Mitochondrial myopathies: deficiencies localized to complex I and complex III of the mitochondrial respiratory chain

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Deficiencies of the mitochondrial respiratory chain or the phosphorylation system are becoming increasingly recognized causes of myopathy and multisystem disease in man. In biochemical terms, the ones most clearly defined have been associated with impaired activities of NADH:ubiquinone reductase (Morgan-Hughes et al., 1979, 1984; Land et al., 1981; Moreadith et al., 1984), ubiquinol:cytochrome c reductase (Spirito et al., 1970; Morgan-Hughes et al., 1977, 1982; Darley-Usmar et al., 1983), cytochrome c oxidase (DiMauro et al., 1985) and ATP synthase (Schotland et al., 1976; Clark et al., 1983). Although in most cases the biochemical error has been identified in isolated muscle mitochondria, myopathy may be overshadowed clinically by major involvement of other organs such as the brain, liver or heart. The morphological hallmark of these disorders is the so called ragged red muscle fibre (Olson et al., 1972), which owes its appearance in frozen sections stained with the modified Gomori trichrome method to large subsarcolemmal collections of structurally abnormal mitochondria (Morgan-Hughes & Landon, 1985).

The diverse clinical expression of defects of this type is illustrated by four examples taken from a series of 30 patients investigated in our laboratories over the last few years. Of these 30 cases, the defect was localized to complex I in 14 and to complex III in another nine. In each case the data presented were obtained by applying classical biochemical methods to purified mitochondria freshly isolated from a sample of the vastus lateralis muscle taken under light general anaesthesia (Morgan-Hughes et al., 1977).

Case 1 was a 21-year-old woman with fatigue-related muscle weakness dating back to early childhood. At the age of 11 years she developed unexplained congestive heart failure, which resolved spontaneously after several months. Her subsequent referral was precipitated by the death of a 23-year-old sister from cardiorespiratory failure and severe lactic acidosis. Examination revealed generalized fatigable weakness of the limb and trunk muscles, but there was no clinical or laboratory evidence of central nervous system, cardiac or retinal involvement. During a standard 10 min exercise test on a bicycle ergometer (Morgan-Hughes et al., 1979) the venous blood lactate rose from 1.3 to 5.7 mM and was still raised at 3.8 mM after 30 min. The blood lactate/pyruvate and β-hydroxybutyrate/acetoacetate ratios rose to 66 and 8 respectively at 20 min. Muscle mitochondria were numerically increased and were markedly elongated with straight transverse cristae and looped ends (Morgan-Hughes & Landon, 1983). Polargraphic studies with a standard oxygen electrode (Morgan-Hughes et al., 1977) showed that O2 uptake rates were low or not demonstrable with all NAD-linked substrates tested but were much higher utilizing succinate or the ascorbate plus NNNN'-tetramethyl-p-phenylenediamine (TMPD) system (Table 1). Additional studies showed that State-3 O2 uptake rates with NAD-linked substrates were greatly enhanced after the addition of TMPD alone (Lee et al., 1967) but succinate oxidation was unaffected by this procedure (Table 1). The mitochondrial cytochromes, intramitochondrial NAD concentration and the specific activities of citrate synthase, pyruvate dehydrogenase and NADH-ferricyanide reductase were within the normal range. We postulated, therefore, that the defect in this case was localized to that part of the respiratory chain between the flavin-mononucleotide-linked NADH-dehydrogenase and coenzyme Q and may have involved one of the Fe–S proteins in complex I.

