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Modulations in Hepatic Nitrogen Metabolism during Short-Term and Prolonged Muscular Stimulations

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The hepatic tissue nitrogen metabolism was studied in frog, *Rana hexadactyla* (Lesson), during short-term and prolonged stimulations. The hepatic tissue of one-day-stimulated animals recorded significant elevation in proteolysis while that of ten-days-stimulated animals had non-significant change. The hepatic tissue of long-term-stimulated animals seems to have developed improved ammonia detoxificatory mechanisms in comparison to that of short-term stimulations.

Key Words: muscular stimulations, hepatic nitrogen metabolism, protease, glutamine, aminotransferases, detoxification

Introduction

In vivo muscle stimulation increases the activity of tissue oxidative enzymes (Reddanna et al. 1978). Heavy exercise induces glycogen depletion in the muscle and liver (Gollnick et al. 1973) resulting in gluconeogenesis in the liver (Goldberg & Odessey 1974, Reddanna & Govindappa 1980). Hepatic aminotransferase activity is elevated during exercise (Schmidt & Schmidt 1969) with a decrease in LDH activity (Novosadova 1969). Since oxidative metabolism is closely associated with nitrogen metabolism, the present study has been undertaken to elucidate the possible changes in hepatic nitrogen metabolism during short-term and prolonged *in vivo* muscular stimulations.

Material and Methods

Frogs, *Rana hexadactyla* (Lesson), were employed for the present study. Right gastrocnemius muscles of intact animals were stimulated with an electronic stimulator. (INCO/CSIO Research stimulator—Ambala) as described earlier (Reddanna et al. 1978). With series of impulses (biphasic) of 5 V at a frequency of 2 C/S for 30 min/day for one day in one batch of experimental animals (short-term stimulations) and for ten successive days in another batch (prolonged stimulations). The duration of each pulse was 100 msec.

The hepatic tissue was isolated for biochemical studies with least injury after pithing the animal and placed in amphibian Ringer. Protein contents of the supernatant (water soluble) and the residue (water insoluble-structural) as obtained by the centrifugation of tissue homogenate at 3000 rpm for 30 min were estimated by the method

of Lowry et al. (1951). Protease activity and free amino acid content (Moore & Stein 1954), free ammonia (Bergmeyer 1974), urea (Natelson 1971), glutamine (Colowick & Kaplan 1967) and the activities of alanine amino transferase (A1AT-EC 2.6.1.2) and aspartate amino transferase (AAT EC 2.6.1.1) (Reitman & Fraenkel 1957) and GDH (EC 1.4.1.3) activity (Lee & Lardy 1965) modified by Reddanna and Govindappa (1978) were estimated.

Results and Discussion

The data presented in the table reveal changes in the ammonia metabolism of hepatic tissue during short-term and prolonged localized muscular stimulations. The total protein content of hepatic tissue significantly declined in animals with one-day muscular stimulations. Further analysis indicated that only the soluble protein fraction of the tissue was considerably depleted while the structural protein level underwent a nonsignificant change. This decrease in the protein content of the tissue could be due to increased proteolysis or enhanced efflux of proteins into the blood. Protease activity showed considerable elevation suggesting the onset of degradative activities in the tissue in response to muscular exercise. Similar elevation in hepatic proteolysis was reported in whole animal exercises (Astrand & Rodahl 1970, Poortmans 1973). Hence proteolysis seems to be responsible for the observed decrease in the tissue protein content. Free amino acid content of the tissue was elevated which might be due to increased proteolysis as indicated above or due to their decreased utilization in oxidative reactions. GDH activity, which represents the oxidative deamination of amino acids showed nonsignificant change. Similarly the activity levels of transaminases revealed either inhibition (AAT) or nonsignificant change (A1AT) indicating least involvement of amino acids in transamination reactions. In view of these observations, elevated tissue, amino acid level could be due to increased proteolysis in the tissue. The levels of free ammonia, glutamine and urea showed nonsignificant changes indicating the probability of least disturbance in the formation of nitrogenous end products of hepatic origin in response to *in vivo* electrical stimulations of muscle on a single day.

