

**1692-Pos****TRP<sup>1</sup>Ing on QPatch in Multi-Hole Mode**

**Rasmus B. Jacobsen**, Hervør L. Olsen, Søren Friis, Rikke Schrøder, M.K. Jensen, Morten Sunesen.

Sophion Bioscience A/S, Ballerup, Denmark.

Transient receptor potential (TRP) channels are non-selective cationic channels that are widely distributed in mammalian tissues. Their specific physiological functions are largely unknown. Proposed functions include responses to pain, temperature, touch, osmolarity, pheromones, and taste. But due to the lack of specific blockers and the full understanding of their mechanisms of activation studies of TRP channels have been difficult and unexpectedly slow.

The emergence of automated patch clamp (APC) systems has increased the number of new targets available for ion channel drug development and has augmented throughput.

In order to facilitate tests of large compounds libraries on e.g. TRP targets, we have recently developed two multi-hole APC systems: QPatch HTX and QPatch 16X. The multi-hole technology allows the simultaneous recording of 10 cells in parallel per recording site thereby increasing the signal to noise ratio and the success rate.

In this study, we have validated several TRP channels for their activation by their appropriate agonist e.g. Menthol, Capsaicin and temperature. We have found that using the QPatch in multi-hole mode significantly increased the volume of electrophysiology data that can be generated. Our results demonstrate that the QPatch multi-hole systems are capable of generating high quality data from a wide range of the channels belonging to the TRP family of receptors.

We believe that the combination of high throughput and high quality data in a single system has more than a "transient potential" to advance the understanding of the complex mechanisms of action exhibited by difficult targets such as the TRP channels.

**1693-Pos****TRPV1 Modulates Acetylcholine Release From Motor Nerve Terminals**

**Baskaran Thyagarajan**, Joseph G. Potian, Vishwendra Patel, Carmen C. Garcia, Joseph J. McArdle.

UMDNJ - New Jersey Medical School, Newark, NJ, USA.

Transient receptor potential (TRP) proteins are expressed ubiquitously throughout the body. Previous studies demonstrated expression of TRPV1, the capsaicin receptor, in sensory neurons. Recently, we reported TRPV1 expression in mouse motor nerve endings (MNEs; *J. Pharmacol. Exp. Ther.* Aug 04, 2009) where we observed that capsaicin protected MNEs from botulinum neurotoxin A (BoNT/A). We hypothesized that capsaicin reduced clathrin coated pit (CCP) dependent endocytosis of BoNT/A and demonstrate the regulatory influence of TRPV1 in exo-endocytic processes of MNEs. Phrenic nerve diaphragm muscle preparations isolated from isoflurane anesthetized adult mice were analyzed for the nerve-evoked twitch and transmitter release (TR). Capsaicin produced a concentration-dependent decline of twitch tension (TT), an effect attributed to suppression of stimulus-evoked acetylcholine release (SEAR) since these capsaicin concentrations reduced the amplitude of endplate currents. These effects of capsaicin were antagonized by capsazepine, the TRPV1 antagonist. To understand the mechanism whereby capsaicin reduced TR, we studied cholinergic Neuro 2a cells. Acute exposure to capsaicin altered the subcellular distribution of clathrin heavy chain (Chc) and AP2, two proteins essential to CCP formation. Wortmannin, (non selective PI3K/PI5K inhibitor), inhibited the TT and SEAR of the isolated nerve-muscle preparations and delocalized Chc and AP2 in Neuro 2a cells. Chlorpromazine, an inhibitor of CCP dependent endocytic pathway [*Cell Mol. Biol. Lett.* 2004; 9 (3): 475-81], mimicked the effects of capsaicin on AP2 delocalization. These data suggest that endogenous TRPV1 proteins are coupled to the exo-endocytic mechanisms that regulate neuromuscular transmission and that activation of TRPV1 with high capsaicin concentrations reduces exocytosis of acetylcholine by down regulating the compensatory CCP dependent endocytic pathways.

