Christine Simmons, Kathryn Swoboda, Kevin Ess, Alternating Hemiplegia of Childhood (The research was supported by grants CZ.1.07/2.3.00/30.0041 and LO1304)

Quercetin is a flavonoid, proposed to be capable of modulating mitochondrial activity and thereby exerting biological effects, including signaling by reactive oxygen species production. These effects are instrumental to ischemia-reperfusion induced changes in the heart. We tested the effect of quercetin on respiration of whole H9c2 cells, a model cell line derived from cardio-myoblasts. Brief treatment with quercetin up to 25μM had no effect, while 24-hour treatment with quercetin caused a decrease in the routine respiration relative to vehicle (<0.05) and an increase in maximum respiration relative to vehicle (<0.01). To measure changes in respiration of H9c2 cells immediately following ischemia, we treated the cells just prior to 60 minutes of simulated ischemia. There was no significant difference between treated and untreated ischemic cells. However, the routine coupling control ratio (CCR) of ischemic and untreated ischemic cells was significantly lower than that of ischemic cells treated with vehicle (<0.05). We also monitored the changes in respiration of intact H9c2 cells in DMEM after 3 hours of simulated ischemia and 3 hours of reperfusion. Quercetin treated ischemic cells demonstrated significantly lower routine CCR (P<0.05). Likewise, quercetin treated ischemic cells showed higher non-phosphorylating resting respiration than vehicle treated ischemic cells (P<0.05). Hence, data suggest that quercetin, below 25μM, behaves as a mild uncoupler of mitochondrial respiration. To test this hypothesis we treated isolated rat heart mitochondria with sequent additions of 4μM quercetin. Quercetin indeed displayed an effect resembling that of an uncoupler. Furthermore, the uncoupling effect of quercetin was diminished by using bongkrekic acid and carboxyatractyloside, two known inhibitors of adenine nucleotide transporter (ANT). Our data support the role of quercetin as a positive regulator of ischemic changes via ANT-dependent uncoupling.

(Received was supported by grants CZ.1.07/2.3.00/30.0041 and LO1304)

Impaired Cell Surface Expression of ATP1A3 Mutations Associated with Alternating Hemiplegia of Childhood

Christine Simmons, Kathryn Swoboda, Kevin Ess, Alfred George

1Pediatrics and Neurology, Vanderbilt University, Nashville, TN, USA.
2Neurology, University of Utah, Salt Lake City, UT, USA.
3Pharmacology, Northwestern University, Chicago, IL, USA.

Mutations in ATP1A3 have been identified as the genetic cause of alternating hemiplegia of childhood (AHC). Loss of Na/K-ATPase activity has been proposed as the likely functional consequence of mutations, but specific mechanisms have not been demonstrated. We performed experiments designed to ascertain the functional and biochemical consequences of the three most common ATP1A3 mutations (D801N, E815K, G947R) associated with AHC. For functional studies, we adapted a fluorescence-based thallium uptake assay system to monitor time-dependent uptake into HEK-293 cells stably expressing either wildtype (WT) or AHC-associated mutant alleles of ATP1A3. A FLAG epitope was added to the distal carboxyl terminus to enable biochemical studies. Thallium update assays demonstrated greatly reduced function for the three AHC-associated mutations compared with WT-ATP1A3. Using cell surface biotinylation and immunofluorescence microscopy, we observed heterogeneity among the four ATP1A3 alleles with regard to plasma membrane expression. WT-ATP1A3 and D801N exhibited similar levels of cell surface expression, whereas both E815K and G947R had greatly reduced plasma membrane expression. These findings suggested that E815K and G947R may be impaired protein trafficking to surface membranes. A screen for small molecules that could correct impaired trafficking and restore cell surface expression of mutant pumps, identified four compounds with activity in the fluorescence-based thallium uptake assay system. In subsequent validation experiments, using cell surface biotinylation coupled with western blot analysis, two of the compounds including one with weak proteasome inhibitory activity exhibited partial rescue of plasma membrane expression. In separate proof-of-concept experiments, we demonstrated that the potent proteasome inhibitor bortezomib restored plasma membrane expression of E815K and G947R to levels similar to WT-ATP1A3. We conclude that reduced cell surface expression may contribute to ATP1A3 loss-of-function in AHC and may be reversible with proteasome inhibition.


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Simulating Free Radical Production from Complex I

Jason Bazili, Venkat Pannala, Ranjan Dash, Daniel Beard

1University of Michigan, Ann Arbor, MI, USA. 2Medical College of Wisconsin, Milwaukee, WI, USA.

