autoimmune diseases, effector memory T cells have a unique ion channel pattern, therefore they are promising therapeutic targets. A number of ion channel inhibitors are known as selective inhibitors of T lymphocyte proliferation, but the data available is contradictory. Our aim was to elucidate this phenomenon by investigating how the blockage of ion channels affects the activation and proliferation of T cells treated previously with different concentrations of mitogens.
In our experiments human peripheral blood lymphocytes from volunteers were activated via monoclonal antibodies affecting the TCR-CD3 complex on the cell surface and the co-stimulator molecule CD28. We applied specific ion channel blockers acting on the major cationic channels of the T cell, the Kv1.3, the KCa1.1 and the CRAC channel, either alone or in combination with rapamycin, the inhibitor of the mammalian target of rapamycin (mTOR). Five days after the stimulus flow cytometry measurements were performed to determine the extent of cellular viability and proliferation.
Our measurements indicated that ion channel blockers and rapamycin had a negative dose-dependent effect on the amount of cell division. Simultaneous application of blockers for each channel along with rapamycin proved to be the most effective, which indicates that they affect independent regulation pathways. Upon increasing the rate of stimulation, the anti-proliferative effect of the blockers diminished. This phenomenon was unknown to date and may prove to be important in understanding the fine-tuning of T cell activation.

2963-Pos Board B393
Proton Channels are Present in Cell Membranes of the Breast Cancer Cell Line MDA MB 231 and Affect Recovery from an Acid Load
Deri Morgan[1], Patrick McIntire[1], Vladimir Cherny[1], Susan Smith[2], Boris Musset[3], Thomas DeCoursey[1].
[1]Molecular Biophysics, Rush University Medical Center, Chicago, IL, USA, [2]Department of Biology & Physics, Kenshaw State University, Kennesaw, GA, USA, [3]Institute of Complex Systems, Forschungszentrum Jülich, Jülich, Germany.
Voltage gated proton channels (HV1) have been implicated in late stage breast cancer (Wang et al, 2012. J. Biol. Chem. 287:13877), where HV1 expression correlated with tumor size and poor prognosis. HV1 knockdown reduced cell proliferation and migration as well as matrix metalloprotease release. However, the presence of functional HV1 on cancer cell membranes has not been demonstrated and the mechanism by which they affect the function of these cells has not been elucidated.
Here we show the definitive presence of functional HV1 on the membranes of MDA MB 231 cells, a highly metastatic triple negative cell line. We performed patch clamp experiments on these cells and were able to detect bona fide voltage- and pH-gated channels that were perfectly selective for protons. The membrane density of the channels in these cells was recorded as 3.5 pA/pF which is roughly 5-fold lower than the 15 pA/pF displayed in neutrophils. In order to show that HV1 expresses at a level sufficient to impact pH regulation within these cells, we acid loaded them using the ammonium prepulse technique and monitored pHi recovery utilizing SEER with SNARF-1. Inhibiting HV1 with 1 mM Zn2+ slowed recovery from an acid load by 3-fold, demonstrating that the expression of HV1 on these cells affects pH regulation in these cells.
We conclude that one mechanism by which HV1 may influence the pathophysiology of breast cancer is by improving the ability of breast cancer cells to regulate their internal pH.
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2964-Pos Board B394
TOK1 Potassium Channels in Phytopathogenic Fungi
William R. Manville[1], Andrew Corran[2], Anthony Lewis[1].
[1]School of Pharmacy and Biomedical Sciences, University of Portsmouth, Portsmouth, United Kingdom, [2]Fungicide Biochemistry, Syngenta Limited, Bracknell, United Kingdom.
Fungal plant pathogens are a significant threat to crop yield and global food security and the search for pathogen-specific agricultural fungicide targets is of high priority. TOK1 is a structurally and functionally unique plasma membrane potassium (K^+) channel with no known homologues in plants or animals and is the only passive K^+ ion efflux pathway in fungi. Activation of TOK1 leads to ion dyshomeostasis and cell death. However, little is known about TOK1 channels in phytopathogenic fungi. Here we describe the distribution, evolution and molecular conservation of TOK1 homologues across plant fungal phyla, and the cloning and characterization of TOK1 channels from two phytopathogens of significant socio-economic importance. *In-silico* bioinformatics identified genes predicted to encode putative TOK1 protein subunits, conforming to the characteristic eight transmembrane domain two pore domain (8TM/2P) structure, in 204/231 sequenced fungal genomes analysed. Molecular conservation of TOK1 primary structure was greatest in both pore domains and flanking pore lining transmembrane domains, TM6 and TM8. MgTOK1 from *Mycosphaerella graminicola* (wheat leaf blotch) and FgTOK1 from *Fusarium graminearum* (wheat head blight) were cloned by RT-PCR into an expression vector. cRNA was transcribed *in-vitro* and injected into *Xenopus laevis* oocytes and ionic currents measured by two-electrode voltage clamp after 24-48 hours. Both cloned TOK1 channels exhibited K^+ selective, non-inactivating, strongly outwardly-rectifying K^+ currents whose activation threshold was strictly determined by the transmembrane K^+ gradient, as revealed by isotonic replacement of extracellular Na^+ with K^+. Channels varied in their voltage-dependent activation kinetics and distinct from the canonical TOK1 isolate from *Saccharomyces cerevisae*, displayed signs of time-dependent deactivation. This appears to be the first reported molecular identification and characterisation of TOK1 K^+ channels from plant pathogenic fungi.

