concentrations in accordance with LD50. Membranes were incubated with venom 10 min before adding ATP (8 mM). The enzyme activity due to Mg$^{2+}$ alone as defined as Mg-ATPase activity is subtracted from that due to Na$^+/K^+$ and Mg$^{2+}$, and due to Ca$^{2+}$ and Mg$^{2+}$ to obtain Na/K-ATPase and Ca-ATPase activity respectively. It was shown that Na$^+/K^+$ ATPase activity in erythrocytes membranes was increased in the presence of the MLO venom (low concentration ~1.81 times, sub-lethal concentration ~3.83 times and lethal concentrations ~4.28 times respectively). Under these conditions Ca$^{2+}$-ATPase activity was decreased (low concentration ~3.37 times, sub-lethal and lethal concentrations ~17.93 times respectively). We also studied Mg$^{2+}$ ATPase activity. In this case Mg$^{2+}$ ATPase activity was not dependent on concentration. These results suggest that ATPase activity is very sensitive to venom components and venom influence leads to possible conformation changes in ATPases.

doi:10.1016/j.bbabio.2014.05.263

S10.P9

Stochastic modelling of neuronal membrane glutamate transporters
Olga A. Kofanova, Stanislav E. Boronovskiy, Yaroslav R. Nartsissov
Institute of Cytochemistry and Molecular Pharmacology, Russian Federation
E-mail: olga.kofanova@gmail.com

The membrane glutamate transporters are expressed in various tissues but are of the most importance in the brain. Thus, they precisely define termination of excitatory neurotransmission in glutamatergic neurons and prevent brain tissue from glutamate induced excitotoxicity. This transporters belong to solute carrier 1 (SLC1) family and appeared to be secondary active transporters, which use cotransport of one Na$^+$ ion and one H$^+$ ion as driving force for taking up one glutamate molecule into the cell against its concentration gradient, as accepted for human transporter subtypes. Specified stoichiometry results in a total movement of two positive charges into the cell for each transport cycle, so transmembrane potential can also act as a driving force and transport process called electrogenic. Appropriate glutamatergic neurotransmission is essential for the most aspects of normal central nervous system functioning, such as cognition, memory and learning. Glutamate concentration maintenance under excitotoxic physiological level also plays major role in the CNS development, including synapse induction and elimination, cell migration, differentiation and death. So, function impairing or reduction of membrane glutamate transporters results on many CNS diseases and disorders. In order to clarify kinetic properties of single neuronal glutamate transporter stochastic modeling algorithm was proposed. It consists of several logical blocks and is based on probabilities of elemental steps during the neurotransmitter transport cycle such as substrate binding and translocation across the membrane. As sequence of these elemental steps is still under discussion, our approach is capable of its varying. Virtual computer experiments can also be carried out under different environmental conditions such as sodium, potassium and glutamate concentrations, and pH. The structural properties of the protein are implicit in a probabilities value, which generally derived from equilibrium constants of each elemental reaction or differences of free energies of substrate binding. That’s why the whole procedure is less time consuming as many other approaches and affords an opportunity to insight into membrane glutamate transporter functioning mechanism in more detail. Thus it becomes possible to evaluate relevance of evidence of transitional sodium binding site existence.

doi:10.1016/j.bbabio.2014.05.264

S10.P10

Two multi-subunit cation/proton antiporters have major roles in the bacterial pathogen *Staphylococcus aureus*
Terry Kruulwich$^a$, Manisha Vaish$^b$, Stephanie Christie$^c$, Victor J. Torres$^3$, Francis Alonzo$^b$, Tamara Reyes-Robles$^b$, Alexa Price-Whelan$^b$, Jun Liu$^c$
$^a$Department of Pharmacology & Systems Therapeutics, USA
$^b$Department of Microbiology, USA
$^c$Department of Pharmacology & Systems Therapeutics, USA
E-mail: terry.kruulwich@mssm.edu