In contrast, repeated muscle stimulations for 10 days showed an entirely different pattern of nitrogen metabolism in hepatic tissue. The total protein content and protease activity of the tissue recorded nonsignificant change suggesting inhibition of exercise-induced proteolysis of hepatic tissue on prolonged muscular stimulations. However, the structural protein level was slightly depleted. The free amino acid content showed considerable decline which might be due to their enhanced utilization into oxidative metabolism. GDH activity was elevated indicating the possibility of increased amino acid oxidations in the tissue. Since the activity levels of both A1AT and AAT showed a significant decline and the total protein content showed nonsignificant change, the observed decline in amino acid content can be accounted only by the activity of GDH. In spite of elevated oxidative deaminations in the tissue, as revealed by GDH activity, ammonia content declined. This decreased ammonia content might be due to its mobilization towards the formation of urea or glutamine as detoxificatory measure. Urea content showed a decline indicating lack of mobilization of ammonia towards urea synthesis. Urea/ammonia ratio was depleted supporting the absence of mobilization of ammonia towards urea formation. But glutamine content was elevated suggesting the diversion of ammonia towards the formation of glutamine.

Table 1 *Levels of total, soluble and structural proteins, protease activity, free amino acids, AlAT, AAT, GDH, ammonia, glutamine and urea in hepatic tissues of control, short-term and prolonged muscular stimulated animals**

Component	Control	Experimental	
		1-day stimulated	10-day stimulated
Total proteins (mg/g wt)	183.95 ±15.95	162.70 ±14.97 −11.55 P < 0.02	176.97 ± 8.44 − 3.79 NS
Soluble proteins (mg/g wt)	109.3 ±10.86	92.24 ± 7.65 −15.61 P < 0.001	111.39 ± 4.35 + 1·91 NS
Structural proteins (mg/g wt)	74.65 ± 5.09	70.46 ± 7.32 − 5.61 NS	65.58 ± 4.01 −12.15 P < 0.001
Protease activity (μ mole tyrosine/mg protein/hr)	0.113 ± 0.005	0.137 ± 0.004 +21.24 P < 0.001	0.114 ±0.007 +0.88 NS
Free Amino Acids (μ mole tyrosine/g wt)	9.44 ± 0.55	17.55 ± 3.2 +85.91 P < 0.001	4.33 ±0.76 −54.13 P < 0.001
AlAT (μ mole pyruvate/mg protein/hr)	0.718 ± 0.14	0.8 ± 0.15 +11.4 NS	0.38 ±0.05 −47.07 P < 0.001
AAT (μ mole pyruvate/mg/protein/hr)	0.55 ± 0.02	0.486 ±0.067 −11.64 P < 0.02	0.306 ±0.017 −44.36 P < 0.001
GDH (μ mole formazan/g wt/hr)	22.81 ± 2.77	22.08 ± 1.37 − 3.1 NS	29.17 ± 1.53 +23.5 P < 0.01
Ammonia (mg/g wt)	1.20 ± 0.04	1.32 ± 0.33 + 9.3 NS	0.8 ±0.012 −33.72 P < 0.001
Glutamine (μ mole/g wt)	15.32 ± 2.16	14.4 ± 1.23 − 4.0 NS	21.88 ± 1.22 +46.0 P < 0.001
Urea (mg/g wt)	0.74 ± 0.038	0.78 ± 0.04 + 5.2 NS	0.36 ±0.048 −51.26 P < 0.001
Urea/ammonia	0.61	0.6 − 1.64	0.45 −26.23
Glutamine/ammonia	12.76	10.99 −13.87	27.35 +114.34

*Values are mean ± S.D. of 6 observations

+ and − indicate percent increase and decrease over normal

Similarly glutamine/ammonia ratio was elevated suggesting such a possibility. The elevated hepatic glutamine content might also be derived from the muscle under work. However, the hepatic ammonia seems to have diverted towards the formation of glutamine rather than urea.

Thus the prolonged *in vivo* muscular stimulations seem to have developed improved ammonia detoxificatory mechanisms, besides inhibiting exercise—induced proteolysis of the tissue. In general it can be concluded that the prolonged muscular stimulations of the present study were responsible for the improved metabolic efficiency of the hepatic tissue.

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