2965-Pos Board B395
KCa1.1 (BK) Channels on Fibroblast-Like Synoviocytes: A Novel Therapeutic Target for Rheumatoid Arthritis
Mark R. Tanner[1], **Zoltan Petho**[2], Rajeev B. Tajhya[1], Redwan Huq[1], Frank T. Horrigan[1], Percio S. Gulco[3], Christine Beeton[1].
[1]Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX, USA, [2]Biophysics and Cell Biology, University of Debrecen, Debrecen, Hungary, [3]Division of Rheumatology, Icahn School of Medicine at Mount Sinai, New York, NY, USA.
Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease attacking principally freely-moveable joints and affecting approximately 1.3 million in the US. Recently, joint-resident fibroblast-like synoviocytes in RA (RA-FLS) have been implicated in disease pathogenesis. We have shown that KCa1.1 is the predominant potassium channel expressed by RA-FLS and by FLS from the pristane-induced arthritis (PIA) rat model of RA. Blocking KCa1.1 with paxilline or iberiotoxin or reducing its expression with siRNA inhibited the production of pro-inflammatory cytokines, chemokines, and proteases, and the invasiveness of both RA- and PIA-FLS. In contrast, the overexpression of KCa1.1 increased the invasiveness of PIA-FLS and induced the invasiveness of healthy rat FLS. These data demonstrate a crucial role of KCa1.1 in regulating the aggressive behavior of FLS during RA.
We induced two models of RA in rats, moderate PIA and severe complete Freund's adjuvant collagen-induced arthritis. Treatment with the small molecule KCa1.1 blocker paxilline, starting after onset of clinical signs, significantly reduced disease severity in both models. However, paxilline can cross the blood-brain barrier and block all variants of KCa1.1 throughout the body, thereby inducing side effects that preclude its use as a therapeutic for human use without significant modification. A strategy to target KCa1.1 on RA-FLS without side effects in other tissues involves the identification of the β and γ regulatory subunits of KCa1.1 in RA-FLS through qPCR, western blotting, and patch-clamp electrophysiology. This has the potential for the development of blockers that selectively target KCa1.1 on RA-FLS and do not enter the central nervous system.

2966-Pos Board B396
Kidney CLC-K Chloride Channels Inhibitors: Definition of Novel Structural Requirements and Efficacy in CLC-K Polymorphism Associated with Hypertension
Paola Imbrici[1], Antonella Liantonio[1], Giuseppe Fracchiolla[1], Giuseppe Carbonara[1], Maria Maddalena Dinardo[1], Michael Pusch[2], Diana Conte[1].
[1]Department of Pharmacy - Drug Sciences, University of Bari, Bari, Italy, [2]Institute of Biophysics, CNR, Genova, Italy.
The human chloride channels CLC-Ka and CLC-Kb play a pivotal role in kidney by controlling chloride and water absorption. Both channels require barttin as an accessory subunit for full activity. Mutations in CLC-Kb and barttin genes lead to severe renal salt loss while CLC-K gain of function polymorphisms could predispose to hypertension. Thus, compounds that selectively bind to CLC-Ka and/or CLC-Kb channels may have a significant therapeutic potential. Recently, we explored the pharmacological profile of CLC-K/barttin expressed in mammalian HEK-293 cells and demonstrated that HEK cells represent a valid biological system to screen CLC-K high affinity blockers (Imbrici et al., Biochim Biophys Acta, 2014). Here, by using molecular modeling and patch-clamp technique, we developed a new series of benzofuran derivatives and evaluated their efficacy on CLC-K channels expressed in HEK 293 cells. Chemical modifications regarding the hydrophobic group at C-5 and C-3 position of the benzofuran nucleus of the lead compounds RT-93 and JBL-44 (IC50 within 10-30 μM range), allowed us to define the structural requirements to ensure an efficacious CLC-Ka block, finally identifying SRA-36 the most potent compound so far described, with an IC50 of 2.6 ± 1 μM. Interestingly,

