8L.4 The impact of mitochondrial ROS formation for epilepsy

Wolfram S. Kunz
Division of Neurochemistry, Department of Epileptology and Life & Brain Center, University Bonn, Germany
E-mail: wolfram.kunz@ukb.unionn.de

Mitochondrial ROS are implicated to be responsible for a large number of brain pathologies including neuronal cell loss observed in various forms of epilepsy. However, brain seizure activity is characterised by intense activation of mitochondrial oxidative phosphorylation. This stimulation of oxidative phosphorylation is in the low magnesium model of seizure-like events accompanied by substantial increase in formation of reactive oxygen species (ROS). It has remained unclear which ROS-generating site can be attributed to this phenomenon. Here, data are provided, which show stimulatory effects of calcium ions and uncouplers, mimicking mitochondrial activation, on ROS generation of isolated rat and mouse brain mitochondria. Since these stimulatory effects are visible with the superoxide sensitive dye hydroethidine, but with the hydrogen peroxide sensitive *p*-hydroxyphenylacetate only in the additional presence of SOD, it can be concluded that the complex redox properties of the 'Qo' center at respiratory chain complex III, delivering superoxide to the mitochondrial inter membrane space, are very likely responsible for these observations. In accordance with this hypothesis redox titrations of the superoxide production of antimycin-inhibited submitochondrial particles with the succinate/fumarate redox couple confirmed for brain tissue a bell shaped dependency with a maximal superoxide production rate at $+10$ mV (pH = 7.4). This reflects the complex redox properties of a semiquinone species which is the direct electron donor for oxygen reduction in complex III-dependent superoxide production. From these experiments it can be concluded that under conditions of increased energy load complex III site can contribute to superoxide production of brain mitochondria, which might be relevant for ROS production to the mitochondrial inter membrane space during epilepsy-related seizure activity. On the other hand, the ROS related damage of mitochondrial DNA detected in hippocampal samples of patients with temporal lobe epilepsy and Ammons horn sclerosis is very likely related to mitochondrial ROS produced by respiratory chain complex I in the mitochondrial matrix space.

doi:10.1016/j.bbabio.2010.04.227

8L.5 Mitochondrial quality control and membrane dynamics

Thomas Langer
Institute for Genetics, University of Cologne, Germany
Max-Planck-Institute for Biology of Aging Cologne, Germany
E-mail: Thomas.Langer@unioeln.de

Dysfunction of mitochondria has severe cellular consequences and is linked to aging and neurodegeneration in human. Several surveillance mechanisms have evolved which prevent the accumulation of non-functional mitochondria and ensure cellular integrity. Whereas irreversibly damaged mitochondria can be selectively removed by autophagy, various intraorganellar proteases degrade non-native mitochondrial proteins and limit mitochondrial damage. These include AAA proteases, conserved ATP-dependent metalloproteases in the inner membrane. AAA proteases exert dual activities within mitochondria: 1) they conduct protein quality control surveillance and degrade misfolded and damaged inner membrane proteins top peptides; and 2) they act as processing peptidases and regulate mitochondrial gene expression and dynamics. Two isoforms of *m*-AAA proteases which differ in their subunit composition have been identified in human mitochondria. AFG3L2 subunits form homooligomeric isoforms or assemble with homologous paraplegin subunits into heterooligomeric proteolytic complexes. Interestingly, heterozygous missense mutations in *AFG3L2* cause dominant hereditary ataxia SCA28, whereas mutations in paraplegin cause a recessive form of spastic paraplegia. The pathogenic mechanisms of these neurodegenerative disorders remained enigmatic. Increasing evidence points to an intimate link of *m*-AAA protease function to mitochondrial dynamics and to the dynamin-like GTPase OPA1. OPA1 is required for mitochondrial fusion, regulates cristae morphogenesis, and protects cells against apoptosis. Knockdown experiments in mammalian cells and the analysis of *Afg3l2*-null mutant mice revealed that the ATP-dependent proteolytic activity of AFG3L2 is required for the balanced formation of long and short forms of OPA1, a prerequisite for mitochondrial fusion. Stress conditions, like low ATP levels, result in the complete turnover of long OPA1 isoforms by the ATP-independent metallopeptidase OMA1, which is accompanied by an inhibition of fusion and a fragmentation of the mitochondrial network. The control of OPA1 stability by different peptidases and stress-induced mitochondrial fragmentation is emerging as an important process during mitochondrial quality control.

