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 elastoelectric 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 ion channels and mitochondrial dysfunction.

**References**


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**S10.P12**

**Antagonists of tubulin–VDAC interaction induce oxidative stress and mitochondrial dysfunction**

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**BACKGROUND:** Mitochondrial oxidative phosphorylation, membrane potential (ΔΨ) 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 ΔΨ, NAD(P)H, ROS, JNK and cell killing.

**METHODS:** Using confocal fluorescence microscopy, ΔΨ was assessed with tetramethylrhodamine methylster (TMRM) and ROS with MitoSOX 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. 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 ΔΨ 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, erasin 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.

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**S10.P13**

**The discovery of functionally diverse membrane pyrophosphatase subfamilies**

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Membrane pyrophosphatases (mPases) 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 α-helical mPase homodimers, formed from ~75 kDa monomers, reside in the membranes of plants, bacteria, archaeabacteria, and protists [1]. Until the discovery of three Na\(^+\)-transporting mPases in 2007 [2], mPases had generally been thought to be H\(^+\)-transporters. To elucidate the previously obscure functional versatility of mPases, we selected representative mPases of different clades based on their phylogenetic relationships and characterized them. We found that Na\(^+\)-mPases form a single clade on the phylogenetic tree, whereas H\(^+\)-mPases constitute multiple branches that diverged from Na\(^+\)-mPases on multiple occasions via subtil amino acid changes [3]. These data provide evidence that Na\(^+\)-mPases 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\(^+\)-mPases are able to transport H\(^+\) at subphysiological Na\(^+\) concentrations [4] and identified a novel mPase 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 mPase transport specificity from the amino acid sequence.

**References**