THE INFLUENCE OF DENERVATION ON THE CO-ENZYME SPECIFICITY OF MALATE AND GLUTAMATE DEHYDROGENASES IN GASTROCNEMIUS MUSCLE OF FROG, *RANA HEXADACTYLA*

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ABSTRACT

Activity levels of nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP)–linked malate dehydrogenase (MDH) and glutamate dehydrogenase (GDH) were assayed in normal and denervated gastrocnemius muscles of frog at 1, 2, 3 and 4 weeks after sciatectomy. The atrophy process evoked progressive decrease of NAD-linked malate and glutamate dehydrogenase activity levels while NADP–linked malate and glutamate dehydrogenases showed progressive increase. This shift in co-enzyme specificity of MDH and GDH in atrophied muscle seems to be one of the fundamental characters responsible for atrophy process. Possible reasons for these changes during atrophic process of skeletal muscle are discussed.

INTRODUCTION

THE prominent features of hereditary and nutritional muscular dystrophies and atrophy, resulting from disuse, denervation and tenotomy are due to progressive decrease of oxidative and glycolytic activity[1–4] with increased proteolytic activity[5, 6]. Further the denervation atrophy in various skeletal muscles was shown to involve disorganisation of mitochondria[4, 7, 8]. The NAD-dependency of oxido reductases decreases, leaving room for NADP-dependency[9, 10]. It was reported earlier that atrophy process evoked progressive decrease of NAD-linked isocitrate dehydrogenase, and progressive increase of NADP-linked isocitrate dehydrogenase[11] and glucose-6-phosphate dehydrogenase[12] in the amphibian gastrocnemius muscle. This shift in co-enzyme specificity of dehydrogenases in atrophied muscle seems to be one of the fundamental characters responsible for atrophy process. In view of the above, an attempt was made to study the activity levels of both NAD and NADP-linked malate dehydrogenase (MDH), which catalyzes a key reaction in citric acid cycle and glutamate dehydrogenase (GDH), which plays a vital role in ammonia metabolism, on progressive denervation of amphibian gastrocnemius muscle which may provide some insight into the relative importance of co-enzyme specificity in atrophied muscle.

MATERIAL AND METHODS

Rana hexadactyla (Lesson) of medium size were denervated by sciatic nerve section about 1 cm from its origin on one side of the leg, while contralateral muscle was considered as control. The frogs were fed *ad libitum* with cockroaches, and water was changed regularly. After 1, 2, 3 and 4 weeks of post-operative period the animals were sacrificed and both the denervated and contralateral control gastrocnemius muscles were excised quickly. Homogenates of tissues (10% wt/vol) were prepared in 0·25 M ice cold sucrose using Potter-Elvehjem type glass homogenizer and centrifuged at 2,500 rpm for 15 min. The supernatant (0·5 ml containing 50 mg tissue) was assayed for malate dehydrogenase and glutamate dehydrogenases by adopting the method of Lee and Lardly[13] with the following modifications: The reaction mixture of 2 ml had 40 μ moles of substrate (sodium malate for MDH and sodium glutamate for GDH), 100 μ moles of phosphate buffer (pH 7·4), 4 μ moles of INT (2-*p*-iodophenyl-3-nitrophenyl-5-phenyl tetrazolium chloride) and 0·1 μ mole of NAD both for NAD-linked malate dehydrogenase (L-malate: NAD^+ oxido-reductase, EC. 1.1.1.37) and NAD-specific glutamate dehydrogenase (L-glutamate: NAD^+ oxido-reductase, EC. 1.4.1.3) or 0·2 μ moles of NADP both for NADP-specific malate dehydrogenase (L-malate : NADP oxido-reductase, EC. 1.1.1.40) and NADP-specific glutamate dehydrogenase (L-glutamate: NADP oxido-reductase, EC. 1.4.1.4) The reaction was initiated by the addition of 0·5 ml of the supernatant. Controls received 0·5 ml of sucrose in place of the enzyme extract. After an incubation for 30 min at 37° C, the reaction was stopped by the addition of 5 ml glacial acetic acid, and the derived formazan was extracted into 5 ml of toluene. After keeping it overnight in cold, the colour was measured in UV-spectrophotometer (Hilger and Watts, England) at 495 nm using sila

cuvettes of 10 mm path length. Individual zero time controls were maintained for all the samples by the addition of glacial acetic acid to reaction mixture prior to the addition of enzyme. Enzyme activities both for MDH and GDH were expressed as μ moles of formazan/mg protein/hr. Protein levels were determined by the method of Lowry *et al.*[14]. Data were subjected to statistical processing according to standard procedures[15].

Results and Discussion

Progressive denervation atrophy induced an initial increment (+ 8·88% after 1st week) in the activity level of NAD-specific malate dehydrogenase followed by a gradual decrease (– 29·4% after 4 weeks) in the gastrocnemius muscle of frog (Table I). Similar decrease in Krebs cycle enzymes like NAD-specific isocitrate dehydrogenase[11] and SDH[16] has been reported earlier. Though the NAD-specific MDH has shown increased activity level one week after denervation when compared to normal control muscle, it is statistically not significant showing that denervation does not affect the NAD-specific MDH at early periods of denervation. The progressive decrease of NAD-specific MDH, in later periods of denervation may be due to the poor respiratory control or due to the low mitrochondrial content in denervated muscle[8]. NAD-specific MDH was known to have dual localization both in mitochondria and cytoplasm[17]. Mitochondria of denervated muscle have decreased ability to oxidize α-ketoglutarate, L-glutamate and pyruvate[18]. Hence on denervation, skeletal muscle metab lism seems to be geared to low level of oxidations. Further, NAD-specific MDH has been shown to be sensitive to the levels of NADH[17]. A high concentration of NADH leads to a decrease of NAD-specific MDH activity. Hence it can be presumed that a prominent role is played by NADH in the inactivation of the enzyme in the denervated muscle, since it is known that the ratio, NADH/NAD, increases in the skeletal muscle of genetically dystrophic and vitamin-E deficient animals[19]. The present observation indicates that similar regulatory phenomenon might exist in the denervated gastrocnemius muscle also.

