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Oxidative Metabolism in the Denervation-induced Atrophy in Gastrocnemius Muscle of Toad *Bufo melanostictus*

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Oxidative metabolism is impaired during denervation-induced muscle atrophy. Succinate, isocitrate and malate dehydrogenase activities decreased in the denervation-induced muscle atrophy. The data further indicated that mitochondria were fragmented during atrophy process.

Key Words: **Denervation,** *Bufo melanostictus,* **Dehydrogenases, Gastrocnemius muscle**

Introduction

Denervation atrophy of the skeleton muscle disturbs their functional activity with altered metabolic patterns (Wagner & Max 1979, Max et al. 1981). An impairment in the energy metabolism was found in the atrophied gastrocnemius muscle of frog (Pramilamma 1976). Though considerable work has been carried out on the effect of progressive denervation atrophy on the gastrocnemius muscle of the frog (amphibious in habitat), the investigations on the terrestrial amphibians, such as toads, are scanty. The swimming movements of the frog require less expenditure of energy because of the floating mode while the hopping habit of toad on land needs greater expenditure of energy. Hence the muscles of toad in general are hard (Anjaneyulu et al. 1982) and the denervation atrophy effects in them could be markedly different.

An attempt has been made here to assess the denervation atrophy effects on the energy metabolism of gastrocnemius muscle of toad by studying few oxidoreductases as indicators of oxidative metabolism.

Materials and Methods

Male *Bufo melanostictus* of body weight range (30 ± 5 g) were collected from localities in and around Tirupati and acclimated to laboratory conditions for a week. The sciatic nerve was sectioned in one leg as described by Swami and Satyanarayana (1976). The toads were fed with earthworms *ad libitum* and kept in clean troughs containing sand which was changed regularly once in a day. The toads were double pithed at 7th, 14th, 21st and 28th day post-operatively and both the denervated and contralateral gastrocnemius (control) muscles were excised quickly. 5% (W/V) tissue homogenates were prepared in cold 0.25 M sucrose medium and centrifuged at 1000 g for 30 min to remove the cell debris. The supernatants were assayed for the succinate (SDH) and malate dehydrogenases (MDH) using the method of Nachlas et al. (1960). NADP dependent isocitrate dehydrogenase (ICDH) activity was estimated by the method of Korenberg and Pricer (1951). The activity levels were expressed as μmoles of formazan formed/mg protein/hr. For extracting mitochondria from the muscle, the method of Ernester and Nordenbrand (1967) using Chappel and Perry (1954) medium was used.

The sucrose homogenate was centrifuged at 800 g for 30 min and the supernatant so obtained was centrifuged successively at 12000, 15000 and

18000 g for 30 min. The succinate dehydrogenase activity levels were determined in the sediments obtained at each centrifugal field. The protein content was determined by the method of Lowry et al. (1951).

Results and Discussion

No significant changes were observed in the activity levels of SDH, MDH and ICDH in the contralateral (control) muscles at all the time points after sciatectomy (table 1). Of the three enzymes, the SDH showed highest activity level whereas the MDH had lowest activity level when compared to ICDH. In the denervated (atrophied) muscle, the activity levels of these dehydrogenases in general showed decreases in activity and this diminution is progressive from 1st week to 4th week. In the case of ICDH, there is slight elevation in the 1st week. But the general pattern seems to be similar at later time points of atrophy. In the one week atrophied muscle, the SDH (−19.8%) and MDH (−12.6%) activities were decreased whereas the ICDH activity was increased (27.5%). During initial stages of the denervation atrophy, the response of the oxidoreductases investigated seems to be different than that of the later stages. In the later stages, i.e., 2nd–4th week, the trend seems to be same in all the oxidoreductases, that is a persistent diminution in activity levels (about 50% reduction). The deprivation of neurotrophic factors as effected by cutting of the nerve seems to affect the citric acid metabolism in different stages (Muscatello & Patriarca 1968).

The rapid decrease of SDH activity levels in the 1st week of the atrophic process is indicative of cessation of the aerobic segment of the carbohydrate metabolism. Similar decrement in MDH activity level was also apparent. The MDH is known to be involved in metabolic pathway other than the citric acid cycle such as Wood-Workman reactions involving CO_2 fixation by pyruvate carboxylase. Probably this side reaction of MDH is partially affected while the citric acid involvement of this enzyme is more affected. The positive modulation of ICDH is indicative of the prevalence of NADP levels in the muscle metabolism. An increase in the NADP level was reported in the amphibian muscles subjected to denervation atrophy (Masthanaiah et al. 1979).

