fibroblasts. The impairment of proton-translocation activity of COX was directly confirmed by mitochondrial membrane potential measurements using TPP⁺ electrode. While proton pumping at complexes I and III in patient fibroblasts was similar to controls, ascorbate + TMPD substrates were unable to support generation of proton gradient. Consequently, mitochondrial membrane potential as estimated by JC-1 staining was lower in intact patient fibroblasts, leading to extremely decreased rates of mitochondrial ATP production to 25% of control values. Such drop in energy provision ultimately resulted in two-fold decrease of ATP/ADP ratio in patient cells grown in galactose medium, when most of ATP must be synthesized by mitochondria. In contrast to profound impairment of mitochondrial energetics, no changes in the production of reactive oxygen species (ROS) or antioxidant defences could be found in patient fibroblasts. This is perhaps due to decreased mitochondrial membrane potential, which may serve as a paradoxical ROS-preventing mechanism. We conclude that unlike to mitochondrial disorders caused by dysfunction of ATPase or complex I, the pathogenic mechanism of COX deficiencies seems to have only single component—impaired mitochondrial energy provision.

This work was supported by grants from Grant Agency of the Czech Republic (303/07/781) and Ministry of Education (1M6837805002, AV0Z S010509) of the Czech Republic.

4P.12 POLG mutations lead to decreased mitochondrial DNA repopulation rates after EtBr-induced depletion in fibroblasts

Susanne Schoeler, Miriam Baron, Wolfram S. Kunz
Division of Neurochemistry, Dept. Epileptology and Life&Brain Center, University Bonn, Germany
E-mail: Susanne.Schoeler@gmx.net

Mutations in nuclear genes encoding proteins that are involved in mitochondrial DNA (mtDNA) maintenance, e.g. POLG, TK2, are associated with various neurodegenerative disorders [1]. All pathogenic mutations in these nuclear genes lead to mtDNA depletion and secondary mtDNA mutations, which cause dysfunction of the oxidative phosphorylation and lead to disease phenotype. Until now it is a major challenge to demonstrate the direct functional consequences of those mutations. To address the issue, whether POLG or TK2 mutations lead to impaired mtDNA maintenance, a kinetic assay for mtDNA replication in primary human fibroblasts was performed. Different fibroblast cell lines were depleted of their mtDNA by treatment with ethidium bromide (EtBr) and the rates of mtDNA repopulation were determined. Here we demonstrate that the rate of mtDNA depletion, induced by EtBr, showed no significant difference between patients and controls. In contrast, the restoration of mtDNA levels is significantly delayed in fibroblasts from patients with POLG mutations, while TK2 mutations have no effect on mtDNA repopulation rates. These findings provide the first in vivo evidence that pathogenic POLG mutations directly influence the mtDNA maintenance in human cells. Furthermore, these results are in line with in vitro data showing reduced catalytic activity and processivity for several pathogenic POLG alleles [2–5].

References

4P.13 Impact of diabetes-associated lipoproteins on oxygen consumption, enzymatic activities of mitochondrial respiratory chain complexes

Subir Roy Chowdhury, Ganesh Sangle, Xueping Xue, Garry Shen
University of Manitoba, Department of Internal Medicine, Canada
E-mail: gshen@ms.umanitoba.ca

Diabetes is a mitochondrial disease. Atherosclerotic coronary artery disease (CAD) is the leading cause of mortality in diabetic patients. Mitochondrial dysfunction and increased production of reactive oxygen species (ROS) are associated with diabetes and CAD. Elevated levels of glycated low density lipoproteins (glyLDL) and oxidized LDL (oxLDL) were detected in patients with diabetes. Our previous studies demonstrated that oxLDL and glyLDL increased the generation of ROS and altered the activities of antioxidant enzymes in vascular endothelial cells (EC). The present study examined the effects of glyLDL and oxLDL on oxygen consumption in mitochondria and the activities of key enzymes in mitochondrial electron transport chain (ETC) in cultured porcine aortic EC. The results demonstrated that glyLDL or oxLDL significantly impaired oxygen consumption in Complex I, II/III and IV of mitochondrial ETC in EC compared to LDL or vehicle control detected using oxygraphy. Incubation with glyLDL or oxLDL significantly reduced mitochondrial membrane potential, the levels of NAD⁺/NADH ratio, and the activities of mitochondrial ETC enzymes (NADH—ubiquinone dehydrogenase, succinate cytochrome c