Although the total carnitine concentration in muscle from case 1 was normal at 1.34 μmol/g wet wt. (control value 2.0 ± 0.7 S.D., n = 28), three other patients with complex I deficiency presented with a carnitine-deficient lipid-storage myopathy (Clark et al., 1984). One of these (case 2) was a 49-year-old man with a 2 year history of progressive weakness, severe exercise intolerance and muscle aches. Plasma carnitine was normal but the total carnitine concentration in muscle was 0.22 μmol/g wet wt. A mitochondrial preparation showed low or negligible State-3 O2 uptake rate with NAD-linked substrates, but much higher rates of oxygen consumption with succinate or ascorbate plus TMPD (Table 1). As in case 1, respiratory rates with NAD-linked substrates were greatly enhanced after the addition of TMPD alone (Table 1). The mitochondrial cytochromes and specific activities of pyruvate

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**Table 1. Respiratory activities of isolated muscle mitochondria**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Conditions</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>State 3</td>
<td>+ TMPD</td>
<td>State 3</td>
<td>+ TMPD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(50 μM)</td>
<td>(50 μM)</td>
<td>(50 μM)</td>
<td>(50 μM)</td>
</tr>
<tr>
<td>5 mM-Pyruvate (+ malate)</td>
<td></td>
<td>103</td>
<td>80</td>
<td>58</td>
<td>467</td>
</tr>
<tr>
<td>10 mM-GL-Glutamate (+ malate)</td>
<td></td>
<td>137</td>
<td>193</td>
<td>81</td>
<td>234</td>
</tr>
<tr>
<td>50 μM-DL-Palmitoylcarnitine</td>
<td></td>
<td>ND</td>
<td>81</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>10 mM-Succinate (5 μM-rotenone)</td>
<td></td>
<td>50</td>
<td>56</td>
<td>57</td>
<td>277</td>
</tr>
<tr>
<td>1 mM-L-Ascorbate (5 μM-rotenone and 10 μg of antimycin A/μl)</td>
<td></td>
<td>110</td>
<td>254</td>
<td>570</td>
<td>449</td>
</tr>
</tbody>
</table>
dehydrogenase, citrate synthase and NADH: ferricyanide reductase were within the normal range.

The mechanism of secondary carnitine deficiency in defects of this type is uncertain. One possible explanation is that carnitine may serve to detoxify the mitochondria of acyl residues which accumulate as a result of the metabolic block. The acylcarnitine esters so formed may then be transported out of the mitochondria and excreted in the urine. This would impose a considerable drain on carnitine stores and may lead to carnitine deficiency, particularly in patients whose dietary intake of carnitine is inadequate. A similar mechanism has been proposed to explain the occurrence of secondary carnitine deficiency in the organic acidurias (Roe et al., 1983) and in defects of fatty acid oxidation (Turnbull & Sheratt, 1985).

The presence of clinical heterogeneity in complex I deficiencies is well illustrated by case 3, a 45-year-old coalminer, who despite having been a rather delicate child had worked at the coal face until his late thirties when his workmates and relatives noticed a change in his personality and in his gait. Over a period of several months he developed increasing unsteadiness on his feet and became irritable, forgetful and at times belligerent. When first seen he was demented and euphoric with a scanning cerebellar dysarthria and a broad based ataxic gait. He had bilateral nerve deafness, a mild sensory peripheral neuropathy and choreic movements in the limbs. A computed tomography brain scan showed extensive cerebral and cerebellar atrophy. Despite the absence of a myopathy on clinical testing, a muscle biopsy showed the typical changes of a mitochondrial myopathy with paracrystalline mitochondrial inclusions. Oxygen uptake rates utilizing NAD-linked substrates were substantially higher than in the first two cases, but they were significantly reduced when compared with those recorded utilizing succinate or ascorbate plus TMPD, and were enhanced almost fivefold by the TMPD shunt (Table 1). As in the previous cases, the mitochondrial cytochromes and specific activities of several mitochondrial enzymes and NADH: ferricyanide reductase were normal. We concluded that the defect of complex I in this patient was not only present in skeletal muscle but also involved the cerebral and cerebellar cortex and basal ganglia. Indirect evidence for this came from studies using $^{15}$O and $^{18}$F-2-deoxy-D-glucose to measure cerebral oxygen and glucose utilization and cerebral blood flow (Rhodes et al., 1983). These studies were carried out in collaboration with Dr. Richard Frackowiak at the Cyclotron Unit, Hammersmith Hospital. The results (Table 2) showed a 25–30% reduction in cerebral blood flow and glucose utilization, possibly due to loss of cerebral tissue, but a disproportionately greater reduction in cerebral oxygen utilization.

