SKELETAL MUSCLE PROTEIN METABOLISM UNDER DENERVATION ATROPHY IN DOG, *CANIS DOMESTICUS*

S. JABEEN BEGUM, M. MUNIRATHNAM REDDY*, O. RAMAKRISHNA,**
K. INDIRA AND K. S. SWAMI

Department of Zoology,
Sri Venkateswara University, Tirupati - 517 502

(Received on August 8, 1986)

Summary : Denervated dog gastrocnemius muscle has shown a progressive decrease in total protein content, alanine aminotransferase (AlAT), aspartate aminotransferase (AAT) and glutamate dehydrogenase (GDH) activity levels and elevation in free amino acid, ammonia, urea, glutamine contents and AMP deaminase activity levels during post-neurectemic days The possible implications of these findings are discussed in relation to denervation atrophy.

Key words : denervation atrophy, protein metabolism, gastrocnemius muscle, dog

INTRODUCTION

Denervation is found to produce structural, chemical and biophysical alterations in the muscle due to metabolic derangement (6). The activities of glycolytic and citric acid cycle enzymes and adenosine triphosphate levels were low (1). Further the denervation atrophy in various skeletal muscles was shown to involve disorganisation and fragmentation of mitrochondria (16). It has been reported that there is an elevation in ammonia content after sciatectomy in frog and ammonia is known to interfere with the energy metabolism (15). Since oxidative metabolism is closely associated with nitrogen metabolism, the present study has been undertaken to elucidate the possible changes in muscle nitrogen metabolism during progressive denervation atrophy in dog.

*For correspondence
**Department of Surgery and Radiology, College of Veterinary Sciences, Tirupati - 517 502

MATERIAL AND METHODS

Two sets of (each contains 6) healthy mongreal dogs *C. domesticus* aged 3-4 years and weighing 11-16 *kg* were selected for the present study. One set served as control while the other set was subjected to unilateral sciatectomy under asceptic conditions as described earlier (3). The right neuroctomized muscle was designated as denervated muscle (DM) and the left non-neuroctomized muscle served as the contralateral muscle (CM). As there was no significant difference observed, between contralateral and normal muscles, the former was taken as the control.

Total proteins (9), free amino acids (10), free ammonia (2), urea (13), glutamine (19) and the activity levels of AlAT, AAT (14), GDH (7) and AMP deaminase (18, 17) were estimated in CM and DM of dogs for I, II, III and IV weeks. The data was analysed by student 't' test to assess the difference between control and experimental.

RESULTS

The data presented in Tables I and II reveal the changes in protein metabolism of control and denervation atrophied muscle of dog, *Canis domesticus.*

Total protein content showed a continuous decrement upto 52.43% at 4th week during denervation atrophy with a significant elevation (74,72%) in free amino acid content (Table I). The activity levels of AlAT (—37.37%), AAT (—28.07%) and GDH (—67.71%) were decreased in the denervated muscles when compared to the controls. A significant elevation in the activity levels of AMP deaminase (82.93%) and the levels of ammonia, (43.18%) urea (32.10%) and glutamine (24.26%) was evinced in the gastrocnemius muscle of dog during progressive denervation atrophy (Table II).

DISCUSSION

The total protein content of muscle significantly declined upto 4 weeks during denervation atrophy indicating increased proteolysis or enhanced efflux of proteins into the blood (11). Free amino acid content of the tissue was elevated which might be due to their decreased utilization in oxidative reactions. GDH activity, which represents the oxidative deamination of amino acids showed a decline trend indicating its lesser contribution to the ammonia pool. Similarly the activity levels of alanine and aspartate aminotransferases were also diminished indicating least involvement of aminoacids in transmination reactions. This observation in dog is in consonance with the earlier reports of denervation indicating decreased activity of GDH, AlAT and AAT in various animal muscles

TABLE I : Changes in the levels of total proteins, Free amino acids, AIAT, AAT and GDH in gastrocnemius muscle of *Canis domesticus* during progressive denervation atrophy. Each value is mean±S.D. of 6 observations. Values in parenthesis represent percent deviation over control. CM, contralateral muscle; DM, denervated muscle. * indicates statistical significance at 0.05 level.

Metabolite/Enzyme	*00 hours*		*I week*		*II week*		*III week*		*IV week*	
	CM	DM	CM	DM	CM	DM	CM	DM	CM	DM
Total proteins (*mg/g*, wet wt)	172.5 ±11.18	169.4 ±10.76 (—1.80)	168.5 ±9.85	134.4* ±12.99 (—20.24)	166.7 ±4.59	116.8* ±7.96 (—29.93)	162.9 ±10.11	92.5 ±7.02 (—43.22)	160.6 ±13.44	76.4* ±6.71 (—52.43)
Free amino acids (*μmoles/g*, wet wt)	15.40 ±1.86	15.61 ±1.01 (1.36)	15.21 ±1.21	20.01* ±1.36 (32.87)	15.32 ±1.47	22.87* ±1.18 (49.28)	15.22 ±1.15	23.89* ±1.08 (56.97)	15.19 ±1.06	26.54* ±1.17 (74.72)
Alanine aminotransferase (*μmoles* of pyruvate formed/*mg* protein/hr)	7.62 ±0.561	7.48 ±0.612 (—1.84)	7.55 ±0.511	6.32 ±0.714 (—16.29)	7.54 ±0.312	5.70* 0.414 (—24.40)	7.54 ±0.515	5.22* ±0.313 (—30.77)	7.52 ±0.710	4.71* ±0.318 (—37.37)
Aspartate aminotransferase (*μmoles* of pyruvate formed/*mg* protein/hr)	4.13 ±0.220	4.07 ±0.118 (—1.45)	4.11 ±0.122	3.66* ±0.317 (—10.95)	4.10 ±0.114	3.48* ±0.291 (—15.12)	4.11 ±0.330	3.26* ±0.214 (—20.68)	3.99 ±0.117	2.87* ±0.210 (—29.07)
Glutamate dehydrogenase (*μmoles* of formazan formed/*mg* protein/hr)	0.612 ±0.014	0.608 ±0.017 (—0.654)	0.611 ±0.051	0.450* ±0.022 (—26.35)	0.609 ±0.041	0.384* ±0.011 (—36.95)	0.608 ±0.031	0.246* ±0.017 (—59.54)	0.607 ±0.009	0.198* ±0.021 (—67.71)

