567-Pos Functional Interactions between HERG and KvLQT1 Downregulate I_{kr} Currents

XIAOQIN REN, GongXin Liu, Gideon Koren
CVRC, RIH, Providence, RI, USA.

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KvLQT1 and HERG code for the α-subunits of ***IKs*** and ***IKr***, the two main repolarizing K^+ currents of most mammalian cardiomyocytes. Whether KvLQT1 and HERG interact with each other is what we are currently concerned. We have created two transgenic rabbit models for long QT syndrome 1 (LQT1) and long QT syndrome 2 (LQT2) by over-expression of human a loss-of-function pore mutant of KvLQT1 (KvLQT1Y315S) and HERG (HERGG628S), respectively in the heart. The result showed that as expected KvLQT1Y315S abolished the endogenous IKs currents, however, it also down-regulated the IKr currents. Similarly, over-expression of HERGG628S transgene abolished the endogenous IKr currents and reduced the IKs currents.

To further investigate the possible interaction between HERG and KvLQT1, we have created a CHO cell line stably expressing HERG with a FLAG epitope on its N-terminus. Transient transfection of either WT KvLQT1 or KvLQT1Y315S resulted in the down-regulation of HERG currents. Immunocytochemistry experiments confirmed that the surface HERG was down-regulated with the transfection of either WT KvLQT1 or KvLQT1Y315S. Coimmunoprecipitation experiment shows that HERG polypeptides could be co-precipitated with either WT KvLQT1 or KvLQT1Y315S. Moreover, KvLQT1 polypeptides could be co-precipitated with either WT HERG or HERGG628S polypeptides. The down regulation observed *in vitro* correlates with our observation in LQT1 and LQT2 rabbits. Collectively, these results suggest that HERG and KvLQT1 polypeptides might specifically and significantly interact with each other (directly or indirectly) and that these interactions could modify the level of expression of the currents encoded by these α subunits.

568-Pos Biochemical and Spectroscopic Studies of Heteromeric Interactions of HERG1a and HERG1b Potassium Channel Subunits

Zeus D. Pendon, Matthew C. Trudeau
University of Maryland, School of Medicine, Baltimore, MD, USA.

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Human Ether-a-go-go Related Gene (HERG) encodes a voltage-gated potassium (K^+) channel that is abundantly expressed in the heart and in nervous tissue. Two isoforms of HERG, 1a and 1b, have been described in heart and neurons. In the heart, 1a and 1b have been shown to form heteromeric channels and underlie the native rapidly activating delayed rectifier K^+ current (I_{Kr}). Here, we examined the direct interaction of 1a and 1b subunits. Single cysteine mutations were introduced to 1a and 1b. Cysteines spontaneously formed disulfide bonds and in the absence of reducing agents were stable through biochemical purification. Western blots of homomeric single cysteine mutants showed two bands corresponding to subunit monomers and dimers. Mixing 1a and 1b cysteine-containing subunits showed a band consistent with 1a-1b dimers, indicating direct 1a–1b subunit interactions. Homomeric double cysteine mutants showed two additional bands which were consistent with trimeric and tetrameric complexes. Coexpression of 1a and 1b subunits with double cysteine mutations showed formation of a 1a-1b dimer as well as multiple bands at high molecular weights. The sizes of these bands were consistent with the formation of multiple combinations of heteromeric complexes. To investigate subunit interactions at the cell surface, HERG1a and HERG1b were fused to the Enhanced Cyan Fluorescent Protein (eCFP) and Citrine fluorescent proteins. We used Forster Resonance Energy Transfer (FRET) to show direct interactions between 1a and 1b subunits at the membrane surface. We used Fluorescence Intensity Ratio studies to measure relative 1a and 1b subunit expression levels at the membrane surface and electrophysiology to measure channel function. Taken together, the results indicate that that the interaction of HERG1a and HERG1b subunits is random in membranes and at the membrane surface.

Anion Channels

569-Pos GaTx1: A New Tool For The Study of ATP-dependent Gating In The CFTR Chloride Channel

Cody S. Freeman[1], Christopher H. Thompson[1], Robert J. French[2], Balázs Sarkadi[3], Nael A. McCarty[1]
[1] *Georgia Tech, Atlanta, GA, USA*
[2] *University of Calgary, Calgary, AB, Canada*
[3] *Hungarian Academy of Sciences, Budapest, Hungary.*

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We recently reported the isolation and initial characterization of a novel 3.7 kDa peptide toxin, GaTx1, which is a potent and reversible inhibitor of CFTR, acting from the cytoplasmic side of the membrane. While many peptide inhibitors of cation-permeable channels have been described, and used as highly specific probes of those channel targets, peptide toxin inhibitors of chloride channels of known molecular identity have not been available. Thus, GaTx1 is the first peptide toxin identified which inhibits a chloride channel of known molecular identity. GaTx1 was prepared in synthetic form using solid-phase chemistry, and was then folded under oxidizing conditions and purified by HPLC. In excised, inside-out patches pulled from oocytes expressing wildtype CFTR, GaTx1 inhibited CFTR channel activity by prolonging the interburst closed duration. GaTx1 exhibited high specificity, showing no effect on a panel of nine transport proteins including Cl^- channels, K^+ channels, and ABC Transporters. To study dose-response relations for activity of synthetic toxin, we used multichannel patch recordings from oocytes expressing Flag-cut-ΔR-CFTR, taking advantage of the insensitivity of this CFTR variant to dephosphorylation-mediated rundown. GaTx1-mediated inhibition of CFTR channel activity is strongly state-dependent; both potency and efficacy are reduced in presence of high [ATP]. When CFTR channels were pre-incubated

with 50 nM GaTx1 for 5 minutes in the absence of ATP, currents measured subsequently in the presence of 1 mM ATP plus toxin were inhibited by 80.2%. These data suggest that GaTx1 may function as a non-competitive inhibitor of ATP-dependent channel gating. This tool will allow the application of new quantitative approaches to study CFTR structure and function, particularly with respect to the conformational changes that underlie transitions between open and closed states.

