicz equation using the appropriate pH-dependent chloride affinity.

The sensor was successfully tested in vivo with two different cell lines cultured in a pH range 5.5–8.5 with chloride concentration up to 200 mM. Applicability to high-throughput screening, range of validity and accuracy of time-lapse maps will be discussed.

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2241-Pos

WITHDRAWN

2242-Pos Automated Internal Solution Exchange On A Planar Patch Clamp Surface

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Board B357

Ion channels have become important targets in academic and pharmaceutical research alike. Understanding how ion channels function is critical in determining underlying disease mechanisms and drug interactions. Patch clamp electrophysiology has been used for decades as a tool for understanding ion channel function. Planar patch clamp platforms have also gained a place in recent years in ion channel research for those requiring higher data throughput. Nanion's planar patch clamp devices, the Port-a-Patch and Patchliner, provide higher throughput whilst maintaining high quality, typically obtaining Giga-seals with a 60–80% success rate.

In addition to providing higher throughput, the planar patch clamp chip also gives unprecedented access to the internal surface of the membrane. Nanion has exploited this feature by designing a device which can exchange the internal solution of the cell in an automated fashion. The device for internal perfusion described in this study is suitable for the rapid administration of up to 4 different solutions directly to the internal side of the membrane. This greatly expands the experimental possibilities open to the ion channel researcher. For example, studies of second messenger systems by exchanging the internal solution become possible. In addition, compounds can be applied internally to examine differences in potency or mechanism when applied to the internal vs. the external surface of the cell.

In this study data will be described for exchanging the internal solution when recording from cells expressing K+ currents. For example, the effect of blockers such as ions or small molecules on the Kv1.3 channel when applied internally will be shown. The data described here are just the beginning of the ever-expanding possibilities of planar patch clamp.

Myosin & Myosin-family Proteins - I

2243-Pos Testing Cargo-induced Dimerization of Myosin VI

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Board B358

The propensity of the unconventional Myosin VI (M6) to form processive dimers through coiled-coil interactions in its tail domain is thought to be initiated by adapter proteins that bind to the globular tail domains of two monomeric M6 molecules. In Drosophila, embryonic neuroblasts target cell fate determinants basally, rotate their spindles to align them apically, and divide asymmetrically. The spindle orientation, and the localization of cell fate determinants and their adaptor proteins such as Miranda are all specified by the same apical complex. As Miranda localization requires M6, our studies test whether the presence of Miranda in vitro could convert an otherwise nonprocessive, single-headed M6 to a processive, two-headed complex.

2244-Pos Probing the Reverse Directionality of Myosin VI

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