(RyR2) function via regulation of both $[Zn^{2+}]_i$ and $[Ca^{2+}]_i$ homeostasis in hyperglycemic cardiomyocytes. We used freshly isolated cardiomyocytes from a rat model of chronic diabetes induced by streptozotocin either treated or untreated with an antioxidant N-acetyl cysteine (NAC; 1.5 mg/kg, daily; for 4 weeks). We used cytosolic Ca^{2+} and Zn^{2+} dyes (Fluo-3AM and FluoZin-3AM, respectively) to measure both Zn^{2+} and Ca^{2+} sparks, their transient changes under electrical or caffeine stimulation, and the resting levels of both $[Zn^{2+}]_i$ and $[Ca^{2+}]_i$ in the loaded cells. We obtained significantly increased resting levels of $[Zn^{2+}]_i$ and $[Ca^{2+}]_i$, impairments in the parameters of both global and transient fluorescence changes in diabetic cardiomyocytes while these were found to be preserved in the *in vivo* and *in vitro* NAC-treated diabetic cardiomyocytes. Free protein-sulfhydryls in isolated diabetic cardiomyocytes were measured to be significantly decreased compared to those of the both controls and NAC-treated diabetics. Furthermore our results have demonstrated that intracellular Zn^{2+} induces marked phosphorylation and dysfunction in the RyR2, while these were preserved with NAC-treatment. Overall, the present data suggest that the changes under hyperglycemia not only in $[Ca^{2+}]_i$ but also $[Zn^{2+}]_i$ play important role in RyR2 function, in part via hyperglycemia-induced increased oxidative stress and depressed antioxidant-defence system in cardiomyocytes. (Supported by TUBITAK-SBAG-109S267&111S042)

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Evaluation of On-Chip Quasi-In Vivo Cardiac Toxicity Assay for Direct Prediction of TdP Occurrence using Closed-Loop-Shaped Cardiomyocyte Network

Fumimasa Nomura, Tomoyuki Kaneko, Tomoyo Hamada, Kenji Yasuda.
Tokyo Medical & Dental University, Tokyo, Japan.

Re-entry of excitation in the heart is one of the abnormal phenomena to cause the lethal arrhythmia such as torsade de pointes (TdP) and is thought to be induced by the looped structure of excitation conduction pathway. For adaptable *in vitro* preclinical strategies to evaluate global cardiac safety, an *on-chip* quasi-*in vivo* cardiac toxicity assay for lethal arrhythmia measurement using circuit-shaped cardiomyocyte network model has been developed. The electrocardiogram (ECG)-like field potential data, which includes the information both of the repolarization and of the conductance abnormality, was acquired from the self-convoluted extracellular field potential (FP) profiles of a lined-up cardiomyocyte network on a ring-shaped electrode in an agarose microchamber. When E-4031 (hERG blocker) or Astemizole (TdP positive but false-negative drug traditional *in vitro* assay) were applied to the closed-loop cardiomyocyte network, self-convoluted FP profile of normal beating changed into an abnormal waveform, including early afterdepolarization (EAD) like or TdP-like abnormal waveform at 1 μM in either case (2 out of 8 samples in E-4031 and 8 out of 10 samples in Astemizole), and especially followed by fibrillation-like arrhythmia and arrest in Astemizole. Self-convoluted FP duration prolongation and its fluctuation increase were also observed according to the increase of E-4031 concentration ($188 \pm 21\%$ prolongation of FPD corrected with beating rate and $168 \pm 42\%$ increase of STV at 1 μM, mean ± SE, n=6). The results indicate that the self-convoluted FPs of the ring-shaped cardiomyocyte network assay could be used for quasi-ECG measurement to estimate QT prolongation and arrhythmic potential including the effects on both repolarization process and the conductance abnormality of cardiomyocyte.

Membrane Transporters & Exchangers II

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Regulation of Ammonium Transport Proteins

Tobias Pflueger, Paula Schleberger, Philipp Lewe, Susana L.A. Andrade.
University of Freiburg, Freiburg, Germany.