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570-Pos Structural characterisation of the *C. elegans* CLIC ion channel - EXC4

Ramya A. Mandyam[1], Samuel N. Breit[2], Paul M.G. Curmi[3], Louise J. Brown[1]

[1] *Macquarie University, Sydney, Australia*
[2] *St Vincent's Hospital and The University of New South Wales, Sydney, Australia*
[3] *The University of New South Wales, Sydney, Australia.*

Board B415

The CLIC (Chloride intracellular channel) protein family represent an unusual class of proteins, which can exist in both soluble and membrane bound states. These proteins are members of the glutathione S-transferase (GST) fold family, highly conserved in most vertebrates, with related proteins in invertebrates including *C. elegans* (EXC4). A functional study of the chloride conductivity of EXC4 across artificial lipid bilayers was performed by chloride efflux assay. The assay showed that EXC4 conducts chloride ions at low pH (Littler *et al*, Proteins, *In press* 2007). The crystal structure of EXC4 was also recently determined, albeit the soluble state, and shows the classic CLIC family GST fold. We are investigating the structure of the membrane-associated form of EXC4 using tryptophan fluorescence spectroscopy and Site Directed Spin Labeling-Electron Paramagnetic Resonance Spectroscopy (SDSL-EPR).

The N-terminal 66 residues of EXC4 contain the putative transmembrane region believed to facilitate the formation of the ion channel. Two single tryptophan mutants of EXC4 were created (N-domain - W221F & C-domain - W43F). Tryptophan fluorescence emission and quenching experiments in the presence of the phospholipid bilayer showed that the N-domain interacts with the membrane while the C-domain does not. This agrees with the property that the first 66 residues are sufficient for membrane localisation of EXC4 in *C.elegans* (Berry et al, J Mol Biol. 2006 *359*:1316).

For SDSL-EPR studies, a cys-less EXC4 was constructed by site-directed mutagenesis of the 4 native cysteines to serines. Structural characterisation of the recombinant cys-less by fluorescence and Circular Dichroism studies showed that it is highly α-helical, similar to the wildtype EXC4. On going experiments of spin-labeled mutants in the transmembrane region of EXC4 are being analysed by EPR spectroscopy.

571-Pos C-terminal Domain Movement Of The CLIC1 Ion Channel Upon Membrane Insertion

Sophia C. Goodchild[1], Michael W. Howell[1], Kenneth A. Sale[2], Paul M.G. Curmi[3], Louise J. Brown[1]

[1] *Macquarie University, Sydney, Australia*
[2] *Sandia National Laboratories, Livermore, CA, USA*
[3] *The University of New South Wales, Sydney, Australia.*

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Conventionally, it was thought that proteins adopt a well-defined tertiary structure. However, it is becoming increasingly apparent that some proteins can exist in two or more stable conformations. The CLIC (Chloride Intracellular Ion Channel) proteins are one such family that can exist in both soluble and integral membrane forms as they can insert into the lipid bilayer to form an active channel in the absence of other cellular proteins. However, the manner and factors controlling CLIC insertion into the bilayer remains unclear. Crystal structures of soluble CLIC1 show that it is capable of undergoing a large-scale structural rearrangement from a glutathione S-transferase fold, under reducing conditions, to a non-covalent all α-helical structure upon oxidation. Additionally, an intramolecular disulphide bond forms between Cys24 and Cys59.

To explore the CLIC1 membrane structure, Fluorescence Resonance Energy Transfer (FRET) and Site Directed Spin Labeling-Electron Paramagnetic Resonance Spectroscopy (SDSL-EPR) were performed on a CLIC1 recombinant construct in which four native cysteines were mutated to serine (C89S, C178S, C191S, C223S). This cys-less CLIC1 exhibited ion channel activity. An EPR spin label was then attached to each of the 4 cysteines and their mobility measured by EPR in the presence and absence of the bilayer. FRET was also performed from the intrinsic tryptophan (Trp35) to each single cysteine labelled with 1,5-IAEDANS. Upon interaction with the bilayer, the FRET distance between Trp35 and Cys223 in the C-domain increased significantly. Furthermore, the EPR mobility of Cys223 also increased, suggesting an unfolding of the CLIC1 as the N-domain is inserted into the membrane. This large conformational change of CLIC1 upon membrane interaction reinforces the notion of the CLIC protein family as dynamic entities and challenges many accepted views of ion channel structure.

572-Pos Effects Of ATP-Related Nucleotides/Nucleosides On The Common Gating Of ClC-1

Pang-Yen Tseng, Tsung-Yu Chen

University of California-Davis, Davis, CA, USA.

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Our recent study revealed that intracellular ATP inhibits ClC-1 common gating, and that this inhibition is enhanced by a low intracellular pH (pH_i). To further examine the interaction of nucleotides (or nucleosides) with ClC-1, we study the effects of various ATP-related reagents in inhibiting the ClC-1 common gating. At $pH_i = 6.2$, 3 mM ATP shifts the P-V curve of the common gate by

~107 mV. The same concentration of ADP and AMP also has a similar effect: the common gate P-V curve is shifted by ~126 mV and ~97 mV, respectively. The ATP half-effective concentration at $pH_i = 6.2$ is 0.12mM. Adenosine also has an effect on ClC-1 common gating at $pH_i = 6.2$ with a similar half-effective concentration as that of ATP. However, the shift of the common-gate P-V curve by adenosine is only ~76mV, indicating that adenosine is an inhibitor with less efficacy. In contrast, adenine and cAMP have no effect on ClC-1 common gating, while GTP and p-ATP, an ATP analogue with N6 modification of adenine, only have a very slight inhibition on ClC-1 common gating. The effects of ATP together with those of other nucleotides and nucleosides suggest that the phosphate group of ATP plays a less important role in the inhibition of the ClC-1 common gating. On the other hand, both the adenine moiety and the ribose ring are critical for the modulation of ClC-1 common gating.

573-Pos Gating of hClC-4 chloride/ proton antiporters

Gökce Orhan, Christoph Fahlke, Alexi K. Alekov

Medizinische Hochschule Hannover, 30625 Hannover, Germany.

