

2958-Pos Board B388**M1 and M2 Microglia Exhibit Significant Differences in their K⁺ Channel Expression**Eva Melanie Grossinger¹, Hai Minh Nguyen¹, Yi-Je Chen¹, Izumi Maezawa², Heike Wulff¹.¹Pharmacology, University of California Davis, Davis, CA, USA,²Department of Pathology and Laboratory Medicine, University of California Davis, Sacramento, CA, USA.

Microglia effector functions are widely associated with specific phenotypes, which are referred to as M1 and M2. Classically activated M1 microglia release pro-inflammatory cytokines and neurotoxic molecules and have been associated with neurological damage in ischemic stroke and Alzheimer's disease. Alternatively activated M2 microglia exhibit beneficial immunological effector functions such as phagocytosis of debris and release of anti-inflammatory and neurotrophic factors. Similar to B- and T-cells, microglia activity is regulated by calcium (Ca²⁺)-signaling, which is maintained by potassium (K⁺) channels. We here investigated whether M1 and M2 microglia differ in their K⁺ channel expression by differentiating neonatal mouse microglia into M1 and M2 phenotypes using lipopolysaccharide (LPS) and/or interferon-gamma or IL-4 and studying the cells by whole-cell patch-clamp. We identified three types of K⁺ channels based on their biophysical and pharmacological fingerprints: a use-dependent, outwardly rectifying current sensitive to the Kv1.3 blockers PAP-1 and ShK-L5, an inwardly rectifying Ba²⁺-sensitive Kir2.1 current, and a Ca²⁺-activated, TRAM-34-sensitive KCa3.1 current. M1 microglia, obtained by stimulation with LPS or a combination of LPS and interferon-gamma exhibited high Kv1.3 current densities (~30-60 pA/pF at 40 mV) and virtually no KCa3.1 and Kir currents, while IL-4 stimulated M2 microglia exhibited large Kir currents (~10 pA/pF at -120 mV). KCa3.1 currents were generally low but moderately increased following stimulation with interferon-gamma or ATP. This differential K⁺ channel expression pattern suggests that K⁺ channel modulators could be used to selectively inhibit detrimental neuroinflammatory functions of microglia.

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2959-Pos Board B389**Atherogenic Very-Low-Density Lipoprotein Shortens Atrial Action Potential Duration by Increasing Potassium Currents and Calcium Transient**
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Background: Compared to that of healthy normal subjects, plasma very low density lipoprotein (VLDL) of patients with the metabolic syndrome (MetS) has been shown to be more electronegative and atherogenic. Given the association between MetS and increased prevalence of atrial fibrillation (AF), we investigated the mechanistic role of VLDL in the AF pathogenesis.

Methods: We extracted VLDL via peripheral blood obtained from normal, healthy volunteers and MetS individuals. The normal-VLDL and MetS-VLDL samples were treated to HL-1 atrial cardiomyocytes respectively for 12 hours before experiments. Whole-cell patch clamp was used for recording the action potentials, voltage-gated potassium currents, and L-type calcium currents. Calcium image with Fura-2-AM Ca²⁺ indicator was applied for the intracellular calcium measurements.

Results: MetS-VLDL treated HL-1 cells exhibited significantly higher densities of repolarizing potassium currents, IKs and IKr. MetS-VLDL shifted the activation curve of ICaL toward more negative membrane potentials. Intracellular calcium signals were significantly enhanced by MetS-VLDL but not by normal-VLDL. MetS-VLDL significantly shortened action potential durations (MetS-VLDL 178.1 ± 32.0 msec vs control 257.2 ± 52.7 msec; P=0.0017). Additionally, frequent occurrences of early after-depolarization on action potentials were noted in MetS-VLDL treated HL-1 cells.

Conclusions: The VLDL of MetS individuals augmented repolarizing potassium currents, increased intracellular calcium release, and significantly shortened action potentials. These changes may contribute in coordination to increased AF vulnerability in MetS.