TABLE II : Changes in the levels of AMP deaminase activity, ammonia, urea and glutamine in gastrocnemius muscle of *Canis domesticus* during progressive denervaton atrophy. Each value is mean ± S.D. of 6 observations. Values in parenthesis represent percent deviation over control. CM, contralateral muscle; DM, denervated muscle. * indicates statistical significance at 0.05 level.

Metabolite/Enzyme	*00 hours*		*I week*		*II week*		*III week*		*IV week*	
	CM	DM	CM	DM	CM	DM	CM	DM	CM	DM
AMP deaminase	1.27	1.29	1.25	1.69*	1.24	1.89*	1.24	2.09*	1.23	2.25*
(*μmoles* of ammonia/*mg*	±0.018	±0.046	±0.034	±0.065	±0.094	±0.076	±0.021	±0.025	±0.054	±0.037
protein/hr)	(1.57)		(35.20)		(52.42)		(68.55)		(82.93)	
Ammonia	0.676	0.679	0.674	0.787*	0.673	0.869*	0.672	0.923*	0.674	0.965
(*μmoles/g*, wet wt)	±0.001	±0.003	±0.004	±0.005	±0.008	±0.006	±0.007	±0.012	±0.004	±0.003
	(0.444)		(16.77)		(29.12)		(37.35)		(43.18)	
Urea	3.28	3.31	3.25	3.64*	3.24	3.86*	3.22	4.08*	3.24	4.28*
(*μmoles/g*, wet. wt)	±0.004	±0.075	±0.218	±0.147	±0.212	±0.031	±0.009	±0.061	±0.072	±0.016
	(0.915)		(12.00)		(19.14)		(26.71)		(32.10)	
Glutamine	5.74	5.81	5.72	6.09*	5.72	6.31*	5.71	6.78*	5.73	7.12*
(*μmoles/g*, wet. wt)	±0.314	±0.217	±0.018	±0.023	±0.064	±0.078	±0.061	±0.014	±0.019	±0.076
	(1.22)		(6.47)		(10.32)		(18.74)		(24.26)	

(11, 16). In view of these observations, elevated tissue amino acid level could be due to increased proteolysis in the muscle. Enhanced proteolysis and elevated free amino acids may be responsible by providing the source for elevation of ammonia content in denervation atrophy (12).

AMP deaminase which regulates the liberation of ammonia from purine nucleotides and aminoacids and thereby adjusts the TCA cycle intermediates is found to be elevated continuously upto 4th week of denervation. The increased AMP deaminase activity in the denervated muscle reflects its physiological role in ammonia production. In support of this speculation, recent studies indicated that the operation of purine nucleotide cycle was mainly responsible for the production of ammonia in muscle (8). The overall metabolic implications of altered deamination systems elucidate that the source of excess ammonia production is mainly from purine nucleotides.

Since the production of ammonia is increased during atrophy, the ammonia detoxifying systems may be triggered. The major mechanisms involved in ammonia detoxification are the synthesis of urea and glutamine. The increased levels of urea in the denervated muscle may be due to the possible activation of arginase (5) in the presence of elevated ammonia levels. In support of this, Narayana Reddy (11) reported elevated levels of ornithine and arginase in the denervated muscle in the presence of excesses of ammonia and also the rapid operation of urea cycle in the denervated muscle, though it is generally restricted to the liver and kidney.

The glutamine levels were also elevated in the denervated muscle since glutamine is the other non-toxic product of ammonia during the process of its elimination. The significant increase in glutamine content of denervated muscle could be due to the decreased oxidation of glutamate by GDH activity and increased glutamine synthetase activity. This gains further support from the reports of Ganda and Ruderman (4) and Narayana Reddy and Swami (12).

Thus the rapid formation of urea and glutamine in the denervated muscle may be presumed as an adaptive mechanism to maintain the metabolic homeostasis in response to the high ammonia concentration and increase in the Co_2 storage capacity.

The overall metabolic changes observed during progressive denervation atrophy suggest that there is an increase in amino acid pool and increased nucleotide based free ammonia which may lead to metabolic derangement in the physiological profiles of gastrocnemius muscle of dog.

ACKNOWLEDGEMENTS

The authors SJB and MMR are grateful to CSIR, New Delhi for financial assistance.

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