Board B418

ClC-3, 4 and 5 form a branch within the ClC family of anion channels and transporters. They are located mainly in vesicles of endocytotic and lysosomal pathways and have been recently shown to function as electrogenic H^+/Cl^- antiporters. We studied hClC-4 gating using standard whole-cell patch-clamp recordings on mammalian cells. ClC-4 currents display a pronounced rectification and time- and voltage-dependent changes of current amplitudes due to variations in the number of active transporting proteins, analogous to ion channel gating. ClC-4 gating resembles fast and slow gating of classical ClC channels. Activation and deactivation are characterized by a bi-exponential time course (τ_{act1}=4.5±0.2ms and τ_{act2}= 33±5ms at +150mV and at −100mV: τ_{deact1}=0.25±0.02ms; τ_{deact2}=3.4±0.3ms). Under many conditions, activation occurs only at voltages far away from physiological values, i.e. $V_{1/2} = +80.2 \pm$ 2.3mV at symmetric pH 7.4, external SCN^- and internal I^-. However, the voltage dependence of channel opening depends on the anion and proton concentrations on both membrane sides. Lowering the external pH or rising the internal pH shifts the open probability to more depolarized potentials. Changes in the external anion concentration have only small effects, while intracellular variations cause pronounced alterations of the voltage dependence. Decreasing internal anion concentration from 95 to 10mM produces a −60mV shift of the activation curve. Since native hClC-4 channels are mainly expressed in the endoplasmic reticulum, the ionic conditions they are exposed will result in voltage-dependent transitions at voltages close to 0 mV. In summary, we demonstrate a surprising similarity of hClC-4 gating behavior to gating of ClC-0, ClC.-1 and ClC-2. Moreover, its anion dependence seems to be adapted to the intracellular localization shifting its major mode of operation into the physiological voltage range.

574-Pos Chloride Ions and the gating mechanism of CLC-2 chloride channel

Jorge E. Sanchez[1], Pablo G. Nieto[1], Jose A. De Santiago-Castillo[2], Jorge Arreola[1]

[1] *Instituto de Fisica, Universidad Autonoma de San Luis Potosi, San Luis Potosi, Mexico*

[2] *Department of Pathology, Anatomy and Cell Biology Jefferson Medical College of Thomas Jefferson University Philadelphia, Philadelphia, PA, USA.*

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ClC-2 is a double barrel, ClC-type inwardly rectifying chloride (Cl^-) channel activated by voltage, extracellular H^+ and intracellular Cl^- Gating of ClC-2 is controlled by a protopore gate (P_p) acting independently on each pore, and by a common gate (P_c), acting simultaneously on both pores. In this work we used the patch clamp technique to study the effects of $[Cl^-]_e$ and $[Cl^-]_i$ on the gating mechanism of ClC-2 heterologously expressed in HEK-293 cells. Increasing $[Cl^-]_e$ from 40 to 200 mM, did not induce appreciable changes in the apparent open probability (P_a) *vs* voltage (V_m) relationship, and the V_m dependence of P_p and P_c. However, increasing $[Cl^-]_i$ in the same range, shifted to the right the P_a vs Vm curve. This shift can be described by the Nernst equation as if the pore sensed $[Cl^-]_i$. Accordingly, the open probability of P_p, a gate located within the pore, increased as $[Cl^-]_i$ increased. In addition, the time constant for the P_c gate was slowed as $[Cl^-]_i$ increased while P_p gating time constant was not altered. To understand the effects of $[Cl^-]_i$ on channel gating, we performed global fits to analyze the kinetics using a model for CLC-2 developed by *de Santiago* (J. Gen. Physiol. 2005, *33*:591). In this model, the open and close transitions are controlled by six kinetic constants ($\alpha_1, \beta_1, \alpha_2, \beta_2$ from P_p, and, λ and μ from P_c). This approach allowed us to determine the Cl^- dependence of these rate constants. Our analysis suggests that, in addition to the effects of $[Cl^-]_i$ on P_p, additional Cl^- dependence resides on the slow kinetics (λ, μ).

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575-Pos Blockade of the Opened Pore of ClC-0 by CPA and Amphiphilic Blockers

Xiao-Dong Zhang, Tsung-Yu Chen

University of California, Davis, CA, USA.

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Previous studies showed that the ClC-0 chloride channel is inhibited by p-chlorophenoxy acetate (CPA) in a voltage-dependent manner, and that CPA blocks a pore-opened mutant, E166A, of ClC-0 with an affinity several hundred-fold higher than that of blocking the wild-type channel. These findings prompted a hypothesis that the CPA block of ClC-0 may be state-dependent, namely, the fast-gating of the channel involves a conformational change in the pore that renders different CPA binding affinities in the opened and closed

states. However, we found that the CPA blocking affinity of another pore-opened mutant, E166Q, is very low. Therefore, unless the gating-associated conformational change is very different between E166A and E166Q, it is difficult to explain the difference in their CPA affinities. To further explore the CPA blocking mechanism, we examined more mutants of E166 in ClC-0. We found that the CPA apparent affinity of these mutants depends on the side-chain volume and hydrophobicity of the introduced residue. These mutations at position 166 can alter the steady-state blocking affinity by ~1000-fold, and the effect appears to mostly come from the change in the off-rate of the CPA block. On the other hand, mutations at the intracellular pore entrance, K519M and K519E, alter both the on- and off-rates of the CPA block, but only modestly change the steady-state blocking affinity. We also found that the pore-opened mutants of ClC-0 can be blocked by fatty acids, with the affinity increasing with the length of the molecule. These results suggest that CPA and fatty acids may block the pore of ClC-0 via a three-state blocking mechanism proposed in the blockade of the voltage-gated K^+ channel by the long-chain QA compounds or by the inactivation ball peptide.

576-Pos High-Throughput Assay Development to Identify High Affinity ClC-ec1 Inhibitors

Andrew E. Howery, Aaron S. Miller, J. Du Bois, Merritt Maduke

Stanford, Menlo Park, CA, USA.

Board B421

CLC "chloride channels" are a widespread molecular family with diverse physiological roles. ClC-ec1, a prokaryotic Cl^-/H^+ antiporter, is currently the only CLC with a fully elucidated crystal structure. The ability to crystallize ClC-ec1 opens up the possibility of determining structures in the presence of inhibitors. However, only a small number of mid-micromolar inhibitors are known for any CLC homolog. A high-throughput assay is currently being developed to identify high-affinity, small-molecule inhibitors of ClC-ec1. The assay takes advantage of a YFP mutant whose fluorescence is quenched by a number of anions with varying millimolar affinities (Galietta et al., FEBS Lett. 2001; Galietta et al., Am J Physiol Cell Physiol 2001), and in particular is quenched more effectively by nitrate than by chloride. The YFP mutant and chloride are trapped inside ClC-ec1-containing liposomes, and YFP fluorescence is monitored over time. Because both nitrate and chloride permeate ClC-ec1, addition of extravesicular nitrate results in a decrease in fluorescence as the nitrate exchanges with intravesicular chloride and quenches the YFP fluorescence. Potent inhibitors will stop nitrate/chloride exchange, resulting in a significantly smaller reduction of fluorescence when nitrate is added. Present work has confirmed the feasibility of this assay. Current experiments are being performed to optimize the assay, after which a library of 100,000 compounds will be screened for inhibition of CLC-ec1. Potential leads will be used as models in SAR (Structure-Activity Relationship) studies to design more potent inhibitors. These inhibitors will be used as probes to further our insight into the mechanisms of the CLC family of channels.

