Purine nucleotide cycle enzymes in dystrophic and normal mouse muscle

Peter S. FITT and Matthew B. PARLIAMENT

Department of Biochemistry, University of Ottawa, Ottawa, Ontario, Canada KIN 9A9

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A comparative study of the operation of the purine nucleotide cycle and of the activity of adenylosuccinase in extracts of muscle from the two strains of dystrophic mouse shows that the cycle is defective in both cases in the conversion of adenylosuccinate to AMP. However, adenylosuccinase activity is only slightly reduced in the standard conditions for its direct assay.

Sanada and Yamaguchi (1979) recently reported that adenylosuccinase (adenylosuccinate AMP-lyase, EC 4.3.2.2) activity in hind leg muscle of C57BL/6J-dy/dy mice was less than 3% of that in phenotypically normal animals while the activities of the other two enzymes of the purine nucleotide cycle, adenylosuccinate synthetase (IMP: aspartate ligase (GDP), EC 6.3.4.4) and adenylate deaminase (AMP aminohydrolase, EC 3.5.4.6), were significantly reduced. These findings were of great interest in view of the hypothesis that human Duchenne muscular dystrophy might arise from a defect in adenine nucleotide metabolism (Thompson & Smith, 1976; Stone, 1979), but measurements of adenylosuccinase levels in muscle from humans suffering from several forms of muscular dystrophy showed no change in its activity (Kar & Pearson, 1981). Kar and Pearson therefore concluded that murine muscular dystrophy is not comparable to any form of the human disease. In contrast, Hamada et al. (1981) found that the serum of Duchenne muscular dystrophy patients contains an aberrant adenylate kinase (ATP: AMP phosphotransferase, EC 2.7.3.2) isoenzyme, suggesting that this disease may indeed be due to an abnormality in nucleotide metabolism, even though the enzyme concerned may not be the same as that affected in murine dystrophy.

In an attempt to clarify the problem we have now compared the operation of the first two steps of the purine nucleotide cycle and adenylosuccinase activity in both the known dystrophic mouse mutants and phenotypically normal controls.

Materials and Methods

Animals. Dystrophic male mice (4-7 weeks old) of the C57BL/6J-dy<sup>2J</sup>/dy<sup>2J</sup> strain and phenotypically normal controls (C57BL/6J-dy<sup>2J</sup>/+ and C57BL/6J-+/+) were a gift from Dr. D. Parry. Similar dystrophic males (6-7 weeks old) of the C57BL/6J-dy/dy strain and controls (C57BL/6J-dy/+ and C57BL/6J-+/+) were purchased from the Jackson Laboratory, Bar Harbor, ME, U.S.A. All were fed ad libitum on Purina Rat Lab Chow.

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Preparation of muscle extracts. Muscle extracts were prepared by published methods (Sanada & Yamaguchi, 1979; Tornheim & Lowenstein, 1972). The mice were killed by cervical dislocation and the hind leg muscles (ca. 1 g wet wt./animal) were removed, immersed in ice-cold 0.18 M KCl, 0.09 M potassium phosphate buffer, pH 6.5 (4 ml), and homogenized with a Brinkman Polytron (PT20ST probe, setting 9, 10 s). Centrifugation at 46 000 g for 10 min followed by further centrifugation at 130 000 g for 45 min gave the crude extract. A sample (1 ml) of the extract was applied to a 0.9-cm x 12-cm column of Sephadex G-25, equilibrated and eluted with 0.28 M KCl, 5 mM EDTA, 0.1 mM dithiothreitol, 15 mM potassium phosphate buffer, pH 6.5. Fractions (2 ml) were collected: the third fraction contained the enzyme activities and was used for the assays.

Assay of the purine nucleotide cycle. The overall operation of the first two steps of the purine nucleotide cycle was assayed by a differential spectroscopic method (Tornheim & Lowenstein, 1972) in which the rates of formation from IMP of adenylosuccinate plus AMP and of AMP alone are used as measures of the activities of adenylosuccinate synthetase and adenylosuccinase, respectively. The reaction mixture contained (final concentrations): 0.52 mM IMP; 0.3 mM GTP; 4 mM L-aspartate; 1.67 mM creatine phosphate; 8.3 mM MgCl₂; 27 mM imidazole-HCl buffer, pH 6.7; and 0.2-0.5 mg/ml of protein. Incubation was at 30°C and controls contained no aspartate. Samples (0.1 ml) were withdrawn at intervals up to 80 min and diluted with water (0.9 ml). The absorbances of the dilutions were determined immediately at 262.5 nm, 270 nm, and 282 nm (1-cm path length) against the diluted controls. The adenylosuccinate and AMP concentrations were calculated as described by Tornheim and Lowenstein (1972).

Adenylate deaminase assay. Adenylate deaminase was assayed as described by Fishbein (1979).

Adenylosuccinase assay. The adenylosuccinase activity was assayed directly by Woodward's (1978) method. One unit of enzyme activity corresponded to the conversion of 1 μmol of adenylosuccinate/ml/min to AMP.

Protein assay. Protein concentrations were determined by the method of Lowry et al. (1951).

Results and Discussion

The dy and dy²3 mutations arose independently in mice and are due to autosomal recessive genes that have been transferred to the C57BL/6J background by crossing (MacPike & Meier, 1976). They are members of a series of multiple alleles (Meier & Southard, 1970), although the dystrophy due to the dy²3 mutation is less severe than that observed in the dy mutants. Both mutants are widely used as models of human dystrophy.

In 1979, Sanada and Yamaguchi described a study of the activity of the purine nucleotide cycle (Lowenstein, 1972) in extracts of muscle from young male C57BL/6J-dy/dy dystrophic mice and phenotypically normal controls (dy/+ and ++/+ ) of the same strain. The overall operation of the cycle was followed by the method of Tornheim and Lowenstein (1972), and it was reported that the amounts of adenine