besides capable of inhibiting CLC-Kb and CLC-K1 isoforms, this compound was also efficacious in blocking A447T CLC-Ka, a polymorphism associated with hypertension. Thus, the SRA-36 molecule could represent a useful probe for exploring CLC-K molecular mechanisms of gating as well as a new potential therapeutic option for hypertensive patients carrying CLC-K gene polymorphisms (Telethon GGP14096).

2967-Pos Board B397

Computational Studies on Bladder Smooth Muscle: Modeling Ion Channels and their Role in Generating Electrical Activity

Chitaranjan Mahapatra, Rohit Manchanda.
Bio science & Bio engineering, Indian Institute of Technology Bombay, IIT Bombay,Powai,Mumbai,Maharastra, India.

Urinary Incontinence (UI) is the involuntary loss of urine that constitutes a social or hygiene problem, which has adverse effects on quality of life. Urinary Bladder Smooth Muscle (UBSM) instability is a major cause of UI. Different ion channels within bladder detrusor smooth muscle (DSM) play a role in generating spontaneous action potentials (sAP) and depolarizations (SD). The aim is establish a mathematical platform of sufficient biophysical detail to quantitatively simulate DSM sAPs and thereby inform future empirical investigations of physiological and pathophysiological mechanisms governing normal and dysfunctional bladder activities. In line with recent experimental evidence, adapting the Hodgkin- Huxley formulation in the NEURON platform, we construct mathematical models for seven ionic currents of UBSM: Ca^{2+} currents (L- and T- type), two voltage gated K^+ currents and three Ca^{2+} - activated K^+ current. The magnitudes and kinetics of each ionic current system in a cylinder - shaped single cell with a specified surface area are described by differential equations, in terms of maximal conductances, electro chemical gradients, voltage-dependent activation/inactivation gating variables and temporal changes in intracellular Ca^{2+} computed from known Ca^{2+} fluxes. These quantifications are validated by the reconstruction of individual experimental ionic currents obtained under voltage clamp. Our integrated model has been validated by comparing the simulated profile of sAPs with experimental recordings and shows good correspondence in terms of amplitude and shape in both control and pharmacologically altered conditions. Stimulation is done using an external current clamp, where somatic current injections were used to generate phasic spike. In summary, our advanced mathematical model provides a powerful tool to investigate the physiological ionic mechanisms underlying the spikes in DSM, which in turn can shed light in genesis of UBSM for a malfunction.

2968-Pos Board B398

Modeling Neurological Disease with Human iPS Cell-Derived Neurons Containing a KCNT1 Mutation

Kile P. Mangan, Michael McLachlan, Tom Burke, Benjamin Meline, Nathan Meyer, Lucas Chase, Brad Swanson, Coby B. Carlson, Susan DeLaura, Eugenia Jones.
Cellular Dynamics International, Madison, WI, USA.

The sodium-activated potassium channel Slack, encoded by the gene KCNT1, is expressed in neurons throughout many brain regions, including the frontal cortex, and mediates a sodium-sensitive potassium current (IKNa). This outward current regulates neuronal excitability and determines how neurons respond to repeated high frequency stimulations, both of which are aspects of memory and learning. Not surprisingly, mutations in KCNT1 and alterations to the IKNa current have patho-physiological consequences. Recent studies have described the emerging role of Slack channels in cognitive deficits, and several reports have found KCNT1 mutations in patients with severe early onset ''childhood'' epilepsies.