577-Pos α1-Adrenergic Receptor-Mediated Depletion of Phosphatidylinositol 4,5-bisphosphate Inhibits Activation of Volume-Regulated Chloride Currents in Mouse Ventricular Myocytes

Kunihiko ICHISHIMA, Shintaro Yamamoto, Tsuguhisa Ehara

Saga University Faculty of Medicine, Saga, Japan.

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Regulation of volume-regulated chloride current ($I_{Cl,vol}$) by α1-adrenergic stimulation was examined in mouse ventricular myocytes with the whole-cell patch clamp. The hypotonicity-induced current was sensitive to chloride channel inhibitors (DIDS, glibanclamide, DCPIB but not CFTRinh-172), and showed the characteristics matching those reported for cardiac $I_{Cl,vol}$. The activation of $I_{Cl,vol}$ was inhibited by α1-adrenergic receptor (α1R) agonist, phenylephrine (PE, 100 μM). This inhibition did not occur in the presence of prazosin (5 μM), α1R antagonist, or when the cells were dialyzed with polyclonal anti-$G_{q/11}$ antibody. U-73122 (5 μM), a phospholipase C (PLC) inhibitor, prevented the PE-induced inhibition of $I_{Cl,vol}$, whereas bisindolylmaleimide-I (100 nM), a protein kinase C (PKC) inhibitor, was without effect. These results show that the effect of PE on $I_{Cl,vol}$ is mediated by α1R-Gq/11-PLC signaling but not by PKC signaling. Interestingly, PE unaffected $I_{Cl,vol}$ when the cells were dialyzed with 10 μM phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2), raising the possibility that PE-induced inhibition of $I_{Cl,vol}$ is related to membrane PI(4,5)P2 depletion. Furthermore, the activation of $I_{Cl,vol}$ was suppressed by intracellular application of wortmannin, which is an inhibitor of phosphatidylinositol kinases, and thus is expected to prevent resynthesis of membrane PI(4,5)P2. However, the inhibitory effect of wortmannin on $I_{Cl,vol}$ persisted after additional intracellular application of PI(4,5)P2, suggesting that PI(4,5)P2 depletion itself did not play a key role in the wortmannin-induced inhibition of $I_{Cl,vol}$. Considering that cell-swelling may activate PI3K, we hypothesize that certain phospholipid component(s) converted from membrane PI(4,5)P2 by the activated PI3K play an essential role in the activation of cardiac $I_{Cl,vol}$, and that α1-adrenergically induced depletion of membrane PI(4,5)P2 level leads to inhibition of $I_{Cl,vol}$.

578-Pos GaTx2: A High Affinity Inhibitor of ClC-2 Chloride Channels

Christopher H. Thompson[1], Denis McMaster[2], Robert J. French[2], Nael A. McCarty[1]

[1] *Georgia Institute of Technology, Atlanta, GA, USA*
[2] *University of Calgary, Calgary, AB, Canada.*

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ClC family proteins form either voltage-gated chloride channels or Cl^-/H^+ exchangers; nine subtypes are found in mammals. Among these, ClC-2 is one of the most widely distributed variants, which is

expressed in several types of epithelial cells as well as glial cells. We have recently reported the isolation from scorpion venom of the first peptide inhibitor of ClC-2 channels, GaTx2. Here we report the initial pharmacological and mechanistic characterization of this toxin. In order to perform these experiments, we created a synthetic form of GaTx2 using solid phase chemical synthesis; synthetic toxin inhibits ClC-2 currents to the same degree as the isolated native toxin. Using multichannel patches from oocytes expressing ClC-2 channels we created a dose-response curve, yielding a K_D of just 12 pM at $V_M = -100$ mV, making GaTx2 the most potent inhibitor available for ClC channels to date. Additionally, inhibition of ClC-2 by GaTx2 was voltage-dependent, with the K_D shifting to the single pM range at more physiological membrane potentials (*e.g.* −60 mV). Single channel recordings of ClC-2 channels performed in the presence of GaTx2 indicated that the single channel amplitude was unchanged in the presence of toxin. However, the latency to first opening was increased by almost 8 fold in the presence of 20 pM toxin. Finally, GaTx2 had no effect on a panel of other Cl^- channels, including voltage-gated, ligand-gated and Ca^{2+}-dependent Cl^- channels, as well as voltage-gated K^+ channels, indicating that GaTx2 is very specific for its target. This high specificity and high affinity suggest that GaTx2 may be a useful probe of ClC-2 channel function in a physiological environment, and a good probe for ClC-2 structural studies into conformation changes resulting from channel gating.

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579-Pos CLC-3 Chloride Channels Associate With A Multiprotein Kinase Signaling Complex In Cardiomyocytes

Elena Kuzmin, Roberto J. Diaz, Taneya Hossain, Alina Hinek, Heather Bilton, Gregory J. Wilson

The Hospital for Sick Children, Toronto, ON, Canada.

Board B424

CL^- channel activity plays a key role in cell volume regulation. There is evidence from isolated cardiomyocytes and whole hearts that activation of CL^- channels by ischemic preconditioning (IPC) limits ischemic cell death by reducing cell swelling thereby preventing cell membrane rupture. CLC-3, a member of the CLC family of CL^- channels, is thought to function as a volume regulated CL^- channel. We have shown that protein kinase C epsilon (PKCε) is associated with Src tyrosine kinase in cardiomyocytes. Inhibition either of PKCε, Src, or phosphatidylinositol-3 kinase (PI3K) has been shown to abolish IPC cardioprotection. Thus, we hypothesize that CLC-3 associates with PKCε, Src, and PI3K forming a multiprotein signaling complex in intact isolated cardiomyocytes. To test this hypothesis, we performed pull-down assays (immunoprecipitation followed by Western blot) using cell lysates from freshly isolated rabbit ventricular cardiomyocytes. Immunocytochemical fluorescence techniques were also used on 24-hour cultured cardiomyocytes to determine co-localization of each pair of associated proteins. PKCε, Src and PI3K were each immunoprecipitated using specific monoclonal antibodies. CLC-3 was detected using specific polyclonal antibodies. Immunoprecipitation assays showed that each of these proteins (CLC-3, PKCε, Src, and PI3K) associates with each other. The presence of a CLC-3/PKCε/Src/PI3K multiprotein complex was also confirmed in the membrane fraction obtained by differential centrifugation. Co-localization between CLC-3 and each of these kinases, and co-localization of each possible pair of kinases, as measured by image analysis, supported the results obtained from the pull-down assays. These results establish, for the first time, the association of PI3K with the PKCε/Src kinase signaling protein complex and also establish the association of this multi-kinase protein complex with CLC-3 channels. These findings provide the basis for a novel working model for CLC-3 mediated volume regulation and cardioprotection.

