Mitochondrial myopathies: defects in mitochondrial metabolism in human skeletal muscle

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An uncommon but increasingly diagnosed clinical situation in humans is mitochondrial myopathy, a condition in which muscle function is impaired by defective mitochondrial metabolism in the generation of energy for muscle contraction. It is also now clear that abnormalities of mitochondrial function are not limited to muscle, but may also underlie multisystem disease, in which other tissues are involved and vision and cerebral function may be impaired (Morgan-Hughes, 1982; Morgan-Hughes et al., 1977, 1979, 1983). The clinical picture is therefore variable, but most patients show a marked fatigability and lactate accumulation even after relatively limited exercise.

Table 1. Classification of mitochondrial myopathies

1. Defects in substrate utilization
   - Monocarboxylate translocase
   - Pyruvate dehydrogenase complex
   - Carnitine-acylcarnitine carrier system, including carnitine palmitoyltransferase

2. Defects in respiratory chain
   - NADH:ubiquinone reductase (Complex I)
   - Cytochromes, e.g. b; b-c; b-aa
   - Respiratory-chain component, e.g. ubiquinone; FeS protein

3. Defects in energy transduction
   - Coupling mechanism (Luft's disease)
   - ATPase

For more details see Morgan-Hughes (1982) and references therein.

Table 2. Respiratory activities of isolated human skeletal muscle mitochondria

Mitochondria were isolated and incubated in the presence of substrates at 25°C as described by Morgan-Hughes et al. (1977). State-3 respiration is defined as being in the presence of ADP (abbreviations: FCCP, carbonyl cyanide p-trifluoromethoxyphenylhydrazone; ND, not determined. The control range is taken from Mackinen & Lee (1968), Max et al. (1972), Scholte et al. (1981) and Scottland et al. (1976).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>State 3</td>
<td>+ FCCP</td>
<td>State 3</td>
<td>+ FCCP</td>
</tr>
<tr>
<td>Pyruvate + malate</td>
<td>0</td>
<td>0</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>Glutamate + malate</td>
<td>14</td>
<td>ND</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Succinate + rotenone</td>
<td>45</td>
<td>ND</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Ascorbate + tetramethylphenylenediamine</td>
<td>90</td>
<td>ND</td>
<td>115</td>
<td>115</td>
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</tbody>
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oxidation of both NADH-linked substrates and succinate was low (Table 2) but coupled. However, in the presence of the uncoupler carbonyl cyanide m-trifluoromethoxyphenyl hydradrazine, there was an approximate doubling of the respiration rate. Cytochrome content and enzymic activities were normal, except for the mitochondrial ATPase, which was 6% (105 nmol/min per mg of mitochondrial protein) of the activity found in three control cases [1800 ± 480 nmol/min per mg (s.d., n = 3)].

Our conclusion in this case was the primary defect lay in the ATP synthase part of the oxidative-phosphorylation machinery rather than with the carnitine deficiency.

Not only do each of these patients represent an interesting metabolic defect, but their cases also highlight a fundamental question in biochemistry, namely the relationship between, and the dependence of, tissues on the provision of energy by oxidative phosphorylation as opposed to anaerobic glycolysis. In order to study this relationship further and to assess possible clinical therapies for the treatment of this condition, an animal model is of obvious importance. Progress towards this has been made by the use of the NADH dehydrogenase inhibitor diphenyleneiodonium (see Bloxam, 1979). The chronic administration of this compound to rats leads to myopathy, which is associated with a defective NADH:ubiquinone reductase activity in the isolated mitochondria from gastrocnemius muscle (Hayes, 1983). In a parallel study, the isometric twitch tension of the same muscle was grossly fatiguable and exhibited a marked delay in recovery on rest (Byrne, 1983). Studies using 31P n.m.r. (see Fig. 1) indicated that the delay in recovery of the muscle twitch tension may be correlated with a decreased rate of recovery of muscle phosphocreatine concentration, which was approx. 8 times slower in the chronic animal model than in controls (Hayes, 1983). A similar delay in resynthesis of phosphocreatine was also observed by 31P n.m.r. in the forearm muscles of two patients (see Fig. 1 and Radda et al., 1982) who had previously been shown to have a Complex-I deficiency (Morgan-Hughes et al., 1979). Further studies using this animal model may therefore be expected to yield useful data for patient treatment as well as for our understanding of the control of cellular energy provision.

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**Application of 31P n.m.r. to inborn errors of muscle metabolism**

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In this paper we report 2 years experience with 31P n.m.r. in the diagnosis in vivo and evaluation of inborn errors of muscle metabolism. Despite the great rarity of these conditions, they repay detailed study for what they may tell us of normal as well as abnormal metabolism. Our group has been able to identify and examine eighteen patients with verifiable genetic disease, as well as a further six subjects in whom the original diagnosis on referral could not be substantiated. Duchenne's and Becker's muscular dystrophy, undoubtedly of genetic origin, are excluded from the present discussion (see Newmann et al., 1982). Thirty-three control subjects, matched approximately for age and sex, form the basis for the definition of a range of normal and abnormal findings with 31P n.m.r. (Taylor et al., 1983). A further seventy or more patients with a wide variety of 'non-metabolic' muscle diseases provide an extensive background against which to consider the findings in genetic muscle disease.

**Patients and method**

Subjects were patients referred from Oxford physicians and from hospitals throughout the U.K., usually with a biopsy-supported or biochemically verified muscle metabolic disease. In several instances, further biochemical tests, including ischaemic lactate test (Strong & Ross, 1983), lactate determinations during bicycle exercise, muscle biopsy and enzyme assay, or mitochondrial respiration studies on isolated organelles (Land & Clark, 1979) were carried out in this laboratory. But, for the most part,