Ammonium, the most reduced form of nitrogen is a primary nutrient for several organisms (archea, bacteria, fungi and plants) and its uptake from the environment is accomplished by dedicated Ammonium Transport (Amt) proteins. In mammals, Amts are crucial for acid-base and pH homeostasis processes in kidney and liver tissues and are generally called Rhesus proteins. Organized as stable trimers, Amt proteins span the membrane with 11 or 12 helices that surround a substrate translocation pore in each of the three monomers [1].
Regulation of assimilatory Amt transport by direct protein-protein interaction involves members of the P(II)-protein family, known as GlnK proteins [2]. GlnK sense the intracellular energy, nitrogen and carbon levels by direct binding effector molecules such as ATP, ADP and 2-oxoglutarate (2-OG) [3-6].
In spite of their highly conserved structure, P(II)-proteins vary strongly in their effector binding kinetics and in the resulting response modulation and interacting partners. The range of downstream target proteins, and the underlying, molecular regulatory processes are still not fully understood [2,7]. We are investigating the role of the three GlnK proteins of the hyperthermophilic euryarchaeon Archaeoglobus fulgidus, each of which is encoded in a transcriptional unit with a distinct amt gene for an ammonium transporter [1,5,6].
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Two Distinct Transport Mechanisms in AmtB and RhCG Proteins

Sefer Baday[1], Shihao Wang[2], Guillaume Lamoureux[2], Simon Bernèche[1].
[1]Biozentrum, basel, Switzerland, [2]Concordia University, Montreal, QC, Canada.

The Amt/Rh family of membrane proteins facilitates the diffusion of ammonium across cellular membranes. Functional data show that Amt proteins, notably found in plants, transport ammonium ion (NH4+) while human rhesus (Rh) proteins transport ammonia (NH3). Comparison between the X-ray structures of the prokaryotic AmtB and the human RhCG reveals important differences at the level of their pore. Despite these important functional and structural differences between Amt and Rh proteins, studies of the AmtB transporter have led to the conclusion that proteins of both sub-families work according to the same mechanism and all transport ammonia. We performed molecular dynamics simulations of the AmtB and RhCG proteins under different conditions. Our free energy calculations suggest that the probability of finding NH3 molecules in the pore of AmtB is negligible in comparison to water and refute a single file diffusion of ammonia in AmtB. The pore lumen of RhCG is found to be more hydrophobic due to the presence of a phenylalanine conserved among Rh proteins. Simulations of RhCG also reveal that one of the signature histidine residues is occasionally exposed to the extra-cellular bulk, which is never observed in AmtB. These quite different hydration patterns are consistent with the idea that permeation in Amt and Rh proteins are not functionally equivalent and take place according to two distinct mechanisms. In both cases, after binding to the histidine dyad, ammonium deprotonates and diffuse down the pore as ammonia. Hydration of the pore in Amt proteins allows proton diffusion, which results in electrogenic transport. Such mechanism cannot take place in Rh proteins due to lack of pore hydration. Our QM/MM simulations rather show that the excess proton is released back to the extracellular bulk through a network of H-bonds, resulting in net NH3 transport.

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Computational Investigation of Charge Transfer Mechanisms in Ammonium Transporters

Shihao Wang[1], Esam A. Orabi[1], Sefer Baday[2], Simon Bernèche[2],
Guillaume Lamoureux[1].
[1]Concordia University, Montreal, QC, Canada, [2]University of Basel, Basel, Switzerland.

The mechanism by which proteins of the Amt/MEP/Rh family transport ammonium remains largely unknown. No convincing mechanistic picture has emerged yet as to whether it is an electroneutral NH3 transport or an electrogenic $NH3/H^+$ co-transport. using free energy calculations with polarizable force fields and with hybrid QM/MM descriptions, we have investigated the binding of ammonium and its transport across the pore of Escherichia coli's AmtB protein. The simulations reveal a novel cotransport mechanism in which the NH4+ substrate binds deeply into the pore and translocates as separate NH3 and H^+ fragments. Critical to the cotransport mechanism is a pair of highly-conserved histidine residues, that enhance NH4+ binding, form a ''proton wire'' for charge transfer, and provide a scaffold for a water chain in the pore lumen.

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Multidrug Binding Properties of the AcrB Efflux Pump Characterized by Molecular Dynamics Simulations

Attilio Vittorio Vargiu[1,2], Hiroshi Nikaido[2].
[1]CNR-IOM, UOS SLACS, Cagliari, Italy, [2]Department of Molecular and Cell Biology, University of California, Berkeley, CA, USA.

Multidrug resistance in Gram-negative bacteria, to which multidrug efflux pumps such as AcrB makes a major contribution, is becoming a major public health problem. Unfortunately only a few compounds have been cocrystallized with AcrB, and thus computational approaches are essential in elucidating the interaction between diverse ligands and the pump protein. We used molecular dynamics simulation to examine the binding of 9 substrates, 2 inhibitors, and 2