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A DEFECT OF PURINE NUCLEOTIDE CYCLE IN THE SKELETAL MUSCLE OF HEREDITARY DYSTROPHIC MICE

Hiroo Sanada and Michio Yamaguchi

Division of Basic Nutrition, National Institute of Nutrition,
1 Toyamacho, Shinjuku-ku, Tokyo 162, Japan

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SUMMARY

The purine nucleotide cycle in the hind leg skeletal muscle of hereditary dystrophic mice (C57BL/6J-dy/dy) was investigated. The amount of adenine nucleotide produced from adenylosuccinate by the muscle extract in the dystrophic group was less than 3 % of that in the control group, while adenine nucleotide plus adenylosuccinate converted from IMP in the dystrophic group was about 70 % of that of the control group. Moreover, the activity of AMP deaminase of the dystrophic group was about 50 % of that of the control group. These results indicate that the purine nucleotide cycle is defective in the dystrophic muscle. This abnormality was suggested to be caused by the considerably low activity of adenylosuccinase.

INTRODUCTION

Genetically dystrophic mouse has been used as an excellent model animal for the study of pathogenesis of muscular dystrophy. The degradation of muscle, especially of myofibril, in dystrophy has been observed to be associated with some biochemical changes as seen in enzymes and components. However, the pathogenesis of muscular dystrophy has not been sufficiently clarified through these observations. We consider that there perhaps is another metabolic defect as a cause of the muscular dystrophy.

In the present experiments, we tried to find more important metabolic changes in the dystrophic muscle that might be caused by inborn errors in metabolism contributing to the pathogenesis. Although the physiological significance of purine nucleotide cycle is still obscure, this cycle has been considered to play an important role in muscular function through ammonia production and AMP degradation (1). We investigated the purine nucleotide cycle in the skeletal muscle of dystrophic mice and demonstrated the defect of the cycle in these animals.

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MATERIALS AND METHODS

Male genetically dystrophic mice (C57BL/6J-dy/dy) and control mice (C57BL/6J-dy/+ and C57BL/6J-+/+) were obtained from the Central Institute for Experimental Animals (Takatsu-ku, Kawasaki-shi, Japan). They were housed in an air-conditioned room with 12-hour light and dark cycles. The composition of their diet was as follows; 20 g of vitamin-free casein (NRC), 68 g of α-cornstarch, 2.8 g of olive oil, 0.2 g of ethyl-linoleate, 3 g of cellulose powder, 5 g of mineral mixture, 1 g of vitamin mixture (0.83 g of cellulose powder and 0.17 g of vitamins), 20 mg of α-tocopherol acetate per 100 g of diet. All the vitamins and minerals necessary for normal mouse were contained in adequate amounts. This diet was given to the animals 3 to 7 days before sacrifice.

Sodium salt of IMP, GTP, creatine phosphate and hexokinase were parchased from Sigma Co. Aspartic acid and 2-deoxyglucose were obtained from Tokyo Kasei Kogyo Co. (Tokyo, Japan).

The assay of purine nucleotide cycle was performed in accordance with the methods described by Tornheim and Lowenstein (2). At the time of experiments, the ages of the dystrophic and control mice were 1 to 1.5 months and 1 to 2.0 months, respectively. The hind leg skeletal muscle of each animal was excised after drawing blood by heart puncture under ether-induced anesthesia. The muscular extract was prepared by homogenizing the muscle with 4 volumes of KCl-phosphate buffer (90 mM potassium phosphate buffer, pH 6.5, containing 180 mM of potassium chloride) by the use of a Polytron homogenizer (Kinematica, Switzerland). After centrifugation at 105,000 x g for 45 min, 1 ml of the supernatant was passed through a Sephadex G 25 column (0.8 x 12 cm) to remove low molecular substances and eluted with 15 mM phosphate buffer (pH 6.5) containing 280 mM potassium chloride, 5 mM EDTA and 0.1 mM dithiothreitol. Protein fractions were collected, and the protein concentration was determined by the method of Kalcker (3). This solution was used as the enzyme sources for the assay of purine nucleotide cycle, creatine phosphokinase and AMP deaminase. In the determination of the purine nucleotide cycle, the reaction mixture contained 0.52 mM IMP, 0.30 mM GTP, 8.3 mM magnesium chloride, 1.67 mM creatine phosphate, 4.0 mM aspartic acid, 27 mM imidazole-HCl buffer (pH 6.7), 4.8 units of hexokinase and enzyme solution equivalent to 2 mg of protein. The total volume of the reaction mixture was 4.0 ml. The reaction mixture was incubated for 2 hours at 30°, and the spectral change (240 - 340 nm) was observed every 20 min by the use of a photo-cell of 1 mm light path. After 80 min of incubation, 20 μl of 2-deoxyglucose (0.292 mM) were added to the reaction mixture to start the AMP deamination according to the degradation of creatine phosphate, ATP and GTP. The assay medium for AMP deaminase contained 27 mM imidazole-HCl buffer (pH 6.7), 100 mM potassium chloride, 0.5 mM AMP and 10 ul of enzyme solution in a total volume of 4 ml. The reaction was started by adding AMP. Then, the reaction mixture was incubated for 5 min at 30°. The initial velocity of AMP degradation was measured at 262.5 nm by the use of a photo-cell of 1 mm light path. To the reference cells, 0.5 mM IMP was added in place of AMP. The concentrations of adenine nucleotide and adenylosuccinate in the reaction mixture were calculated from the optical densities at 262.5 and 282 nm. Creatine phosphokinase activities in serum and muscular extract were determined with a commercially available assay kit (CPK-monotest kit from Boehringer Mannheim). Protein was determined by the method of Lowry (4).

