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Disturbances in mitochondrial transport systems leading to encephalomyopathies

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1. Introduction

During the last two decades we performed biochemical investigations in more than one thousand muscle biopsies obtained from patients suspected to suffer from a mitochondrial (encephalo)myopathy. In several hundreds of them evidence was obtained for the presence of a disturbance in the mitochondrial energy generating system. Muscle samples from these patients clearly exhibited reduced oxidation rates of substrates, like pyruvate, malate and succinate. Besides, production rates of ATP and CrP from these substrates were reduced too. In nearly 75% of these patients measurement of the activities of the complexes I–IV of the respiratory chain and of the pyruvate dehydrogenase complex, revealed a deficiency of one or more of these complexes to a highly varying degree. However, in the remaining 25% of these patients no clear-cut defect in the aforementioned complexes could be established. From this observation it can be concluded that other defects must account for the established defect in the mitochondrial energy generation in muscle. Earlier we focussed our attention on the possible deficiency of mitochondrial creatine kinase as a cause of the disturbed energy production. However, investigation of more than one hundred of muscle samples with such a disturbed oxidative capacity of mitochondria did not reveal a patient with a MiCK deficiency. Another so-called post-respiratory chain enzyme which might be involved in the pathogenesis of mitochondrial myopathies concerns mitochondrial ATPase (complex V). At present only a few patients with an ATPase deficiency have been described. These observations prompted us to look at other possible causes of a disturbed mitochondrial energy generation. We speculated that malfunctioning of transporting systems in the mitochondrial inner or outer membrane might be the primary cause of the biochemical aberrations in some of these patients. At the moment at least 15 mitochondrial carriers have been identified. Some of these carriers are present only in a limited number of tissues. In this presentation special attention will be paid to those carriers which are directly involved in the process of oxidative phosphorylation. Among these the ATP/ADP translocator (ANT), the phosphate carrier (PiC), the pyruvate carrier and the 2-oxoglutarate carrier will be considered. Besides, alterations of the voltage-dependent anion channel (VDAC) and the malate-aspartate shuttle will be discussed as a possible cause of mitochondrial myopathies.

2. The ATP/ADP translocator (ANT)

ANT is an integral inner membrane protein of 30 kDA. It catalyzes the exchange of cytosolic ADP and matrix ATP. Three isoforms of ANT are known in human. In 1993 the first patient with a deficiency of ANT has been reported [1]. The patient had a severe myopathy with lactic acidosis. Increased activities of complexes I, II, II+III, IV, V, pyruvate dehydrogenase complex, 2-oxoglutarate dehydrogenase complex, citrate synthase and of MiCK were found in muscle tissue to a degree varying between 2- and 20-fold. The oxidation rate of [1-^{14}C] pyruvate + malate in the presence of ADP was in the low normal range, as was the production of ATP plus phosphocreatine from these substrates. The oxidation rate of pyruvate was only 2.7 times stimulated by ADP compared with 7 times in controls. Remarkably, the pyruvate oxidation was strongly increased by the uncoupler CCCP. Immunostaining of western blots, using polyclonal antiserum against ANT, revealed a 4-fold decrease in muscle ANT content. The defect was not present in fibroblasts and lymphocytes pointing to a muscle-specific deficiency of ANT.

3. The phosphate carrier (PiC)

There are two systems available to supply the mitochondrion with inorganic phosphate (Pi) for oxidative phosphorylation. The Pi/dicarboxylate carrier mediates the exchange of dicarboxylate against Pi. The phosphate carrier (PiC) mediates the transport of Pi into the matrix in exchange with protons. Two isoforms of the PiC are known. Proper functioning of this carrier is essential for a normal function of the oxidative phosphorylation by supplying Pi for ATP synthesis from ADP. We investigated more than 100 muscle specimens from patients with a mitochondrial cytopathy. So far no deficiency of PiC has been detected.

4. The pyruvate and 2-oxoglutarate carrier

We did not detect a patient with a deficiency of the pyruvate carrier among several hundreds of patients from whom a fresh muscle specimen has been investigated. This conclusion has been drawn from the observation that in all patients in whom only the pyruvate oxidation rate was reduced and not the malate and succinate oxidation, the impairment could be ascribed to a defect in the pyruvate dehydrogenase complex. We investigated those patients for a deficiency of the 2-oxoglutarate carrier, who showed a 2-oxoglutaric acidemia not due to a deficiency of 2-oxoglutarate dehydrogenase. Up to now we did not find a patient with a deficiency of this carrier. However, the applied method has to be improved before a definite conclusion can be drawn.

5. The voltage-dependent anion channel (VDAC)

The voltage-dependent anion channel is a pore-forming protein in the outer mitochondrial membrane. The VDAC pore is open at low transmembrane voltage for anions such as phosphate, chloride and adenine nucleotides. At higher voltage VDAC functions as a selective channel for cations and uncharged molecules. Four genes encoding different human (H)VDAC isoforms have been reported [2]. Until now, only HVDAC 1 and 2 have been shown to be expressed at the protein level, HVDAC 1

being the most abundantly expressed. Recently, we reported the first patient with a VDAC deficiency in muscle tissue [3]. The patient presented with high birth weight and dysmorphic features. Biochemical studies on muscle mitochondria showed impaired rates of pyruvate oxidation and ATP production. However, no deficiency of one of the respiration chain complexes nor of PDHc was found. Western blotting experiments indicated an almost complete deficiency of VDAC in skeletal muscle but not in fibroblasts. The VDAC deficiency might cause an abnormal composition and/or altered osmolarity within the mitochondrial matrix, which could lead to impairment of the mitochondrial energy metabolism in our patient.

6. The malate-aspartate shuttle

The malate-aspartate shuttle mediates the transport of NADH from the cytosol to the mitochondrion. The first and, hitherto, only patient with a defect in this shuttle was reported by Hayes et al. [4]. The patient presented with exercised-induced muscle pain. There was an abnormally rapid decrease in phosphocreatine during aerobic exercise as detected by NMR. Recovery from exercise, measured by following phosphocreatine resynthesis, was delayed. A primary defect in mitochondrial respiration was excluded, since isolated mitochondria oxidized all substrates tested at a normal rate. The defect was localized at the level of the malate-aspartate shuttle. The activity of this shuttle was less than 20% of that in controls.

Summarizing, it can be stated that disturbances in mitochondrial transport systems should be considered as a primary cause of a mitochondrial myopathy.

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