580-Pos Inhibition of Cl^- Currents, Electrical Activity and Insulin Release from INS-1E Rat Insulinoma Cells by Resveratrol

Christian Langelueddecke[1], Martin Jakab[1], Zuzana Bacova[2], Sibylle Lach[1], Sabine Schmidt[1], Markus Ritter[1]

[1] *Paracelsus Medical University, Salzburg, Austria*
[2] *Slovak Academy of Sciences, Bratislava, Slovakia.*

Board B425

Stimulus-secretion coupling (SSC) in pancreatic beta-cells is metabolically regulated by the closure of ATP-sensitive K^+ (K_{ATP}) channels, due to increased $[ATP/ADP]_i$ upon glucose uptake. The resulting depolarization activates voltage-dependent Ca^{2+} channels (Ca_v) which induces Ca^{2+} influx and insulin exocytosis. Cl^- currents activated by beta-cell swelling shown for glucose uptake or extracellular hypotonicity contribute to the SSC by causing membrane depolarization due to Cl^- efflux. Under hyperglycaemic conditions, like observed in type-2 diabetes (T2DM), where the metabolic coupling *via* closure of K_{ATP} channels is ineffective, this alternative pathway might be important. We performed whole-cell perforated-patch clamp experiments in INS-1E cells and found that Cl^- currents elicited by exposure to high extracellular glucose or hypotonicity were inhibited by the swelling-dependent Cl^- current-inhibitor DIDS and by the structurally related phytostilbene *trans*-resveratrol. The resveratrol block was dose-dependent (10 to 100 μM) and reversible. Membrane depolarization and electrical activity evoked by hypotonicity, high glucose or the sulfonylurea tolbutamide were completely and reversibly suppressed by resveratrol (50 μM) and the anion-channel blocker NPPB (100 μM). Moreover resveratrol from the start suppressed hypotonicity-induced membrane depolarization and action potentials. In the presence of 10 mM Ba^{2+} we observed T-type and L-type Ca^{2+} currents. Both currents were inhibited by resveratrol with different drug sensitivity: T-type (10 μM) and L-type currents (50 μM). Resveratrol (50 μM) completely blocked the glucose-induced insulin release from INS-1E cells as measured by RIA. Our data imply that the inhibition of insulin release by resveratrol is due to the inhibition of Cl^--and Ca^{2+} currents, which are crucially involved in the regulation of the electrical activity of insulin secreting cells. This effect might be responsible for the delayed beta cell exhaustion observed in animals under resveratrol treatment.

581-Pos Regulation Of Gating And Kinetics Of Clc-2 By Extracellular Protons

Pablo G. Nieto[1], Jorge E. Sanchez[1], Jose A. de Santiago-Castillo[2], Jorge Arreola[1]

[1] *Instituto de Fisica UASLP Mexico, San Luis Potosi, Mexico*
[2] *Department of Pathology, Anatomy and Cell Biology Jefferson Medical College of Thomas Jefferson University Philadelphia, Philadelphia, PA, USA.*

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ClC-2 is a double barrel chloride (Cl^-) channel, widely expressed, inwardly rectifying, and activated by voltage (Vm), $[Cl^-]_i$ and H^+. Recently it has been proposed that intracellular H^+ contribute to ClC channels gating (Miller. Nature 2006, *440*:484). In each pore open to close transitions are controlled by a protopore or fast gate that is H^+-sensitive as well as a common or slow gate closing the two pores simultaneously. In this work we have studied the effects of both $[H^+]_i$ and $[H^+]_e$ on gating of ClC-2 recorded from transfected HEK cells using the patch-clamp technique. Miller's model predicts that increasing $[H^+]_i$ should increase the apparent open probability (Pa). When pH_i was changed between 4.8 and 9.1 no effects were observed on Pa or Vm dependence of fast and slow gates. In contrast, varying pH_e in the range of 4.8 to 9.1 induced bell-shaped changes on Pa and $V_{0.5}$ (Vm needed to reach half maximum Pa). As $[H^+]_e$ increases Pa increased until reaching a maximum around pH_e 6.4 and then decayed to nearly 0 at high $[H^+]_e$. To explain the bell shape effect of H^+, we assume the presence of two H^+ binding sites with distinct Vm dependence. Site 1 is an activating site associated with the opening of the fast gate. pK_1 of Site 1 decreased as the Vm became positive, indicating a strong interaction between external H^+ and the gate within the electrical field. In contrast, pK_2 of blocking Site 2 displayed little or no Vm dependence. Thus, H^+ enhances ClC-2 open probability by interacting with the fast gate from the external side but not the internal side of the membrane.

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582-Pos ICln-ICln interactions assessed by Fluorescence Resonance Energy Transfer (FRET)

Sabine Schmidt[1], Ivano Costa[1], Martin Jakab[1], Johannes Fuerst[2], Markus Paulmichl[3], Markus Ritter[1]

[1] *Paracelsus Private Medical University, Salzburg, Austria*
[2] *Innsbruck Medical University, Innsbruck, Austria*
[3] *University of Milan, Milan, Italy.*