The decrease in the SDH, ICDH and MDH activity levels indicated the impaired mitochondrial oxidation of succinate, isocitrate and malate in the denervated atrophied muscle. The TCA cycle as represented by these enzymes showed progressive diminution in the time-course of denervation atrophy. The atrophy caused by the denervation or by disuse of muscle leads to the fragmentation of the muscle fibre mitochondria (Carafoli et al. 1964,

Table 1 *Activity levels of oxidoreductases in the contralateral control and denervated atrophied gastrocnemius muscle of toad,* Bufo melanostictus *during progressive denervation atrophy. Values are expressed in μ moles of formazan/mg protein/hr. Values are means of six observations*

Muscle	Enzyme	Period after denervation			
		Week 1st	2nd	3rd	4th
Contralateral control	SDH	3.08	3.25	3.21	3.29
	SD±	0.07	0.38	0.22	0.27
	MDH	0.11	0.10	0.11	0.09
	SD±	0.02	0.01	0.02	0.02
	ICDH	1.31	1.27	1.18	1.41
	SD±	0.02	0.01	0.02	0.02
	SDH	2.47	2.05	1.84	1.56
	SD±	0.01	0.24	0.27	0.23
	% Change over contralateral control	−19.81	−36.92	−42.68	−52.58
	't' test	$P<0.005$	$P<0.001$	$P<0.001$	$P<0.001$
Denervated atrophied	MDH	0.11	0.08	0.05	0.03
	SD±	0.01	0.02	0.02	0.02
	% Change over contralateral control	−4.55	−16.00	−53.64	−64.44
	't' test	NS	NS	$P<0.001$	$P<0.001$
	ICDH	1.67	0.97	0.79	0.67
	SD±	0.02	0.02	0.01	0.01
	% Change over contralateral control	+27.48	−23.62	−33.05	−52.48
	't' test	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$

Muscatello & Patriarca 1968) resulting in decreased activities of some mitochondrial enzymes (Romanul & Hogan 1965). Such an event results in low energy output in the atrophied muscle. The decreasing high energy phosphates in the hearts of dystrophic hamsters (Fedelesova & Dhalla 1971) in the skeletal muscle of vitamin E deficient rats (Dhalla et al. 1971a), in genetically dystrophic hamsters (Dhalla et al. 1971b) indicated the impairment of energy metabolism.

The mitochondrial enzymes showed inactivation in the denervation atrophy and the mitochondria are known to undergo distintegration (Muscatello & Patriarca 1968). Hence, an attempt was made to study the impact of sciatectomy on the functional morphology of mitochondria in the gastrocnemius muscle. The sediments of differential centrifugation at 12000, 15000 and 18000 g for 30 min is expected to sediment large, medium and small sized mitochondria respectively into the pellets. Since the SDH is a marker enzyme for mitochondria, its activity levels are expected to represent the mitochondrial content in the medium. In the contralateral control muscle, the larger mitochondrial fractions obtained at 12000 g showed relatively higher activity than the smaller mitochondrial fractions. In the denervated atrophied muscle the SDH activity levels were comparatively lower than the contralateral control muscles. However, the SDH activity levels seemed to increase with decrements in mitochondrial size and maximum levels of activity were seen in 16000 g sediment (table 2).

The present study indicates that impairment of the operation of TCA cycle might be due to the diminished mitochondrial content in the denervated muscle of toad. Mitochondrial population consisting of smaller types was prominent in the denervated atrophied toad muscle unlike the contralateral control muscle where the large sized mitochondria are more in number. This suggests the possibility of fragmentation or dissolution of mitochondria in the denervation atrophy process. From the results it appears that the hopping and nocturnal habits and terestrial habitat of toad do not have any influence on the denervation effects in muscle because similar changes were reported in the denervated gastrocnemius muscle of frog (Pramilamma 1976).

Table 2 *Succinate dehydrogenase activity in the mitochondrial components of the contralateral control and denervated gastrocnemius muscles of toad,* Bufo melanostictus. *Mitochondrial SDH activity expressed as μmoles of formazan/mg protein/hr. Values are means of six observations*

Source of the muscle enzyme	Centrifugal sediments obtained at		
	12000 g	15000 g	18000 g
Contralateral control	23.11	19.36	21.13
SD ±	1.72	1.89	2.03
Denervated atrophied	12.72	13.84	16.28
SD ±	1.56	1.68	1.49
% Change over contralateral control	−44.96	−28.51	−22.95
't' test	$P<0.001$	$P<0.001$	$P<0.001$

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