**1694-Pos****Direct and Indirect Effectors of the TRPM2 Cation Channel**

**Balázs Tóth**, László Csanády.

Semmelweis University, Budapest, Hungary.

TRPM2 is a Ca<sup>2+</sup> permeable cation channel which plays a role in physiological and pathophysiological processes linked to oxidative stress. TRPM2 channels are co-activated by intracellular Ca<sup>2+</sup> and ADP ribose (ADPR). In addition, in intact cells, a large number of compounds appear to modulate TRPM2 activity. Superfusion of TRPM2-expressing cells with hydrogen-peroxide (H<sub>2</sub>O<sub>2</sub>) activates TRPM2 currents, just as intracellular dialysis of cyclic ADPR (cADPR) or nicotinic acid adenine dinucleotide phosphate (NAADP). Importantly, H<sub>2</sub>O<sub>2</sub>, cADPR, and NAADP enhance ADPR-induced TRPM2 whole-

cell currents. Finally, in intact cells AMP acts as a TRPM2 inhibitor. Because in whole-cell recordings the entire cellular machinery involved in nucleotide- and Ca<sup>2+</sup>-homeostasis is in place, compounds might affect TRPM2 activity either directly, by binding to the TRPM2 protein, or indirectly, by altering the local concentrations of the primary ligands ADPR and Ca<sup>2+</sup>. To identify direct modulators of TRPM2 activity, we have studied the effects of H<sub>2</sub>O<sub>2</sub>, AMP, cADPR, NAADP, and nicotinic acid adenine dinucleotide (NAAD) in inside-out patches excised from *Xenopus* oocytes expressing human TRPM2, by directly exposing the cytosolic faces of the patches to these compounds. H<sub>2</sub>O<sub>2</sub> (1 mM) and enzymatically purified cADPR (10 μM) failed to activate, while AMP (200 μM) failed to inhibit TRPM2 currents. NAADP acted as a partial agonist (maximal efficacy ~50%) while NAAD was a full agonist, but both with low affinities (K<sub>0.5</sub>=104 and 35 μM). Neither of H<sub>2</sub>O<sub>2</sub>, cADPR, and NAADP enhanced activation by ADPR. Thus, in a physiological context the above compounds do not directly affect the TRPM2 channel protein. [OTKA grant F68143]

**1695-Pos****TRPC3-Mediated Electrical Remodeling of Cardiac Myocytes**

Zora Saad, Klaus Groschner.

University of Graz, Graz, Austria.

Recent evidence suggests a key role of transient receptor potential (TRP) channels in cardiac pathophysiology with TRPC3 as one potential key player in cardiac remodeling. TRPC3 is typically up-regulated by hypertrophic stimuli and may be involved in distorted Ca<sup>2+</sup> signaling that drives pathological remodeling. As TRPC proteins generate non-selective cation conductances, we hypothesized that these channels may not only govern Ca<sup>2+</sup>-mediated gene expression but exert in addition a severe impact on basic electrical properties and excitability of the myocardium. Utilizing the patch clamp technique we characterized membrane currents and electrical properties of cardiomyocytes in response to enhanced TRPC3 expression in the murine HL-1 model. Stimulation of TRPC3-overexpressing HL-1 cells with endothelin-1 [100 nM] (ET-1) as a Gq-PLC-activator gave rise to a conductance with features distinctly different from the properties described for TRPC3 conductance in expression systems. In HL-1 cells, the TRPC3 over-expression-induced conductance in physiological solutions reversed at about -50 mV, displayed profound outward rectification and was suppressed by the TRPC3-inhibitor Pyr3 [10 μM] (ethyl-1-(4-(2,3,3-trichloroacrylamide)phenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxylate). As a result of this conductance, action potential duration was effectively shortened by ET-1 in HL-1 myocytes over-expressing the TRPC3, while this effect was minute in wild-type myocytes. Moreover, TRPC3 over-expression enabled significant depolarizing effects of ET-1 along with action potential shortening and reduction of refractory period. Our results suggest that increased expression of TRPC3 in cardiomyocytes may significantly contribute to electrical remodeling in hypertrophic hearts, generating changes in action potential morphology that are likely to promote arrhythmias.