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ICln is a highly conserved, ubiquitously expressed protein which is primarily localized as a water-soluble protein in the cytosol, but some portion is found within or close to the cell membrane. It plays a critical role in regulatory volume decrease and is translocated from the cytosol to the plasma membrane during cell swelling where it is believed to function as an anion channel. Indeed reconstitution into artificial bilayers has shown that the protein forms functional ion pores. The putative channel model predicts the association of at least two ICln molecules to form a functional ion-conducting pore. Truncated ICln ($ICln_{159}$) lacking major parts of the C-terminus and the third acidic domain (AD3) did not form homomultimers as shown in NMR studies. In this study we investigated ICln-self-interactions in living cells using the FRET technique. ECFP and EYFP were used as donor and acceptor dye, respectively and fused either to the C-terminus (ICln-CFP/ICln-YFP), N-terminus (CFP-ICln/YFP-ICln) or both C- and N-terminus (CFP-ICln-YFP) and (co)expressed in NIH3T3 fibroblasts. FRET was assessed from the increase of CFP-fluorescence after 2 minutes of acceptor photobleaching. FRET signals were detected in cells expressing CFP-ICln-YFP, indicating C-N-terminal intramolecular and/or intermolecular interactions. FRET was also evident in cells expressing ICln-CFP/ICln-YFP but not when using CFP-ICln/YFP-ICln, indicating that intermolecular interactions occur between two or more ICln molecules with their C-termini, but not with their N-termini. Strong FRET appears in cells expressing CFP-$ICln_{159}$/YFP-$ICln_{159}$, indicating that the absence of the C-termini allows for N-terminal interactions. Weak FRET-signals can also be detected using $ICln_{159}$-CFP/$ICln_{159}$-YFP. No FRET signals could be measured neither between the C- nor between the N-termini, using ICln truncated at position 134 ($ICln_{134}$) lacking AD3 and AD2, indicating that the presence of AD2 is necessary for ICln-ICln-interactions in living cells.

583-Pos In the Presence of ATP, Low pH Markedly Inhibits Skeletal Muscle ClC-1 Chloride Channel Activity

Brett Bennetts[1], Michael W. Parker[1], Brett A. Cromer[2]

[1] *St. Vincent's Institute, Melbourne, Australia*
[2] *Howard Florey Institute, University of Melbourne, Melbourne, Australia.*

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Skeletal muscle acidosis during exercise has long been thought to be a cause of fatigue but recent studies have shown that acidosis maintains muscle excitability and opposes fatigue by decreasing the sarcolemmal chloride conductance. ClC-1 is the primary sarcolemmal chloride channel and has a clear role in controlling muscle excitability but recombinant ClC-1 has been reported to be activated by acidosis. Following our recent finding that intracellular ATP inhibits ClC-1, we investigated here the interaction between pH and ATP regulation of ClC-1. We found that in the absence of ATP, intracellular acidosis from pH 7.2 to 6.2 inhibited ClC-1 slightly by shifting the voltage dependence of common gating to more positive potentials, similar to the effect of ATP. Importantly, the effects of ATP and acidosis were cooperative, such that in the presence of ATP, the inhibition of ClC-1 activity by acidosis was greatly increased. Adenosine had a similar effect to ATP at pH 7.2 but acidosis did not potentiate this effect, indicating that the phosphates of ATP are important for this cooperativity, possibly due to electrostatic interactions with protonatable residues of ClC-1. A protonatable residue identified by molecular modelling, His847, was found to be critical for both pH and ATP modulation and may be involved in such electrostatic interactions. These findings are now consistent with, and provide a molecular explanation for, acidosis opposing fatigue

by decreasing the chloride conductance of skeletal muscle via inhibition of ClC-1. The modulation of ClC-1 by ATP is a key component of this molecular mechanism.

584-Pos Modulation Of hClC-4 Anion/ Proton Exchanger By External Anions

Alexi K. Alekov, Christoph Fahlke

Medizinische Hochschule Hannover, Hannover, Germany.

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hClC-4 is an anion/proton antiporter belonging to the ClC family of anion channels and transporters. We expressed hClC-4 in mammalian cells and used a combination of whole-cell patch-clamp recordings with ratiometric BCECF fluorimetry to separate anion and proton fluxes. ClC-4 mediates anion and proton currents that both display identical voltage dependence and rectification. The anion to proton transport ratios depend on the external anion, i.e1.6±0.2 for Cl^-, 4.7±0.8 for NO_3^- and 11.0±2.8 for SCN^-. Determining anion and proton currents at varying external concentration of Cl^-, NO_3^- and SCN^- revealed a correlation between the external anion binding affinity and transport stoichiometry. Stronger anion binding results in higher proton to anion transport ratios. In mixtures of Cl^- and SCN^-, steady-state whole-cell currents decrease with increasing concentrations of Cl^-. The current decrease was accompanied by an increase in the proton transport with the same Cl^- concentration dependence. To investigate the mechanism underlying these effects, we performed power spectra and non-stationary noise analysis on hClC-4 mediated ionic currents. Noise analysis revealed two transport modes with dramatically different transport rates, in agreement with the existence of distinct transporter and channel modes. The observed chloride block of SCN^- currents is not caused by a change of the single channel amplitude of the channel mode, but by an increased number of hClC-4 proteins operating as transporter and a decreased number of proteins functioning as channel. Our results suggest a dynamic switch between channel and transporter mode of hClC-4 operation modulated by the binding affinity of the transported anion.

585-Pos Sarcolemmal-restricted Localization of Functional ClC-1 Channels in Mouse Skeletal Muscle

John D. Lueck, Ann E. Rossi, Robert T. Dirksen

University of Rochester, Rochester, NY, USA.

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The voltage-dependent chloride channel ClC-1 stabilizes the skeletal muscle resting membrane potential. ClC-1 channels are hypothesized to prevent myotonia by "short-circuiting" depolarization that results from potassium accumulation in the t-tubule during repetitive action potentials. To determine the subcellular localization of ClC-1, we used the whole-cell patch clamp technique paired with formamide detubulation of flexor digitorum brevis (FDB) muscle. Sarcolemmal localization of ClC-1 channels was confirmed by paired experiments whereby ClC-1 currents were measured in 15–16 day naïve FDB fibers before and after detubulation by the addition and rapid removal of 2M formamide. Despite a 41 ± 3% decrease in total cell capacitance following formamide removal, peak steady state ClC-1 current magnitude was not different between naive (14.6 ± 2.0 nA, n=10) and detubulated (14.6 ± 1.7 nA, n=10) FDB fibers. Unpaired populations of naïve and detubulated 15–16 day FDB fibers demonstrated a similar 35% reduction in total cell capacitance in the absence of a change in ClC-1 instantaneous (−38. 4 ± 4.5 nA and 42.0 ± 5.1 nA, respectively) and steady state (7.8 ±1.3 nA and 8.4 ± 1.4 nA, respectively) current magnitude, the voltage dependence of channel activation (V1/2 was −60.0 ± 7.5 mV, n=20 and −64.6 ± 13 mV, n=18, respectively) or deactivation kinetics. The ability to detubulate FDB fibers was confirmed by loading fibers with di-8-ANEPPS. Naïve fibers exhibited robust sarcolemma and t-tubule di-8ANEPPS fluorescence while primarily only sarcolemmal fluorescence was observed following detubulation with formamide. In contrast to previously published studies, these results suggest that the majority of functional ClC-1 channels in mouse FDB fibers are localized to the sarcolemma. The sarcolemmal restricted localization of functional ClC-1 channels has profound implications for the role of ClC-1 conductance in stabilizing skeletal muscle membrane excitability.