doi:10.1016/j.bbabio.2010.04.228

8L.6 Parkinson's disease-associated genes and mitochondrial integrity

A. Kathrin Lutz, Anna Pilsl, Lena Bouman, Jörg Tatzelt, Konstanze F. Winklhofer
German Center for Neurodegenerative Diseases (DZNE) & Adolf Butenandt Institute, Neurobiochemistry, Ludwig Maximilians University Munich, Germany
E-mail: konstanze.winklhofer@med.uniuenchen.de

Parkinson's disease (PD) is the most common movement disorder and the second most common neurodegenerative disease after Alzheimer's disease, affecting an increasing number of patients due to the demographic trend towards an aged population. Oxidative stress, mitochondrial dysfunction and protein aggregation are pathophysiological alterations consistently found in the course of the disease, however, the etiology of sporadic PD still remains enigmatic. Thus, the identification of genes which are reponsible for familial variants was a major breakthrough. Importantly, several PD-linked gene products have a direct or indirect impact on mitochondrial integrity, emphasizing a crucial role of mitochondria in the pathogenesis of PD. Loss-of-function mutations in the E3 ubiquitin ligase parkin or the mitochondrial kinase PINK1 are associated with autosomal recessive parkinsonism. Our previous work revealed that parkin is a stress-responsive protein with a remarkably wide neuroprotective capacity, preventing cell death under various stress conditions. An early consequence of parkin or PINK1 silencing in human cells is a decrease in mitochondrial membrane potential and ATP production and increase in mitochondrial fragmentation. Remarkably, parkin can increase the clearance of dysfunctional mitochondria by mitophagy in a PINK1-dependent manner. We will discuss the underlying mechanisms and present data indicative of a regulatory crosstalk between the autophagic machinery and mitochondrial dynamics.

doi:10.1016/j.bbabio.2010.04.229

Posters

8P.1 Initiating CoA biosynthesis system in brain mitochondria

Andrey G. Moiseenok, Valery A. Gurinovich, Inna N. Katkovskaya
Institute of Pharmacology and Biochemistry NASB, Grodno Branch, Belarus
E-mail: val@biochem.unibel.by

Studying the pathogenesis of neurodegeneration due to the genetic defect of pantothenate kinase, the key enzyme of coenzyme

A (CoA), classified as Pantothenate Kinase Associated Neurodegeneration (PKAN), resulted in identification of the enzyme mitochondrial form, PANK 2, that was distinguished in regulatory properties from the cytosolic CoA synthesis from pantothenic acid (PA). Coenzyme biosynthesis in CNS structures is determined by PA uptake from circulating blood and biotransformation to intermediate products, such as 4′-phospho-PA (PPA) and 4′-phospho-pantetheine (PPN). For the mitochondrial neuronal compartment, the pathway remains to be open for CoA biosynthesis from pantetheine (PN), exactly, the oxidized form, pantethine (PT). The experiments with intraperitoneal injections of [^{3}H]-PA or [^{3}H]-PT at dose of 1.1 mCi/kg to albino Wistar rats showed active transport (uptake) of both forms of CoA precursors by the large hemispheres that reached a maximum by 180 min (PT treatment) and 12 h (PA administration). The PT treatment was accompanied by radionuclide accumulation predominantly in the postmitochondrial fraction in which HPLC identified PPA as the main form (other PA components, PN, PPN and low amounts of CoA). The predominant metabolite in perchlorate mitochondrial extracts was PPA and, probably, dephospho-CoA. The [^{3}H]-PA administration caused biotransformation of the radionuclide to CoA whose fraction reached 46% after 30 min and 72% after 12 h of the total radioactivity level in perchlorates, whereas the radionuclide was not deposited in the mitochondrial PPA and PPN fractions and the post-mitochondrial supernatant. The PT administration can be suggested to cause PPA accumulation while the PA treatment — to form CoA in the CNS, which reflects the peculiarities of their neuroprotective activity. A high level of uptake and biotransformation of CoA biosynthetic precursors was found in the hippocampus.

doi:10.1016/j.bbabio.2010.04.230

8P.2 Analysis of mitochondrial DNA deletions in epileptic hippocampus

Viktoriya M. Peeva, Gábor Zsurka, Wolfram S. Kunz
Division of Neurochemistry, Dept. Epileptology and Life & Brain Center, University Bonn, Germany
E-mail: vik_peeva@hotmail.com