*Staphylococcus aureus*, a major pathogen, has two 7-subunit Mrp-type cation/proton antiporters, Mnh1 and Mnh2, which function as proton motive force-dependent antiporters. Mnh1 was earlier shown to catalyze Na$^+$/Li$^+$ antiport in membrane vesicle assays using antiporter-deficient *E. coli* strain KNabc. Mnh2 was noted later and has not been analyzed for catalytic capacity or physiological roles. We show that Mnh2 exhibits both Na$^+$/Li$^+$ and K$^+$/H$^+$ antiport in vesicle assays, with greatest activity at pH ≥ 8.5; higher than the Mnh1 optimum of ~pH 7.5. No mnh1 gene disruptions were found in a recent screen by Fey et al. for non-essential *S. aureus* genes, using strain JE2, whose progenitor strain is *S. aureus* CA-MRSA USA300 LA clone (LAC). This raised the possibility that Mnh1 is essential, but we found a possible confounding issue, i.e., a transposition in LAC and JE2 that inactivates Mnh2, and concomitantly produces deficits at high K$^+$ and/or elevated pH relative to reference strain *S. aureus* FPR3757. We used *S. aureus* SH1000 and Newman strains, which have identical antiporter sequences but different lineages, to test whether viability depends on at least one functional Mnh antiporter. In SH1000, the double mutant is viable but is completely inhibited by low sodium concentrations, and its sensitivity to high osmolarity and pH are increased beyond those of each single mutant. In *S. aureus* Newman, only single deletions could be made, but neither Mnh1 nor Mnh2 are “essential”. The Δmnh1 strain exhibits large deficits in salt- and alkali-tolerance, and is highly attenuated in vivo in a murine model of bloodstream infection. In contrast, the ΔmnhA2 strain exhibits deficits in osmore-tolerance in vitro, but no detectable phenotype in the bloodstream infection model. Together, these findings support the notion that the Mnh1 and Mnh2 antiporters of *S. aureus* are critical for the physiology of this organism and at least Mnh1 contributes to the pathogenesis process.

doi:10.1016/j.bbabio.2014.05.265

S10.P11

Ion channel path of cellular transduction
Elena Lacatus
Polytechnic University of Bucharest, Romania
E-mail: elena.lacatus@upb.ro

A better understanding of the selective responses of the ion channels to the multiple concurrent influential parameters of both internal and external environments, can address, complete and refine the existing ion channels models. Widely distributed class of P-type ATPase sodium pump is responsible for the active transport of a variety of cations across cell membranes. The main basic function of the sodium pump is to maintain the Na$^+$ and K$^+$ gradients across the plasma membranes. Thus, membrane potential, nutrient uptake, intracellular volume and pH are regulated by the proper function of the sodium pump. Consequently, the wide varieties of ion channels are related to their functional source of energy, opportunistically harvesting all available stimuli. One of these
stimuli is the acoustic vibration mechanically exciting the entire body, but differentiates for each type of tissue, structure or system. Considering that the acoustic stimuli are nonlinear and the body exposure to acoustic field is differentiate on report of time, density of energy and structural acoustic impedance of tissues, than a more realistic human exposure to acoustic stimuli model could be obtained. This paper, consistent with the Fröhlich theory [1] aims to define a Molecular Dynamics (MD) model of Na\(^+\), K\(^+\)-ATPase designed to predict not only the coherent elasto-electric oscillations of electric polar cellular structures but the influence of the environmental stimuli (acoustic and thermal vibrations) as well. The transducer role is played by the excitable amino acid chains of the proteins from the ion channels. Their density of energy is continuously changing according to the weak vibrations and rotations of these basic live modules during the harvesting energy process. The MD model and the in vivo and in vitro validation experiments [2] reveal quantifiable similarities between the periodic characteristics of voltage activation of ionic pumps (Na\(^+\)–K\(^+\)) through ion channels and the periodic acoustic wave propagation throughout the cells. The rhythm mimetic behavior of heart rate to nonlinear acoustic stimuli (music) is largely known, and this study reveals that it may actually originate within the cellular and neuronal mecanoelectric transduction.

