S7.P12

Novel data on electron transport under conditions of vitamin K3 shunt functioning: The influence of transmembrane potential and NADH synthesis in intermembrane space

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In our previous reports we demonstrated that presence of oxidized form of CoQ or another exogenous quinone is crucial for the concrete path of electron transfer between complexes II and III. Our new results are devoted to the problem of large respiration rate decrease after uncoupler addition under conditions of hydrophilic quinone shunt functioning. This effect has no strict dependence on nature of uncoupler or hydrophilic quinone. TPP+ uptake and hydrogen peroxide production were estimated. The data gives new important information about electron transfer between hydrophilic quinol and CoQ in P-center of bc1-complex. In addition, some speculations on a problem of NQO1 supplementation with NAD(P)H in intermembrane space are made.

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S7. P13

Investigation of the energy metabolism of microglial cells

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Microglial cells play a key role in the pathomechanism of neurodegenerative disorders. They can enter into metabolically different compartments in the CNS. We investigated which compounds can serve as metabolic fuels for these cells. The oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured on primary microglial cells and on the BV-2 microglial cell-line with Seahorse Extracellular Flux Analyzer (Seahorse). ECAR was considered as a parameter of glycolytic activity. Cells were incubated in Artifical Cerebrospinal Fluid (ACSF) supplemented with those substrates, which are available for the cells in the CSF: glutamine, glucose, lactate, or pyruvate. All of the substrates applied supported the metabolism of the cells and none of them influenced their viability negatively. In the presence of glutamine the basal rate of respiration was increased. However in the presence of glucose the OCR was decreased, the ECAR raised and the addition of a lactate dehydrogenase inhibitor after glucose was able to reverse this effect. Adding a mitochondrial fatty acid transporter inhibitor further increased the ECAR in the presence of glucose. We conclude that microglial cells show high metabolic plasticity, using a wide range of substrates. From the ECAR results we claim, that these cells show high glycolytic capacity. Furthermore it was found that besides glucose glutamine is the most preferred substrate for microglial cells. Supported by: OTKA (NK 81983), TAMOP (4.2.2.-B/09-1), MTA (MTA TKI 2013) and Hungarian Brain Research Program – (Grant no. KTIA_13_NAP-A-II/36).

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S7.P14

A novel mutation in the SLC25A12 gene causing mitochondrial aspartate/glutamate carrier 1 (AGC1) deficiency


The mitochondrial aspartate/glutamate carrier isoform 1 (AGC1) catalyzes a Ca2+-regulated entry of glutamate into mitochondria in exchange for internal aspartate. In men AGC1 is an essential component of the malate–aspartate shuttle that transfers NADH reducing equivalents into mitochondria [1]. Defects in the SLC25A12 gene encoding AGC1 cause AGC1 deficiency (OMIM 612949), a novel neurological disorder that manifests with psychomotor development delay, epileptic seizures and hypomyelination associated with a reduced N-acetylaspartate (NAA) content in the CNS [2]. Here we report a novel homozygous SLC25A12 mutation (c.1058G > A, p.R353Q) identified in two Indian consanguineous siblings. R353Q in AGC1 is highly conserved in the aspartate/glutamate carrier subfamily (and also in other mitochondrial carrier subfamilies), is located just below the m(matrix)-gate of the carrier and is thought to participate in closing and opening the carrier on the matrix side through an interaction with the highly conserved E383 [3,4]. To assess the pathogenic potential of the c.1058G > A mutation, wild-type and R353Q mutant AGC1 were overexpressed in Escherichia coli, purified and reconstituted into liposomes. Despite normal insertion of the mutant protein in the liposomal membrane, R353Q AGC1 caused 85% inhibition of the initial transport rates of [14C]aspartate/aspartate and [14C]glutamate/aspartate exchanges. Although the activity is not completely inhibited, the phenotype of the patients resembles those of the Slc25a12 knockout mice [5] and of the first identified patient affected by AGC1 deficiency, where the mutant Q590R AGC1 was totally inactive [2]. NAA produced in neurons is known to undergo transaxonal transfer to oligodendrocytes where it supplies acetyl groups for the synthesis of myelin lipids. It is suggested that AGC1 is essential to supply cytosolic aspartate for the production of NAA and subsequent myelin synthesis.

References

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