The development of better therapies for neurological disorders has been hindered by limited access to clinically-meaningful cell models for research and drug development. The advent of induced pluripotent stem (iPS) cell technology provides a platform to facilitate increased understanding of disease mechanisms in a physiologically-relevant environment. We have leveraged this technology to generate human neurons that express the KCNT1 P924L mutation in the Slack channel. To introduce this alteration, we genetically engineered a ''control'' iPS cell line from an apparently healthy donor with no family history of neurological disorders and generated highly purified (>95% TUJ1-positive), terminally differentiated cortical neurons from the two separate but isogenic iPS cell lines.

Here, we present data on the characterization of these human neurons, including expression of a standard set of neuronal markers at both the genetic and protein level. We also highlight functional testing of the cells, with a specific focus on electrophysiological readouts such as multi-electrode array (MEA) analysis. The ability to model neurological disorders through editing the genome of an iPS cell and subsequently produce previously inaccessible human neurons has revolutionized the way we approach studying and treating diseases of the central nervous system.

2969-Pos Board B399

Novel Mutation of SCN1A in a German Family Presenting with both Hemiplegic Migraine and Epilepsy

Chunxiang Fan, Frank Lehmann-Horn, Karin Jurkat-Rott.
Division of Neurophysiology, University ULM, ULM, Germany.

Familial hemiplegic migraine (FHM) is a rare autosomal dominant migraine subtype with aura associated with reversible hemiparesis. Three causative genes encoding ion-channels / transporters have been identified: CACNA1A (FHM1), ATP1A2 (FHM2) and SCN1A (FHM3). SCN1A is a well-known epilepsy gene with over 150 known mutations, while until now only few FHM3 mutations have been described. Here we report a novel SCN1A L1624P mutation in a three-generation family with four patients who present with both FHM and epilepsy. To explore the underlying mechanism, SCN1A was transiently transfected in human TSA 201 cells together with the auxiliary subunits β1 and β2. Whole-cell patch clamp was employed for the electrophysiological characterization. L1624P produced similar current density and similar voltage dependence of activation as those in wild-type channel. It displayed a positive shift in the steady-state inactivation, a faster recovery from inactivation, an increase of non-inactivated depolarization-induced sodium current near threshold potentials, and a decrease of use-dependent block. Our gain of function findings in L1624P are consistent with consequent neuronal hyperexcitability, and might be involved in the pathomechanism of the phenotype.

2970-Pos Board B400

Possible Role of STIM1 Sensor Signal in Memory Loss Connected with Familial Alzheimer's Disease

Maria Ryazantseva[1], Ksenia Skobeleva[1], Anna Goncharova[2], Nikolai Kamyshev[2], Elena Kaznacheyeva[1].
[1]Institute of Cytology RAS, St. Petersburg, Russian Federation, [2]Pavlov Institute of Physiology RAS, St. Petersburg, Russian Federation.

Familial Alzheimer's disease (FAD) which leads to memory impairments is caused by mutations in presenilin-1 (PS1) gene in approximately 40% of cases. PS1 is well known as a component of the gamma-secretase enzyme which cleaves APP to A-beta. To become a catalytic part of enzyme PS1 undergoes an endoproteolysis. It was shown that mutations in PS1 gene disrupt endoproteolysis increasing uncleaved protein level in brain tissue of FAD patients. In our study we found effects of FAD PS1 mutants (PS1DE9, PS1 D247A) on activity of store-operated calcium (SOC) channels in mice hippocampal neurons and Neuro2a cell line. Increased uncleaved PS1 levels led to SOC channels hyperactivities detected with direct single-cell electrophysiological measurements and calcium imaging experiments with fura2-AM. The effects were caused by impaired signal transduction from ER to SOC channels in plasmatic membrane. The impaired intracellular signal transduction by STIM1 sensor was revealed in live confocal imaging experiments and proved with STIM1 knock-down. Moreover, a feeding of Drosophila melanogaster transgenes expressing human mutated PS1 in cholinergic nervous system with pharmacological inhibitor of STIM sensor signal transduction 2APB led to rescue of the memory loss detected by courtship based assay with aged animals. Therefore hyperactive STIM1 signal transduction leads to increased SOC channels activity which could be the reason for memory loss in FAD.

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