References

doi:10.1016/j.bbabio.2014.05.266

S10.P12

Antagonists of tubulin–VDAC interaction induce oxidative stress and mitochondrial dysfunction
David N. DeHart\(^a\), Monika Gooz\(^b\), Tatiana K. Rostovtseva\(^b\), Kely L. Sheldon\(^a\), John J. Lemasters\(^a\), Eduardo N. Maldonado\(^a\)
\(^a\)Medical University of South Carolina, USA
\(^b\)National Institute of Child Health and Human Development, USA
\(^c\)Johns Hopkins University, USA
E-mail: JJLemasters@musc.edu

BACKGROUND: Mitochondrial oxidative phosphorylation, membrane potential (\(\Delta\Psi\)) formation and generation of reactive oxygen species (ROS) require flux of metabolites into mitochondria through voltage dependent anion channels (VDAC). Free tubulin reversibly blocks VDAC both in vitro and in cells. Erastin, a small molecule lethal to cancer cells, antagonizes blockade of VDAC by tubulin and upregulates mitochondrial metabolism. We hypothesized that erastin and related “erastin-like” compounds open VDAC, increase mitochondrial metabolism and ROS formation, and activate JNK, which in turn cause mitochondrial dysfunction and cell death. Our AIM was to evaluate the effects of erastin/erastin-like compounds on \(\Delta\Psi\), NAD(P)H, ROS, JNK and cell killing.

METHODS: Using confocal fluorescence microscopy, \(\Delta\Psi\) was assessed with tetramethylrhodamine methylester (TMRM) and ROS with Mitotracker Red and chloromethyl dichlorofluorescein (cmDCF). Autofluorescence of mitochondrial NAD(P)H was assessed by multiphoton microscopy. Total and phosphorylated JNK was determined by immunoblotting; Cell death was monitored by propidium iodide fluorescence microscopy.

RESULTS: In lipid bilayers, erastin reversed and prevented tubulin inhibition of VDAC. In HepG2 human hepatocarcinoma cells, erastin increased \(\Delta\Psi\) by 46% and NAD(P)H by 30%, beginning within 30 min. Subsequently, mitochondria depolarized (3–4 h), indicating mitochondrial dysfunction. Erastin-like compounds X1 and X2 were identified in a high-throughput screening and similarly caused mitochondrial hyperpolarization/depolarization. As mitochondria hyperpolarized, ROS formation increased, which was then followed by mitochondrial depolarization and cell death. In addition, erastin activated JNK (maximal pJNK at 60 min). JNK activation and ROS formation both preceded mitochondrial depolarization and cell death.

CONCLUSION: Erastin and erastin-like compounds reverse tubulin-dependent inhibition of VDAC conductance, leading to mitochondrial hyperpolarization, increased ROS production and activation of the stress kinase JNK. These events appear to induce mitochondrial dysfunction, onset of the mitochondrial permeability transition, and ultimately cell death.

doi:10.1016/j.bbabio.2014.05.267

S10.P13

The discovery of functionally diverse membrane pyrophosphatase subfamilies
Heidi H. Luoto\(^a\), Erika Nordbo\(^b\), Alexander A. Baykov\(^b\), Reijo Lahti\(^c\), Anssi M. Malinen\(^d\)
\(^a\)Department of Biochemistry, University of Turku
\(^b\)Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Russian Federation
E-mail: hhluot@utu.fi

Membrane pyrophosphatases (mPPases) transport H\(^+\) and Na\(^+\) through membranes by harnessing the energy of pyrophosphate hydrolysis, thus creating gradients of these ions across membranes that enable ATP synthesis and secondary transport. Predominantly \(\alpha\)-helical mPPase homodimers, formed from ~75 kDa monomers, reside in the membranes of plants, bacteria, archaeabacteria, and protists [1]. Until the discovery of three Na\(^+\)–transporting PPases in 2007 [2], mPPases had generally been thought to be \(\alpha\)-translocators. To elucidate the previously obscure functional versatility of mPPases, we selected representative mPPases of different clades based on their phylogenetic relationships and characterized them. We found that Na\(^+\)-PPases form a single clade on the phylogenetic tree, whereas H\(^+\)-PPases constitute multiple branches that diverged from Na\(^+\)-PPases on multiple occasions via subtle amino acid changes [3]. These data provide evidence that Na\(^+\)-PPases are an ancestral form of the transporter and support the theory that Na\(^+\)-based bioenergetics evolved before H\(^+\)-based bioenergetics. Furthermore, we discovered that Na\(^+\)-PPases are able to transport H\(^+\) at subphysiological Na\(^+\) concentrations [4] and identified a novel mPPase subfamily capable of transporting both Na\(^+\) and H\(^+\) at physiological Na\(^+\) concentrations [5]. Functional and mutational analyses, together with structural information, allowed us to pinpoint Glu and Lys as a specificity-determining gate. Based on the available data, we created an algorithm to predict mPPase transport specificity from the amino acid sequence.

References