Short report

Normal mitochondrial malic enzyme levels in Friedreich's ataxia fibroblasts

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SUMMARY Normal levels of mitochondrial malic enzyme were found in fibroblasts from three patients with Friedreich's ataxia.

The biochemical basis for the pathological changes found in the central nervous system in Friedreich's ataxia is unknown. The well-established clinical association of Friedreich's ataxia and diabetes mellitus1,2 has suggested that abnormal carbohydrate metabolism may be present in this disease, while a number of biochemical observations have raised the possibility of a cellular defect in oxidative mechanisms. Inconsistent alterations in pyruvate decarboxylation3 and in lipoamide dehydrogenase, a component of the pyruvate dehydrogenase complex4 have been reported. Most recently, Stumpf et al5 have described disturbance of pyruvate-malate metabolism in a study of muscle mitochondria in Friedreich's ataxia. Pursuing this observation, they found a severe reduction of mitochondrial malic enzyme (MEm) in Friedreich's ataxia skin fibroblasts, the reduction being to a level of 10% of control enzyme activity. In a pilot study, Friedreich's ataxia fibroblasts were examined for evidence of reduced MEm activity.

Methods

Fibroblasts from forearm skin biopsies obtained with informed consent were cultured from primary explants maintained in Eagle's minimum essential medium supplemented with 10% newborn calf serum, penicillin (25 IU/ml), streptomycin (25 μg/ml) and glutamine (2 mM). Cells from three patients with Friedreich's ataxia (two male, one female, age range from 14–31 years) and three normal controls (one male, two female, age range 10–43 years) were used. Fibroblasts were grown to confluence in roller bottles, three bottles being used for each assay, and were harvested with 0.25% trypsin. The cells were resuspended in 0.5 ml of medium (250 mM mannitol, 2 mM EDTA, 0.3% bovine serum albumin, 1 mM mercaptoethanol, 1 mM ATP in 10 mM Hepes buffer, pH 7.4). Five microlitres of Sigma Type VII protease (11 U/mg) were added and the mixture was incubated at 0°C for 7 min. The cells were then homogenised and centrifuged at 600 g for 10 min, followed by 8000 g for 15 min. Mitochondria were isolated by resuspending the resulting pellet in 250 μl of 300 mM sucrose and 2 mM EDTA at pH 7-0 and sonication on ice. Malic enzyme activity was assayed by following NADP+ reduction at 340 nm on a Unicam SP 1800 spectrophotometer. Each assay had final concentrations of 6 mM malate, 0.2 mM NADP+, 10 mM MgCl2, 1 mM mercaptoethanol, 235 mM tris-HCl at pH 7-4, the assay being started by the addition of the organic acid base. Protein concentrations in mitochondrial and cytosolic fractions were assayed by the method of Lowry et al.6

Results

Mitochondrial plus cytosolic levels of malic enzyme activity are shown in the table.

The mean values for the levels of activity in Friedreich's ataxia cells (MEm = 2.07 × 10⁻² U/min/mg protein; MEc = 1.729 × 10⁻² U/min/mg protein) are influenced by the high values obtained from cells from DW, a controlled diabetic. Nevertheless, there is no apparent deficiency in enzyme activity in any of the Friedreich's ataxia cells compared to the mean values obtained for controls (MEm = 1.412 × 10⁻² U/min/mg protein; MEc = 1.309 × 10⁻² U/min/mg protein).
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Table Malic enzyme activity in Friedreich's ataxia and control fibroblasts

<table>
<thead>
<tr>
<th></th>
<th>Mitochondrial (ME_m)</th>
<th>Cytosolic (ME_c)</th>
</tr>
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<tbody>
<tr>
<td>Friedreich's</td>
<td>(no of experiments)</td>
<td></td>
</tr>
<tr>
<td>MH (2)</td>
<td>1.671</td>
<td>1.293</td>
</tr>
<tr>
<td>CT (2)</td>
<td>1.688</td>
<td>1.495</td>
</tr>
<tr>
<td>DW (2)</td>
<td>2.851</td>
<td>2.400</td>
</tr>
<tr>
<td>Controls</td>
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<td></td>
</tr>
<tr>
<td>IM (2)</td>
<td>1.355</td>
<td>1.273</td>
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<tr>
<td>IG (1)</td>
<td>1.592</td>
<td>1.648</td>
</tr>
<tr>
<td>AP (2)</td>
<td>1.288</td>
<td>1.007</td>
</tr>
</tbody>
</table>

Values represent mean enzyme activity in 10^{-2} U/min/mg protein.

Discussion

Malic enzyme is present in two forms in mammalian tissue, cytosolic (ME_c) and mitochondrial (ME_m), the latter being present in its highest concentrations in the nervous system and heart; it catalyses the decarboxylation of malate to pyruvate, with the formation of NADPH. The recent study of Stumpf et al. produced evidence for a reduced level of mitochondrial malic enzyme activity in Friedreich's ataxia cells, the activity being reduced to 10% of that of normal control cells. We have been unable to confirm the presence of this reduction in our pilot study.

The spectrophotometric assay of malic enzyme in fibroblasts involves estimation of low levels of activity, with inevitable experimental variation. Furthermore, in this preliminary study we did not estimate the amount of contamination with cytosolic malic enzyme by measurement of "marker" enzymes. However, the "uncorrected" levels reported by Stumpf et al. were still reduced in Friedreich's ataxia to 27% of the control cell levels, so it seems unlikely that cytosolic contamination could cause us to record normal enzyme levels in Friedreich's ataxia cells.

Preliminary studies of malic enzyme activity in leukocytes from Friedreich's ataxia patients failed to find a difference in total activity between patients and controls. It is to be hoped that extended studies on fibroblasts, leukocytes and other cells can be carried out to determine the reproducibility of the important observations of Stumpf et al. Our own failure to confirm reduced ME_m in Friedreich's ataxia cells is disquieting, but such factors as small sample size, experimental methodology, and possible biochemical heterogeneity of Friedreich's ataxia patients cannot be dismissed.

This work was supported by the Friedreich's Ataxia Group.

References