Mitochondrial Creatine Kinase Activity in Patients with Disturbed Energy Generation in Muscle Mitochondria

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Summary: Eleven patients with an established disturbance in muscle mitochondrial energy generation, in whom no defect in the pyruvate dehydrogenase complex or in the complexes of the respiratory chain could be detected, were investigated for a possible deficiency of mitochondrial creatine kinase (Mi-CK) (EC 2.7.3.2). Four patients with a defect in one of the complexes of the respiratory chain were also investigated for Mi-CK activity. In none of the investigated patients was Mi-CK deficiency found. Surprisingly, two of the four patients with a defect in one of the respiratory chain complexes showed enhanced activity of Mi-CK. It is concluded that Mi-CK deficiency is not frequently found as a primary defect in patients with disturbance in mitochondrial energy generation, but more patients should be examined to allow a definite conclusion.

A mitochondrial myopathy can be defined as a muscle disease characterized by structurally or numerically abnormal mitochondria and/or abnormally functioning mitochondria (Sengers et al 1984). Clinically such disorders should be considered in all floppy infants and in those babies with perinatal problems such as failure to thrive, seizures and cardiomyopathy (Trijbels et al 1988). The most important symptoms in older infants and adults are muscle weakness and exercise intolerance (Trijbels et al 1988).

The biochemical approach for diagnosis of mitochondrial myopathies, as applied in our department, consists of metabolic screening in body fluids (lactate, amino acids and organic acids), measurement of mitochondrial substrate oxidation rates and ATP plus PCR (phosphocreatine) production rates by muscle preparations. In this way a defect in mitochondrial energy metabolism can be established (Trijbels et al 1988). A more precise location of a defect is based on enzymatic procedures (e.g.

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Fischer et al. 1986), including measurement of the activity of the pyruvate dehydrogenase complex (PDHc) and of the complexes of the respiratory chain.

Applying the aforementioned diagnostic approach regularly, patients have been detected with a defect in mitochondrial energy production in whom no specific enzyme defect could be established in the PDHc or in the respiratory chain. Clinical symptoms such as fatigue, exercise intolerance and muscle weakness with lactic acidosis and/or an increased lactate/pyruvate ratio, found in most patients suffering from a mitochondrial myopathy, can theoretically be caused by a Mi-CK deficiency. Therefore the diagnostic programme was extended by measurement of the Mi-CK activity as a possible cause of the disturbance in energy generation. The results of these investigations are presented.

MATERIALS AND METHODS
Substrate oxidation rates, ATP plus PCr production rates, PDHc activity, single enzyme activities of the respiratory chain and Mi-CK activity were measured in skeletal muscle samples (m. quadriceps) of 15 patients (11 males/4 females; age range 1 month to 35 years) who were biopsied after informed consent.

In all patients a muscle biopsy was performed because of the suspicion, based on clinical examination and laboratory investigations, that they suffered from a mitochondrial myopathy.

The oxidation rates of the various radiolabelled substrates and production rates of ATP plus PCr were measured in supernatants of fresh muscle homogenates as described previously (Bookelman et al. 1978; Fischer et al. 1986). Cytochrome c oxidase (COX) and citrate synthase (CS) activities were determined according to Cooperstein and Lazarow (1951) and Srere (1969), respectively. NADH:O2 oxidoreductase, NADH:Q1 oxidoreductase and succinate:cytochrome c oxidoreductase activities were measured according to Fischer et al. (1986). In the NADH:O2 oxidoreductase assay Mg2+ was not added because omission results in higher activities in most supernatants.

Measurement of total PDHc activity in muscle homogenate was performed as described earlier (Sperl et al. 1990). Mitochondrial and total creatine kinase activity was determined according to Smeitink et al. (1992). Protein was determined by the method of Lowry et al. (1951).

RESULTS
The most important clinical data and the maximum measured blood lactate concentrations are summarized in Table 1.

All patients investigated showed a disturbance in mitochondrial energy generation. The [1-14C]pyruvate oxidation rate in the presence of malate ranged in our patients from 0.27 to 2.7 nmol/h per mU CS (control range 3.6–7.5; n = 14) (Table 2). The ATP plus PCr production rates with pyruvate + malate as substrates ranged from 0 to 30 nmol/h per mU CS (control range 42–81; n = 16) (Table 2).

Four patients exhibited a decreased rate of pyruvate oxidation caused by a deficiency in one of the complexes of the respiratory chain (Table 3). Patients 1, 3