Progressive increase of NADP-specific malate dehydrogenase (malic enzyme) activity level was found in the muscle subjected to denervation, the increase being 36% after 4 weeks (Table I). Similar

Table I

Malate and glutamate dehydrogenase activities in the amphibian gastrocenemius muscle
(Values are mean ± S.D. of 10 observations. Activity expressed as μ molcs of formazan/mg protein/hr)

Weeks after denervation	NAD-specific Activity				NADP-specific Activity			
	I	II	III	IV	I	II	III	IV
				Malate dehydrogenase				
Control muscle	0·146 ± 0·026	0·138 ± 0·022	0·139 ± 0·020	0·142 ± 0·022	0·197 ± 0·022	0·198 ± 0·021	0·193 ± 0·019	0·196 ± 0·020
Denervated muscle	0·159 ± 0·024	0·130 ± 0·020	0·114 ± 0·012	0·100 ± 0·007	0·214 ± 0·024	0·232 ± 0·010	0·253 ± 0·031	0·267 ± 0·010
% Change on atrophy	+ 8·88	– 5·8	–17·75	–29·4	+ 8·63	+17·68	+31·77	+36·22
'P' Values	NS	NS	< 0·005	< 0·001	NS	< 0·001	< 0·001	< 0·001
				Glutamate dehydrogenase				
Control muscle	0·108 ± 0·005	0·098 ± 0·005	0·100 ± 0·003	0·101 ± 0·003	0·149 ± 0·013	0·155 ± 0·008	0·147 ± 0·010	0·144 ± 0·009
Denervated muscle	0·097 ± 0·012	0·075 ± 0·006	0·068 ± 0·007	0·055 ± 0·007	0·165 ± 0·007	0·176 ± 0·003	0·185 ± 0·006	0·189 ± 0·005
% Change on atrophy	–10·09	–23·47	–32·0	–45·55	+10·73	+13·55	+25·85	+31·25
'P' Values	NS	< 0·001	< 0·001	< 0·001	< 0·005	< 0·02	< 0·001	< 0·001

increase in NADP-dependent malate dehydrogenase was found in Duchenne muscular dystrophy and other myopathies and neuropathies[20]. McCaman suggested that a common feature of dystrophic and denervated muscle has been a general elevation in the activities of NADP-dependent dehydrogenases, whereas the activities of NAD-dependent dehydrogenases show a decrease[9, 10]. This is obvious since the major function of NADP-specific malate dehydrogenase appears to be the provision of reduced NADP required for fatty acid synthesis[17]. Further, the increased lipogenesis observed in muscular dystrophy[20, 21] requires NADPH for their synthesis and consequently there is enhanced synthesis of NADP in atrophied muscle which in turn may result in elevated levels of NADP-specific enzymes. It was reported earlier that NADP-specific isocitrate dehydrogenase[11] and glucose-6-phosphate dehydrogenase[12] have shown an increment in their activity levels on denervation and the present finding of enhancement in the level of NADP-specific malate dehydrogenase in denervated frog gastrocnemius muscle is in agreement indicating the presence of synthetic process.

NAD-specific glutamate dehydrogenase activity was also found to fall progressively in the denervated gastrocnemius muscle, the decrease being 45·55% after 4 weeks (Table I). Similar decrease in NAD-linked GDH in atrophic muscle has been reported earlier[16]. This decrease in NAD-specific GDH may be due to the low availability of co-enzyme NAD, since it is known that the ratio, NADH/NAD, increases in dystrophied muscle[19]. Another reason for the decreased glutamate dehydrogenase activity is the general increase in the free ammonia level[22]. Due to decreased citric acid cycle oxidation, keto acids formation is less, so also its utilization. With increase in the ammonia pool, the available keto acids are probably aminated to amino acids by the glutamate dehydrogenase reacting system in the reverse direction, Hence estimation of NAD–specific GDH levels using glutamate may show low level activity. Further, the decrement in the level of NAD-specific GDH activity may be due to product inhibition since ammonia which is the product for this enzyme catalysis was found to increase[22] on denervation in gastrocnemius muscle. Contrastingly, the NADP-linked glutamate dehydrogenase showed progressive increase (31·25% after 4 weeks) (Table I). The contents of glutamate, aspargine and aspartate were found to increase in the denervated muscle[22]. Since most of the biological reactions operate in the first order kinetic region, the elevation of glutamate invariably elevates the glutamate dehydrogenase activity. Under this circumstance, the increase in the activity level of NADP-specific glutamate dehydrogenase as against the decreent in the activity level of NAD-specific GDH is in accordance with the elevated level of ammonia in the denervated frog gastrocnemius muscle.

Thus the shift in co-enzyme specificity of malate and glutamate dehydrogenase systems in atrophied muscle seems to be one of the fundamental characters, switching over of the metabolism from the physiological activity to synthetic activity.

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