The clinical features in the 14 patients with complex I deficiency are summarized in Table 3. There were seven cases with myopathy only and seven with multisystem disease. Six cases had a positive family history. Two patients with multisystem disease were identical twins and a mother and daughter are included in the myopathy only group (Clark et al., 1984). Two other patients in the myopathy only group each had an affected sibling who was not investigated biochemically. It is interesting that degeneration of the retinal pigment epithelium was the commonest associated feature in the multisystem disease cases as these cells are known to be rich in mitochondrial and to have a high demand for ATP (Young & Bok, 1979).

Of the nine patients with a defect localized to complex III, two had a deficiency of reducible cytochrome $b$ (Morgan-Hughes et al., 1977, 1982), one had a combined deficiency of cytochromes $b$ and $c_1$ (Hayes et al., 1984), and in six the spectrum and content of the mitochondrial
cytochromes were normal. One of these (case 4) was a 38-year-old woman with a 14 year history of fatiguable limb weakness, progressive drooping of the eyelids (ptosis) and intermittent double vision. Her daughter was similarly affected. A muscle mitochondrial preparation showed very low or negligible State-3 O₂ uptake rates with NAD-linked substrates and with succinate which were markedly enhanced after the addition of TMPD alone (Table 1). Ascorbate plus TMPD respiration rates were within the normal range, as were the spectrum and content of the mitochondrial cytochromes and the specific activities of several mitochondrial enzymes and NADH: ferricyanide reductase. The combined ubiquinone and ubiquinol (Q₀) concentration in this patient's mitochondria, as measured by h.p.l.c. (Katayama et al., 1980), was 4.65 nmol/mg of mitochondrial protein, which was comparable with concentrations found in other cases studied in which complex III activity was unimpaired. We concluded, therefore, that the complex III deficiency in this case may have been due to a defect involving one of the Fe–S proteins in this part of the respiratory chain.

The clinical features of the nine patients with complex III deficiency are summarized in Table 3. Of the two patients with a positive family history, one (case 4) had a clinically affected daughter and the other had an affected nieces. There was a higher incidence of extraocular muscle involvement in complex III deficiency cases but, as in complex I deficiency, pigmentary retinal degeneration was the commonest associated feature in multisystem cases (Table 3).

What of the origin of these disorders? Current evidence suggests that in the majority of cases defects of this type are the consequence of inborn genetic errors, although the mechanisms of inheritance are uncertain. Of the 22 cases in the present series, eight patients belonging to six different pedigrees had a positive family history. Affected members belonged to the same generation in three families and to different generations in the remaining three. In the latter cases, there was evidence for maternal transmission.

On theoretical grounds, defects of the oxidative phosphorylation pathway could arise from mutations affecting either the mitochondrial or nuclear genetic systems. Major differences in phenotypic expression even in members of the same pedigree (Boustany et al., 1983) would be compatible with non-nuclear inheritance as the pattern of organ involvement may depend upon the way in which mutant mitochondrial DNA is allocated during somatic cell division. Little is known about the partitioning of mitochondria during cell division, but there is some evidence to suggest that it may not be an entirely random process (Birky, 1983). However, of the 67 polypeptides identified in the different respiratory complexes (Capaldi, 1983) only five are known to be coded for by mitochondrial DNA (Anderson et al., 1982) and even if the products of the eight unassigned reading frames are shown to be included, the contribution of the mitochondrial genome is still relatively small. Because of the many polypeptides involved, and the complexity of events leading to their processing and assembly within the mitochondrial inner membrane (Hay et al., 1984), it is difficult to determine the genetic mechanisms involved from clinical studies alone. It seems likely, therefore, that the solutions to these questions will require more accurate characterization of these deficiencies at a molecular level and the systematic application of modern DNA techniques to the study of affected individuals and their asymptomatic relatives.

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