2960-Pos Board B390**New Insight into the Involvement of Large-Conductance Calcium-Activated-Potassium-Channel(BK) in Cell Viability: Pathophysiological Implications in Neuromuscular Disorders**

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The large conductance Ca²⁺-activated K⁺-channel (BK) is involved in several pathophysiological conditions including periodic paralysis (PP) and myotonia. Acetazolamide (ACTZ) a carbonic anhydrase inhibitor used in these conditions, acts targeting BK in PP. Here we investigated the involvement of BK channel in neuronal viability (SH-SY5Y cell) by combining patch-clamp technique and cell proliferation assays. We performed these measurements in the presence or absence of the selective BK channel blocker Iberitoxin (IbTX) (10-400x10⁻⁹M), the unselective K⁺-channels blocker Tetraethylammonium (TEA) (0.01-1x10⁻³M), and the BK channel openers NS1619 (10-100x10⁻⁵M) and ACTZ (0.1-200x10⁻⁶M). Patch-clamp recordings showed that at +30mV (Vm) IbTX and TEA reduced whole cell K⁺-current in a concentration-dependent manner with an I_{max} of -46% and -90% respectively. NS1619 enhanced K⁺-current of +141% at -10mV (Vm). Acetazolamide, that in muscle acts as a BK opener, in neurons caused a concentration-dependent block of K⁺-current at +30mV(Vm) with an IC₅₀ of 1.73x10⁻⁷M an I_{max} of -40% (slope=-0.37) (Number of patches=12). These drugs exert their effects also on neuronal cell viability, enhancing it: IbTX showed a maximal proliferative effect (MPE) of +46% at 10⁻⁸M concentration, reducing it at higher concentrations; TEA showed a concentration-dependent increase of cell proliferation with a MPE of +34% at a 10⁻⁴M concentration; NS1619 and ACTZ showed a MPE of +181.6% at 5x10⁻⁵M and +135% at 100x10⁻⁶M concentration respectively. Staurosporine (STS) (2x10⁻⁶M), a broad spectrum protein kinases inhibitor, prevented the IbTX and TEA proliferative action. These results suggest that BK channel may play a role in the regulation of neuronal viability through an intracellular pathway that involves STS-sensitive protein kinases. These findings may have relevance in the cellular repair mechanisms in the neuromuscular disorders. Supported by Telethon GG14096.

2961-Pos Board B391**Long-Term Modulation of Ion Channels by Aldosterone in Adult Rat Atrial Myocytes**

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In recent years, both aldosterone and mineralocorticoid receptor (MR) have drawn attention as important factors that promote structural remodeling of the atrium. Here, we investigated effects of chronic aldosterone treatment on both intracellular Ca²⁺ and ion channels. Atrial myocytes were cultured in either the absence or presence of aldosterone, and then the activity of ion channels was studied, under whole-cell patch-clamp conditions. Aldosterone increased both the cell membrane capacitance (Cm) and the maximal conductance of ion channels that give rise to IK_s, I_{Na}, ICaT, and ICaL (30-100%). Except for inactivation curves of I_{Na}, which were shifted by -10 mV, aldosterone produced no major alterations in the biophysical properties of the channels. Interestingly, at resting membrane potentials the increase in I_{Na} was cancelled by a greater fraction of inactivation. The onset and recovery of the changes in ICaT, ICaL, and Cm- were also assessed. In general, they required 2d to be noticeable, reached their maximal value in 6d, and returned to basal values after 1-3d of aldosterone removal. The effects on both Cm and ICaL were further studied -to explore both a potential dose-response relationship and a possible implication of the MR. In fact, co-incubating with 10 μM of spironolactone (an MR antagonist) abolished both effects. Furthermore, their corresponding magnitudes fitted well with the Hill equation, being the EC₅₀ values for Cm and ICaL 20 and 130 (nM), respectively. Interestingly, aldosterone did not alter expression levels of Cav1.2, suggesting that the action on ICaL arises from a stimulus in open probability. The hormone also produced a 40% increase in the amplitude of Ca²⁺ transients along with a higher proportion of arrhythmic cells (2.5-fold increase). These results contribute to understanding the role of the MR and aldosterone in atrial electrophysiology.

2962-Pos Board B392**The Anti-Proliferative Effect of Cation Channel Blockers on T Lymphocytes Stimulated by Anti-CD3 and Anti-CD28**

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The ion channels of T lymphocytes form a crucial part of the healthy immune system, as they are important for cellular activation and proliferation. In

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.

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Proton Channels are Present in Cell Membranes of the Breast Cancer Cell Line MDA MB 231 and Affect Recovery from an Acid Load

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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 pH 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. Supported by the Bears Care and the Brian Piccolo fund.

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TOK1 Potassium Channels in Phytopathogenic Fungi

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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 con-

servation 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 cerevisiae*, 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.

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KCa1.1 (BK) Channels on Fibroblast-Like Synoviocytes: A Novel Therapeutic Target for Rheumatoid Arthritis

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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 ibertoxin 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 over-expression 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.

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Kidney CLC-K Chloride Channels Inhibitors: Definition of Novel Structural Requirements and Efficacy in CLC-K Polymorphism Associated with Hypertension

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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 μ M 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 $2.6 \pm 1 \mu$ M. Interestingly,