586-Pos DIDS Hydrolysis Products Inhibit Eukaryotic and Prokaryotic CLCs

Kimberly Matulef, Andrew E. Howery, Li Tan, Justin Du Bois, Merritt Maduke

Stanford University, Stanford, CA, USA.

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DIDS (4,4-diisothiocyanatostilbene-2,2′-disulfonic acid) has been used as an anion transport inhibitor for nearly half of a decade. DIDS has previously been reported to inhibit the eukaryotic chloride channels ClC-0 and ClC-Ka and the prokaryotic homolog ClC-ec1. In our investigation of the stability of DIDS in aqueous solution, we found that DIDS hydrolyzes completely to form several different products. To our surprise, we found that this hydrolyzed DIDS mixture inhibits both ClC-0 and ClC-ec1 better than fresh DIDS. We purified and putatively identified the five major peaks of the hydrolyzed DIDS solution. Using the patch-clamp technique and chloride flux assays, we determined the effects of each purified peak on ClC-0 and ClC-ec1. For both proteins, we found that Peak 1 does not inhibit, Peaks 2 and 3 weakly inhibit, and Peaks 4 and 5 act as potent inhibitors. Inhibition of ClC-0 by Peaks 4 and 5 bears similarity to the inhibition observed with DIDS: it occurs specifically from the intracellular side and is not reversible within 5 minutes of washing. This brings up the interesting possibility that small amounts of the more potent DIDS hydrolysis products (Peaks 4 and 5) might in fact be responsible for previously reported inhibition of ClC-0 (and perhaps other anion transporters) by DIDS. We are currently working to test whether these DIDS hydrolysis products also inhibit ClC-Ka. These new inhibitors may be the most potent inhibitors yet known for the CLC proteins.

587-Pos High-Throughput Screen for Activators and Inhibitors of the Skeletal Muscle Chloride Channel ClC-1

Aaron S. Miller, Merritt Maduke

Stanford University, Stanford, CA, USA.

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There is currently a dearth of high-affinity inhibitors and activators of the CLC type chloride channel family. Specific inhibitors have played an integral role in the characterization of cation channels and would be powerful pharmacological tools for characterizing CLCs. A high-throughput screen (HTS) is being designed to discover novel, high-affinity modulators of ClC-1 activity. ClC-1, which is the best studied of the nine mammalian CLC homologs, provides the chloride conductance required to stabilize the skeletal muscle membrane potential and repolarize the cell after an action potential. Genetic defects in ClC-1 lead to myotonia, a condition in which the ability of muscle membrane to repolarize after cessation of voluntary contraction is hindered, thus delaying muscle relaxation. Compounds that activate ClC-1 may reverse such defects and thus may provide lead compounds for development of novel therapeutics to treat myotonia.

Our HTS-compatible assay of ClC-1 activity takes advantage of a mutant version of yellow fluorescent protein (YFP) as an anion sensor (Galietta et al., FEBS Lett. 2001; Galietta et al., Am J Physiol Cell Physiol 2001). The fluorescence of this YFP mutant is quenched by anions, but is more sensitive to nitrate than to chloride. Since ClC-1 is permeable to both chloride and nitrate, this YFP mutant can be used to monitor exchange of chloride and nitrate through ClC-1. We have generated a CHO cell line that stably expresses YFP and ClC-1. Channel function will be measured in 96-well plates by monitoring the decrease of YFP fluorescence upon addition of nitrate to the extracellular solution. The results from preliminary experiments will be presented.

588-Pos Modelling The Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)

Anthony Ivetac[1], Chris Alexander[2], Xuehong Liu[2], David C. Dawson[2], Mark S. Sansom[3]

[1] *University of California, San Diego, La Jolla, CA, USA*
[2] *Oregon Health & Science University, Portland, OR, USA*
[3] *University of Oxford, Oxford, United Kingdom.*

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The cystic fibrosis transmembrane conductance regulator (CFTR) is a mammalian anion-selective channel, the dysfunction of which leads to cystic fibrosis or secretory diarrhoeas. A member of the ATP-binding cassette (ABC) transporter family, CFTR mediates the passive movement of chloride ions across epithelial cell membranes and is crucial for salt transport. Despite a wealth of experimental data on CFTR, there is an unfortunate lack of three-dimensional structural information to help interpret the results and define the physical basis for anion conduction. The aim of this study is to develop an atomic-scale three-dimensional structural model of CFTR which one can relate to existing experimental results and which can serve to prescribe new studies. We describe the generation of a CFTR model, by constructing a homology model based on the bacterial ABC transporter template of SAV1866. This model has been evaluated by comparison with a range of experimental data and computational analysis (including electrostatics calculations). The results reveal encouraging correlations with an experimental characterisation of a putative pore-lining helix and confirm the anion-selectivity of the model. Further examination of the model reveals an asymmetrical electrostatic potential which suggests anions may be attracted to one side of the pore and identifies a possible extracellular cation binding site.

589-Pos Effects of W401 Mutations on The Stability of the Open State in CFTR Gating

Ming-Feng Tsai, Hiroyasu Shimizu, Yoshiro Sohma, Min Li, Tzyh-Chang Hwang

University of Missouri, Columbia, MO, USA.