RESULTS

In the reaction system used in these experiments, adenylosuccinate was produced from IMP by the enzyme adenylosuccinate synthetase, followed by its

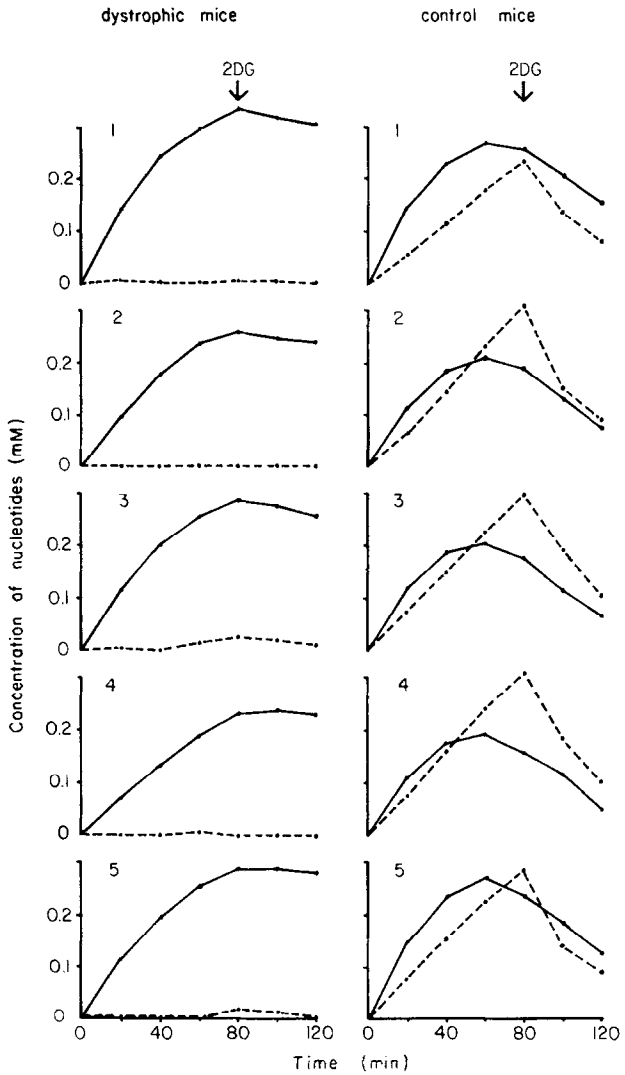

Figure 1. Concentrations of adenylosuccinate (solid line) and adenine nucleotide (broken line) in the reaction mixture. Animal No. is indicated in each case. 2-Deoxyglucose was added at the times indicated by arrows. The dystrophic and the control mice were 1.0-1.5 and 1.5-2.0 months old, respectively.

conversion to AMP and fumarate by adenylosuccinase. After 80 min of incubation, 2-deoxyglucose was added to the reaction mixture to exhaust creatine phosphate and ATP, resulting in the stop of adenylosuccinate synthesis and the start of AMP deamination. The accumulation or degradation of adenylosuccinate and adenine nucleotide in each reaction mixture is shown in Fig. 1. The concentration of adenine nucleotide in the reaction mixture of dystrophic mice

Table 1. Activity of creatine phosphokinase and amount of nucleotide produced from IMP

Group	Activity of creatine phosphokinase		Nucleotide produced in 60 min		
	Serum (U/l)	Muscle (U/mg protein)	Adenine nucleotide	Adenylosuccinate + Adenine nucleotide	Ratio*
			(μmoles/ mg protein)		
Dystrophic mice	375 ± 36	31 ± 2	0.01 ± 0.01	0.55 ± 0.09	0.02 ± 0.01
Control mice	84 ± 21	44 ± 1	0.38 ± 0.03	0.79 ± 0.07	0.49 ± 0.03
P**	< 0.001	< 0.001	< 0.001	< 0.05	< 0.001