**1696-Pos****Critical Role of Pertussis Toxin Sensitive G Proteins in the Activation of TRPC4 and TRPC5 Channels**

Rui Xiao<sup>1</sup>, Jinbin Tian<sup>1</sup>, Jisen Tang<sup>1</sup>, Alexander V. Zholos<sup>2</sup>, Michael X. Zhu<sup>1</sup>.

<sup>1</sup>The Ohio State University, Columbus, OH, USA, <sup>2</sup>Queen's University Belfast, Belfast, United Kingdom.

Canonical transient receptor potential 4 and 5 (TRPC 4 and TRPC5) are non-selective cation channels. Their activation causes membrane depolarization and intracellular Ca<sup>2+</sup> increases. TRPC4 has been implicated in neurotransmitter release, endothelial-dependent regulation of vascular tone, endothelial permeability, and excitation-contraction coupling of intestinal smooth muscles while TRPC5 has been shown to be important for neurite extension and growth cone morphology and behavior responses to fear conditioning. The activation mechanisms of TRPC4/C5 remain unresolved. Most studies have indicated that stimulation of phospholipase C activates TRPC4/C5 channels. Using whole-cell patch clamp recording and fluorescence membrane potential measurements, we show here that G<sub>q/11</sub> signaling pathway alone is insufficient for the full activation of TRPC4/C5. Channel activities are greatly enhanced with co-stimulation of G<sub>i/o</sub>-coupled receptors, including μ opioid, 5-HT<sub>1A</sub> serotonin, M2 muscarinic, and D2 dopamine receptors. Stimulation of the G<sub>i/o</sub>-coupled receptors alone also activates TRPC4/C5 in a pertussis toxin-sensitive manner. We further show that the effect of G<sub>i/o</sub> proteins cannot be attributed to the stimulation of phospholipase C-β through Gβγ subunits as activation of the G<sub>i/o</sub>-coupled receptors induced no detectable intracellular Ca<sup>2+</sup> signal unless TRPC4/C5 are co-expressed. In addition, the activated form of Gα<sub>i/o</sub> rather than Gβγ appears to be involved in the TRPC4/C5 activation. A

more direct role of  $G_{i/o}$  proteins in TRPC4/C5 activation is supported by the demonstration that  $G_{\alpha_i}$  and  $G_{\alpha_o}$  subunits physically bind to the C-terminus of TRPC4. We suggest that a concerted action of  $G_{q/11}$ - and  $G_{i/o}$ -mediated signaling pathways is required for the full activation of TRPC4/C5, making these channels coincident detectors of multiple environmental cues. We have tested the co-dependence of native TRPC4/C5-like currents in smooth muscle cells, endothelial cells and neurons. (Supported by AHA Grant-in-Aid 0755277B and NIH grant RO1 DK081654)

#### 1697-Pos

##### Function of Ion Channels in Cell Migration

Albrecht Schwab, Albrecht Schwab.

Universität Münster, Münster, Germany.

The prognosis of tumor disease is greatly influenced by the formation of metastases. A critical step of the so-called metastatic cascade is the ability of tumor cells to migrate away from the primary tumor. Ion channels and transporters are an integral part of the cellular migration machinery that complement and regulate other components of the cellular motor. KCa3.1 channels for example are upregulated in many tumor cells. Their inhibition slows down migration and prevents its chemokinetic acceleration. KCa3.1 channel activity supports migration among others by inducing localized changes of cell volume at the rear part of crawling cells. TRPC1 channels are involved in coordinating the movement of the protruding front with the retracting rear part of migrating cells. Thus, genetic ablation of channels leads to a marked impairment of persistent migration. In addition, TRPC1 channels are also part of the cellular compass during directed migration in a chemotactic gradient. Silencing of TRPC1 activity by means of RNA interference or pharmacologically with GsMTx-4 impairs chemotaxis towards FGF-2. Reducing TRPC1 channel activity blocks directed cell migration as efficiently as does inhibition of particular steps of growth factor-induced signaling like phospholipase C or phosphatidylinositol-3-OH kinase. Taken together, ion channels are crucial for multiple aspects of tumor cell motility.