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Opening of CFTR chloride channels by ATP is associated with dimerization of CFTR's two NBDs in a head-to-tail configuration. Our previous studies have shown that two NBDs play distinct roles in CFTR gating kinetics. ATP binding to NBD2 is critical for channel opening while ATP binding to NBD1 stabilizes the open state. We further tested this latter idea by using pyrophosphate (PPi), which locks open the channel in a stable open state presumably by preventing ATP hydrolysis at NBD2. Macroscopic CFTR currents in excised inside-out patches were activated by cytoplasmic application of PKA and ATP. Subsequent application of ATP (2 mM) and PPi (2 mM) further increased the current. Upon removal of ATP and PPi, both WT and W401G channel currents showed biphasic relaxations. The fast phase and slow phase time constants of WT-CFTR are significantly larger than those of W401G mutants (τ_{fast}=619 +/− 155ms τ_{slow}= 31.5 +/− 2.1s for WT and τ_{fast}=270 +/− 50ms τ_{slow}= 14.3 +/− 1.6s for W401G). In addition, the fraction of the slow component for W401G is significantly smaller than that of WT (0.81+/−0.05 for WT and 0.27+/−0.042 for W401G). When ATP was replaced with N^6-phenylethyl-ATP (50 μM), a high-affinity ATP analogue, the time constants for current relaxation are increased (τ_{fast}= 1.34+/−0.23s τ_{slow}= 64.7 +/− 4.2s for WT and τ_{fast}=747+/−85ms τ_{slow}= 42.3 +/− 4.4s for W401G). Moreover, P-ATP also increased the slow component fraction of W401G by twofold (0.46 +/− 0.046). These results confirm the importance of nucleotide binding at NBD1 in stabilizing the open state. We are currently characterizing other W401 mutations.

590-Pos CFTR Structural Dynamics During Channel Gating

Andrei A. Aleksandrov, J. R. Riordan

University of North Carolina, Chapel Hill, NC, USA.

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There are limited means to detect the allosteric transitions postulated to occur during CFTR channel gating. While CFTR catalyses the irreversible hydrolysis of its physiological ligand (ATP), there is disagreement about whether CFTR channel gating is a reversible process (Csanady et al., 2006; Aleksandrov et al. 2007). Linear Free Energy Relationship (LFER) analysis which is applicable only to processes exhibiting microscopic reversibility has been successfully applied to ligand gated channels (Grosman et al., 2000). Not only can this approach provide information about conformational motion associated with gating activity but it can also serve as an independent criterion of microscopic reversibility. To test the feasibility of the application of Φ value analysis to CFTR gating, we first analyzed gating of single CFTR channels stimulated with different nucleotide ligands. The log-log graph of opening rate constants versus the equilibrium constants is linear, confirming that CFTR channel gating occurs via reversible pathway. An irreversible mechanism with different pathways for opening and closing should yield a curved rather than a linear relationship. The fact that the Φ value is approximately 1 indicates that all conformational transitions in the binding site are essentially complete before channel opening. Different CFTR structural domains also were tested by LFER analysis. Comparison of the Φ values in each case enabled postulation of a conformational wave propagated from the ligand binding site to the channel pore. This approach can complement other methods of characterizing the dynamic events involved in the allosteric coupling between CFTR's two principal activities.

References

Aleksandrov et al., 2007 Pflugers Arch. *453*(5):693–702;
Csanady et al., 2006 J. Gen. Physiol. *128*(5):523–33;
Grosman et al., 2000 Nature*403*:773–6.

Mechanosensitive Channels

591-Pos Effect Of High Hydrostatic Pressure And Trimethyl N-oxide On Gating Of The Mechanosensitive Channel Of Small Conductance Of *E. Coli*

Evgeny Petrov, Paul R. Rohde, Boris Martinac

The University of Queensland, Brisbane, Queensland, Australia.

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The activity of MscS, the bacterial mechanosensitive ion channel of small conductance, has been investigated under high hydrostatic pressure (HHP) using the "flying-patch" patch-clamp technique. In inside-out excised patches of giant spheroplasts of E. coli, MscS was activated by negative pipette voltage to allow for open probability measurement at different levels of HHP up to 90 MPa. MscS open probability was found to gradually decrease on increasing HHP. To determine the extent that the cytoplasmic and transmembrane domains of the channel may contribute to this effect, the osmolyte methylamine N-oxide (TMAO) was applied to the cytoplasmic side of the excised spheroplast membrane patches. In the presence of TMAO the inhibitory effect of HHP on MscS activity was suppressed at pressures of up to 50 MPa. Above 50 MPa, channel open probability decreased similarly in absence or in presence of TMAO indicating that at pressures higher than 50 MPa, TMAO at concentrations used in this study could not counteract the effect of HHP on the MscS channel activity. The change in the reaction volume calculated in the presence of TMAO differs significantly from the reaction volume calculated in absence of TMAO. Our study suggests that TMAO can stabilize the open state of the MscS channel at HHP, most likely by interacting with the cytoplasmic domain of MscS.

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592-Pos Non-channel Function Of Bacterial Mechanosensitive Channel MscS

Wojciech Grajkowski[1], Marcin Balcerzak[1], Ehud Y. Isacoff[2], Andrzej Kubalski[1], Piotr Koprowski[1]

[1] *Nencki Institute of Experimental Biology, Warsaw, Poland*
[2] *University of California, Berkeley, CA, USA.*

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MscS is an *E.coli* mechanosensitive (MS) channel with homologs found in other bacteria, archea and plants. MS channels in bacteria jettison osmolytes and *E.coli* cells lacking them lyse under osmotic downshocks. MscS is a homoheptamer with a large cytoplasmic domain of unknown function. The domain is mobile upon gating, suggesting that it may undergo state-dependent interaction with binding partners. In effort to identify possible interaction partners, we fused a part of the cytoplasmic region (residues 171–266, $\alpha\beta_3\alpha$ domain) to GST. Using a GST pull-down assay and mass spectrometry we identified FtsZ as a protein that binds specifically to $\alpha\beta_3\alpha$.

FtsZ is a main component of cell division apparatus and forms Z-rings. FtsZ assembly plays an important role in the control of cell division, and its inhibition leads to cell filamentation. Recently, it has been shown that FtsZ is also involved in cell wall synthesis. We found that overexpression of the $\alpha\beta_3\alpha$ domain of MscS, but not of the full-length MscS, results in cell filamentation. Similarly, overexpression of a truncated MscS channel (MscSΔ266) results in cell elongation. MscSΔ266 was previously identified as a channel with impaired inactivation-closure transition (Schumann *et al.*, 2004, FEBS Letters *572*, 233–237). We hypothesize that FtsZ interacts with MscS, via the channel's cytoplasmic domain, and that this interaction occurs selectively in the open or inactivated conformation of the channel. We propose a model in which MscS transiently arrests progression of cell division in osmotically unfavorable conditions.