The dystrophic and the control mice were 1.0-1.5 and 1.5-2.0 months old, respectively. The amount of adenine nucleotide or adenylosuccinate produced from IMP during 60 min of incubation is indicated. Protein was determined as described by Lowry et al. (4). Each value is the mean of five animals ± s.e.m.
* Ratio = adenine nucleotide / adenine nucleotide plus adenylosuccinate.
** P values as calculated by Welch's test for differences between the two groups.

was observed to be almost 0 or extremely low throughout the incubation period. On the other hand, that of the control mice was shown to reach about the same level as that of adenylosuccinate after 60 min of incubation. The sum of the adenine nucleotide and adenylosuccinate produced was considered to roughly correspond with the activity of adenylosuccinate synthetase, and the amount of adenine nucleotide alone was considered to demonstrate the activity of adenylosuccinase. Therefore, as shown in Table 1, production of an extremely small amount of adenine nucleotide in the dystrophic group suggested the considerably low activity of adenylosuccinase in the dystrophic muscle. The sum of adenine nucleotide and adenylosuccinate also decreased in this group, indicating the low activity of adenylosuccinate synthetase. The nucleotide ratio (i.e. adenine nucleotide/ adenine nucleotide plus adenylosuccinate) of dystrophic mice was also significantly small. This fact indicates that the activity of adenylosuccinase per unit of adenylosuccinate synthetase activity may be low in these animals. Slow degradation of adenylosuccinate in the dystrophic mice after the addition of 2-deoxyglucose (as indicates in Fig. 1) was again considered to be caused by the low activity of adenylosuccinase. The activity of AMP deaminase of the dystrophic muscle was shown to be about

Table 2. Activities of AMP deaminase of the dystrophic and the control muscle extracts

Group	Activity of AMP deaminase (μmoles/min/mg protein)	Adenine nucleotide produced from IMP (μmoles/h/mg protein)
Dystrophic mice	0.64 ± 0.09	0.00 ± 0.00
Control mice	1.20 ± 0.05	0.28 ± 0.03
P*	<0.01	<0.01

All animals were 1.0 month old.
Each value is the mean of four animals ± s.e.m.
* P values as calculated by Welch's test for differences between two groups.

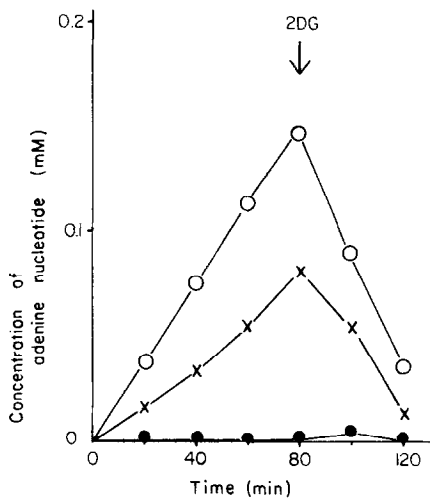

Figure 2. Accumulation of adenine nucleotide in the reaction mixture containing mixed extracts of the dystrophic and the control muscles. Each incubation medium (4 ml) contained the control muscle extract (2 mg of protein) (o—o), the dystrophic muscle extract (2 mg of protein) (●—●), or the control muscle extract (1 mg of protein) + the dystrophic muscle extract (1 mg of protein) (x—x).

50 % of that of the control (Table 2.). To eliminate the possibility of the presence of adenylosuccinase inhibitor in the dystrophic muscle, the amount of adenine nucleotide produced from IMP was measured in the mixed extracts of the dystrophic and the control muscles (Fig. 2). The extract of the dystrophic muscle (2 mg of protein) converted an extremely small amount of IMP to adenine nucleotide. The mixed extracts (1 mg of protein from each extract) produced an amount of adenine nucleotide about half its amount formed by the

control extract (2 mg of protein). This result indicates that there is no inhibitor in the dystrophic muscle.

DISCUSSION

The present data indicate the defect of purine nucleotide cycle in the hind leg skeletal muscle from genetically dystrophic mice, where the considerably low activity of adenylosuccinase is suggested to account for this abnormality. Although the role of purine nucleotide cycle in muscle was not sufficiently clarified, some investigators indicated that the release of ammonia by way of this metabolic cycle was associated with the physiolosical change of the skeletal muscle (5). Therefore, it is conceivable that the ammonia produced by this cycle may play an important role in the muscular function. There also are various interesting reports on the inhibitory effect of ammonia on the activity of lysosomal protease (6,7,8). Schultz and Lowenstein demonstrated that the activity of adenylosuccinase in rat liver and spleen was affected by diet, but was not affected in skeletal muscle, brain and kidney (9). Therefore, the low activity of adenylosuccinase in the dystrophic skeletal muscle was not considered attributable to the small amount of their food intake. Further investigation is necessary to clarify the relation between the defect of purine nucleotide cycle and the pathogenesis of muscular dystrophy.

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