#### 1698-Pos

##### A Combined Spectroscopic and Biochemical Approach to Counting MscL Subunits

Chris S. Gandhi<sup>1</sup>, Troy A. Walton<sup>1,2</sup>, Douglas C. Rees<sup>1,2</sup>.

<sup>1</sup>California Institute of Technology, Pasadena, CA, USA, <sup>2</sup>Howard Hughes Medical Institute, Pasadena, CA, USA.

The mechanosensitive channel of large conductance (MscL) is a homo-oligomeric, stretch activated membrane protein responsible for regulating osmotic pressure in bacteria and archaea. Increasing membrane tension activates the protein resulting in a ~2.9 nS non-selective pore. Two MscL crystal structures have been solved in distinct conformations and oligomeric states. *M. tuberculosis* MscL is a non-conducting pentamer while *S. aureus* MscL is a partially expanded tetramer. The primary sequences of these proteins are 38% identical and 57% similar. Given their high relatedness, the structures raise interesting questions regarding the assembly and activation of MscL homologs. We have been interested in understanding the molecular determinants responsible for the differences between the two structures, specifically the switch between tetrameric and pentameric species. Using a combination of multi-angle light scattering and mass tagging followed by Blue Native PAGE, we have characterized the oligomeric states of several MscL homologs and chimeras. We find that the two methods are in good agreement with each other and single molecule measurements. Potential reasons for the differences in oligomeric states will be discussed.

#### 1699-Pos

##### TREK Channel Pore Probed by Cysteine Scanning Mutagenesis and Structural Modelling

Paula L. Piechotta<sup>1</sup>, Phill J. Stansfeld<sup>2</sup>, Murali K. Bollepalli<sup>1</sup>, Markus Rapedius<sup>1</sup>, Isabelle Andres-Enguix<sup>3</sup>, Lijun Shang<sup>3</sup>, Hariolf Fritzenschaft<sup>1</sup>, Mark S.P. Sansom<sup>2</sup>, Stephen Tucker<sup>3</sup>, Thomas Baukrowitz<sup>1</sup>.

<sup>1</sup>Institut für Physiologie II, Friedrich-Schiller-Universität Jena, Jena, Germany, <sup>2</sup>Structural Bioinformatics and Computational Biochemistry Unit, Department of Biochemistry, University of Oxford, Oxford, United Kingdom, <sup>3</sup>Clarendon Laboratory, Department of Physics, University of Oxford, Oxford, United Kingdom.

The TREK channel belongs to the superfamily of two-pore-domain potassium channels (K2P-channels) that are made up of four transmembrane segments (TM1 - TM4) and two pore-forming domains that are arranged in tandem. The activity of these channels is directly regulated by the intracellular pH, heat, polyunsaturated fatty acids, phospholipids and mechanical stretch. Cur-

rently little is known about the pore structure and how these different stimuli gate the pore in structural terms. To this end we employed systematic cysteine scanning mutagenesis on the four TM domains and functionally characterised these mutants in several respects: I) using chemical cysteine modification we identified pore lining residues, II) by measuring detailed pH dose response curve we identified residues involved in the pH gating mechanism and III) by studying different pore blocking compounds we identified potential blocker interacting residues. These sets of functional data will be evaluated in the context of structural models of the TREK channel pore in the closed and open state.