Mitochondrial dysfunction is emerging as a key cause factor for therapy-resistant forms of severe epilepsy [1]. A broad variety of mitochondrial DNA mutations [2] is associated with mitochondrial respiratory chain failure, consequent mitochondrial dysfunction and different epileptic phenotypes. It is not yet clear whether dysfunction of mitochondrial oxidative phosphorylation has a causative role in temporal lobe epilepsy (TLE) with hippocampal sclerosis. In this study we compared amount of multiple deletions of the mtDNA in four different hippocampal subfields (CA1, CA3, AD and PH) in patients with TLE, by using a long range PCR technique. The samples were obtained after epilepsy surgery of 20–40 years old patients, belonging to two different TLE subgroups. The first group is represented by 21 TLE patients whom developed Ammon's horn sclerosis — the most common type of neuropathological damage in individuals with TLE, characterized by neuronal cell loss in the hippocampus. The second group includes 8 TLE patients, with identified brain lesion as primary cause of epilepsy. It is well known that these patients usually do not develop hippocampal degeneration. We found a significant difference in the mtDNA deletion amounts between the two groups of patients, in each of the four hippocampal subfields. Our findings demonstrate that, in difference to lesion TLE patients, mtDNA deletions are typical for patients with AHS, especially, in the CA3 region. Though, it is known that multiple deletions are accumulating with age, in this study we demonstrate that in AHS patients multiple deletions can be detected in early stage of life. We propose that mtDNA deletions might be relevant for seizure generation in AHS patients.

References

[1] Kudin AP *et al.* (2009) *Exp. Neurol.* **218:** 326–332.
[2] Zsurka G *et al.* (2010) *Neurology* **74:** 507–512.

doi:10.1016/j.bbabio.2010.04.231

8P.3 Abnormalities of mitochondrial physiology and phenotype in sensory neurons in diabetes

Subir K. Roy Chowdhury[1], Eli Akude[1,2], Elena Zherebitskaya[1], Darrell R. Smith[1,2], Rick T. Dobrowsky[3], Paul Fernyhough[1,2]
[1]*St. Boniface Hospital Research Centre, Division of Neurodegenerative Disorders, Winnipeg, MB, Canada*
[2]*University of Manitoba, Department of Pharmacology & Therapeutics, Winnipeg, MB, Canada*
[3]*University of Kansas, Department of Pharmacology & Toxicology, Lawrence, KS, USA*
E-mail: skr_chowdhury@yahoo.ca

Diabetic sensory neuropathy is a major complication of type 1 and 2 diabetes that leads to distal loss of nerve fibers. Impairments in mitochondrial function have been proposed to play a role in the etiology of the neurodegeneration. We tested the *hypothesis* that mitochondrial dysfunction in sensory neurons in type 1 diabetes is due to abnormal activity of the respiratory chain and an altered mitochondrial proteome. Rates of oxygen consumption in mitochondria from dorsal root ganglia (DRG) of age-matched control, 12–22 week streptozotocin (STZ)-induced type 1 diabetic rats and diabetic rats treated with insulin were measured by OROBOROS oxygraph. Rates of coupled respiration with pyruvate + malate (P + M; full respiratory chain) and with ascorbate + TMPD (Asc + TMPD; Complex IV) in lumbar DRG were unchanged after 12 weeks of diabetes. By 22 weeks of diabetes, respiration with P + M was significantly decreased by 31–44% and with Asc + TMPD by 29–39% compared to control. Attenuated mitochondrial respiratory activity of STZ-diabetic rats was significantly improved by insulin treatment that did not fully correct other indices of diabetes. Enzymatic activities of mitochondrial complexes I and IV and the Krebs cycle enzyme, citrate synthase, were decreased in mitochondria from DRG of 22 week STZ-diabetic rats compared to control. Quantitative proteomic analysis using $^{13}C_6$-Lys and $^{13}C_6$,$^{15}N_4$-Arg labeled mitochondria as isotope tagged internal standards indicated that proteins associated with oxidative phosphorylation and the citric acid cycle were significantly down-regulated. Western blotting of DRG samples confirmed the proteomic analysis results for a specific subset of proteins and revealed reduced activation of AMP kinase (AMPK) coupled with diminished expression of peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α). In vitro confocal imaging studies with neurons from diabetic rats using TMRM+ in sub-quench mode showed mitochondria in axons to be depolarized and to exhibit an aberrant hyperpolarization in response to oligomycin. Mitochondrial dysfunction in sensory neurons in type 1 diabetes was associated with impaired rates of respiratory chain activity and modified adaption to hyperpolarization. The abnormal mitochondrial activity correlated with a down-regulation of an array of mitochondrial proteins that was associated with lowered activation status of the up-stream regulators of mitochondrial biogenesis, AMPK and PGC-1α.

doi:10.1016/j.bbabio.2010.04.232