Complex I is a respiratory pump (NADH:quinone oxidoreductase) in the electron transport chain of mitochondria that can produce a significant amount of reactive oxygen species (ROS) in the form of superoxide and hydrogen peroxide (H2O2) at the flavin mononucleotide (FMN), but the location of superoxide production is less certain. A computer model fit to kinetic data is used to determine the location and rates of ROS production. The model includes the major redox centers in the complex: the FMN, iron-sulfur cluster N2, and semiquinone. The analysis identifies that the fully reduced FMN and semiquinone are the major sources of superoxide with little to no production from the iron-sulfur cluster N2. Also, the FMN radical only produces superoxide when turnover at the quinone-reductase site is blocked. When electron flow through the complex is reversed, ROS production is maximized with the FMN and semiquinone producing similar amounts. Moreover, high membrane potentials and alkaline matrix pH stimulate ROS production. Of all the ROS produced, the majority originates from the FMN. The Complex I model is integrated into a larger model of the respiratory chain that includes ROS production from Complex III, and the relative rates of ROS production from each complex is determined under a variety of simulated conditions.

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Preliminary Characterization of VDAC3, an Elusive Member of the Outer Mitochondrial Membrane Porin Family

Simona Reina, Vanessa Guarnieri, Francesco Guarniro, Carlo Guardiani, Ramona Belfiore, Matteo Cecerelli, Ildiko Szabo, Vito De Pinto

1Biological Sciences, University of Catania, Catania, Italy. 2Biology, University of Padua, Padua, Italy. 3Istituto Officina dei Materiali, CNR, Cagliari, Italy. 4Physics, University of Cagliari, Catania, Italy. 5Biologo, University of Padua, Catania, Italy.

The VDAC (Voltage Dependent Anion selective Channel) family of proteins is composed of three evolutionary related isoforms in vertebrates [1-2]. Despite the sequence similarity, the functional data existing nowadays point to different functions for them. The isoforms are expressed at different levels [3], and show a different ability to complement the growth defect in yeast S. cerevisiae devoid of the endogenous porin [4], indicating that either they have different capacities to be translocated in the OMM or they have really different pore-forming activity, at least when expressed in S. cerevisiae [4-5]. We are trying to elucidate the functional role of VDAC3, the least abundant and most elusive member of the family, with various molecular and predictive approaches. In our hands the reconstitution of recombinant human VDAC3 gave origin to an unexpected pore-formation activity, with pores by far smaller than those of other isoforms [5]. The molecular dynamics analysis of the three isoforms does not support such activity in terms of predicted diameters [6]. The definition of the VDAC3 interactome [7] shows that various cytosolic or cytoskeletal proteins can influence the activity of the protein. Other evidence will be reported to explain this puzzling behavior of the VDAC3 isoform.

Acknowledgments: PRIN 2010CSJX4F (VDP, IS, MC).


1559-Pos Board B510

Molecular Origin of Ion Selectivity in Phaseolus Coccineus Mitochondrial VDAC

Eva-Maria Krammer, Hayet Sadani, Martine Prévost, Fabrice Homblé

Université Libre de Bruxelles, Brussels, Belgium.

The mitochondrial voltage-dependent anion-selective channel (VDAC) is the major permeation pathway for small ions and metabolites through the mitochondrial outer membrane. The deciphering of the mechanism underpinning ion selectivity among the four VDAC isoforms in vertebrates [1-2] is of great interest. While sequence similarity, at least when expressed in S. cerevisiae, among the four VDAC isoforms is high, their ionic selectivity are directly involved in respiration. Although a wealth of electrophysiological data points to a different ability to complement the growth defect in yeast S. cerevisiae devoid of the endogenous porin [4], indicating that either they have different capacities to be translocated in the OMM or they have really different pore-forming activity, at least when expressed in S. cerevisiae [4-5]. We are trying to elucidate the functional role of VDAC3, the least abundant and most elusive member of the family, with various molecular and predictive approaches. In our hands the reconstitution of recombinant human VDAC3 gave origin to an unexpected pore-formation activity, with pores by far smaller than those of other isoforms [5]. The molecular dynamics analysis of the three isoforms does not support such activity in terms of predicted diameters [6]. The definition of the VDAC3 interactome [7] shows that various cytosolic or cytoskeletal proteins can influence the activity of the protein. Other evidence will be reported to explain this puzzling behavior of the VDAC3 isoform.

Acknowledgments: PRIN 2010CSJX4F (VDP, IS, MC).