#### 1700-Pos

##### Coupling of Water Permeation with Mechano-Gating In the E-Coli Mechanosensitive Channel MscL

Yasuyuki Sawada<sup>1</sup>, Masahiro Sokabe<sup>1,2</sup>.

<sup>1</sup>Nagoya University Graduate School of Medicine, Nagoya, Japan.

<sup>2</sup>ICORP/SORST Cell Mechanosensing, JST, Nagoya, Japan.

The bacterial mechanosensitive channel of large conductance MscL is constituted of homopentamer of a subunit with transmembrane inner and outer  $\alpha$ -helices, and its 3D structure of the closed state has been resolved. Understanding the gating process driven by tension in the membrane is one of the major subjects in MscL study. Although several models for its opening process have been proposed based on molecular dynamics (MD) simulations, as they do not include MscL-lipid interactions, it remains unclear which amino acids sense membrane tension and how the sensed force induces channel opening. We performed MD simulations for the mechano-gating of MscL embedded in the lipid bilayer. Upon tension generation in the bilayer, Phe78 in the outer helix was dragged by lipids, leading to a tilting of the helices. Among amino acids in the outer helix, Phe78 at the water-lipid interface showed the strongest interaction with lipids, thus may work as a major tension sensor. Neighboring inner helices cross each other in the inner leaflet, forming the most constricted part of the pore. In the closed state of MscL, Leu19 and Val23 in the constricted part form stable hydrophobic environment. As tension increased, the crossings moved toward the cytoplasm associated with an expansion of the constricted part and the hydrophobic environment was broken followed by water penetration and permeation. It seemed that water penetration and permeation accelerated the pore opening probably by decreasing the hydrophobic interaction between Leu19 and Val23. We performed MD simulations of the GOF mutant G22N with almost the same pore size of the wild type, and found that G22N mutant permeated water molecules without tension increase in the bilayer. This spontaneous water permeation seemed to be mediated by hydrogen bonds between Asn22 and water molecules.

#### 1701-Pos

##### Significance of the *Corynebacterium Glutamicum* YggB Protein in Fine-Tuning of Compatible Solute Accumulation

Kirsten Börngen<sup>1</sup>, Andrew R. Battle<sup>2</sup>, Nina Möker<sup>3</sup>, Susanne Morbach<sup>3</sup>, Kay Marin<sup>3</sup>, Boris Martinac<sup>4</sup>, Reinhard Krämer<sup>3</sup>.

<sup>1</sup>University of Cologne, Köln, Germany, <sup>2</sup>University of Queensland, Brisbane, Queensland, Australia, <sup>3</sup>University of Cologne, Köln, Germany,

<sup>4</sup>Victor Chang Cardiac Research Institute, Sydney, New South Wales, Australia.

Based on structural similarity, the *yggB* gene product of *Corynebacterium glutamicum* belongs to the family of MscS-type mechanosensitive channels. In order to clarify its physiological significance in response to osmotic shifts in detail, we studied the properties of YggB using both patch-clamp techniques and betaine efflux kinetics. After heterologous expression in an *E. coli* strain devoid of mechanosensitive channels, in patch-clamp analysis of giant *E. coli* spheroplasts YggB showed the typical pressure dependent gating behavior of a stretch-activated channel with a current/voltage dependence indicating a strongly rectifying behavior. Apart from that, YggB is characterized by significant functional differences with respect to conductance, ion selectivity and desensitization behavior as compared to MscS from *E. coli*. Deletion and complementation studies in *C. glutamicum* showed a significant contribution of the YggB protein to betaine efflux in response to hypoosmotic conditions. As a novel finding, detailed analysis of concomitant betaine uptake (by the betaine transporter BetP) and efflux (by YggB) under hyperosmotic conditions revealed that YggB acts as a key player in osmoregulation in *C. glutamicum* by fine-tuning the steady state concentration of compatible solutes in the cytoplasm which are accumulated in response to hyperosmotic stress.