SKELETAL MUSCLE CARBOHYDRATE METABOLISM DURING SHORT-TERM AND PROLONGED *IN VIVO* ELECTRICAL STIMULATIONS

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Summary : Electrical stimulations were applied to the gastrocnemius muscle of intact frog, *Rana hexadactyla* (Lesson) for short-term (SMS) and prolonged (PMS) periods. Short-term muscular stimulations resulted in decreased glycogenolysis and glycolysis with depleted glycogen and lactic acid contents in the muscles and increased lactic acid content in the blood. The activity levels of SDH and MDH were decreased in SMS muscles. Prolonged muscular stimulations, on the other hand, increased glycogenolysis and glycolysis with accumulation of lactic acid. Higher residual glycogen inspite of elevated glycogenolysis was suggested to be due to stepped-up glycogenesis. Tissue oxidative metabolism was also correlated with regulation of activities of enzymes concerned with anaerobic pathways.

Key words : electrical stimulation, fatigue, glycogenolysis, glycolysis, glycogenesis

INTRODUCTION

Muscular contractions for prolonged periods impair the contractile function leading to fatigue (9, 19) which was described as a protective device to prevent permanent damage to the structural and physiological organization of the muscle fibres (21, 33). Exhaustion of substrates (2, 13, 18) and accumulation of products (11, 19, 24, 31) have been related to the onset of fatigue. But the actual metabolic steps and causative factors leading to muscular fatigue were not yet clearly understood. The improvements in physical fitness achieved through training programmes (8, 16, 30) suggested the development of mechanisms in the muscles for delaying the onset of fatigue. However, it was not clear whether the training adaptability lies in a better tolerance in local and general acidosis or changes in the regulation of enzymes. The information pertaining to the induction of training effects into the muscle metabolism through localized electrical stimulations is scanty. Our pre-

vious findings (23) demonstrated the induction of training effects into the muscle through localized electrical stimulations. However, the details pertaining to muscle carbohydrate metabolism have not been elucidated during the muscular stimulations. Hence the present study is aimed to understand the alterations in the muscle carbohydrate metabolism during short-term and prolonged electrical stimulations.

MATERIALS AND METHODS

Male frogs, *Rana hexadactyla* (Lesson) of 35 ± 2 *g* were collected from ponds near Tirupati. They were maintained in clean glass aquaria and fed with cockroaches *ad libitum*. Water in the aquaria was changed regularly once in a day. The frogs were acclimatized to laboratory conditions for two weeks prior to experimentation. Frogs were divided into three groups, each group consisting of 8 animals. First group of animals were used as controls, second group was subjected to short-term muscular stimulations and third group to prolonged muscular stimulations.

(a) Experimentation procedures : The right gastrocnemius muscle of intact conscious frog was Stimulated by placing directly the animal in a specially designed plastic chamber with two platinum electrodes placed at a distance of 1 cm apart using electronic stimulator (INCO/CSIO Research Stimulator – Ambala) as described earlier (23, 28, 29). The entire portion of the gastrocnemius muscle underwent contraction as a result of applied electrical stimulations. Biphasic, rectangular pulses (5V, 2 c/s, 100 ms duration) were applied for a period of 30 min/day for 1 day in one batch of experimental animals (Short-term Muscular stimulated - SMS) and for 10 successive days in another batch (Prolonged Muscular Stimulated – PMS) after due standardization (29). Muscles from unstimulated animals were taken as controls. Blood from control and experimental animals was collected by puncturing the heart.

(b) Homogenate preparations : Control frogs and experimental frogs immediately after electrical stimulations were killed by double pithing. and thrown into the freezing mixtures (–4°C). The right gastrocnemii from frozen animals were isolated in the walk–in–cooler (10°C). Tissues were minced and then homogenized in a glass homogenizer in walk–in–cooler. Homogenates to be used for the estimation of glycogen, lactic and pyruvic acids were prepared in 10% (W/V) trichloroacetic acid, in acqueous medium containing 0.1 M NaF and 0.037 M EDTA of pH 6.5 for the assay of phosphorylase activity as recommended by Guillary and Mommaerts (12). Homogenates to be used for the estimation of aldolase activity were prepared in all glass distilled water and those used for the assay of dehydrogenases in 0.25 M sucrose solution. The homogenates used for the assay of enzyme activities were made uniform by homogenizing the tissues using acid

washed sand and by centrifuging at 6000 rpm.

(c) Assay methods: Glycogen (5), lactic acid (3, 17) and pyruvic acid (10) in control and experimental muscles were estimated. Protein content in the homogenates was estimated by the method of Lowry *et al.* (22).

Glycogen phosphorylase (EC 2.4.1.1) was assayed by the method of Cori *et al.* by the determination of the amount of inorganic phosphate (Pi) formed from glucose-1-phosphate (6). 0.4 *ml* of the diluted enzyme was incubated with 2 *mg* of glycogen for 20 min at 37°C. The reaction was started by the addition of 3.2 *µmols* of glucose-1-phosphate to one of the tubes, and to the other a mixture of 1.6 *µmols* of glucose-1-phosphate and 0.4 *µmols* adenosine-5-monophosphate for the assay of active phos-

TABLE I : Levels of glycogen (*mg* glucose/g dry wt), phosphorylase 'a', 'ab' and 'b' activities (*µmols* Pi formed/*mg* protein/hr) and glucose (*mg*/*g* wet wt) in experimental muscles in comparison to control.

Each value represents mean of eight observations. Mean±S.D.; + and − indicate percent increase and decrease over control.

S. No.	Component	Control	Experimental	
			SMS	PMS
1	Glycogen	16.51±2.95	4.63±0.45 −71.96 P <0.001	10.60±2.56 −35.8 P <0.01
2	Phosphorylase 'a'	26.58±0.98	21.42±2.16 −19.28 P <0.001	28.08±4.95 +5.87 NS
3	Phosphorylase 'ab'	68.95±2.77	58.79±4.21 −14.88 P <0.001	73.43±3.68 +6.57 P <0.02
4	Phosphorylase 'b'	42.37±2.39	37.38±5.55 −11.78 P <0.05	45.35±4.87 +7.03 P <0.05
5	Glucose	2.32±0.27	1.54±0.21 −33.62 P <0.001	1.05±0.21 −54.74 P <0.001

TABLE II : Levels of aldolase activity (*µmole* of FDP cleaved/*mg* protein/hr), FDPase activity (*µmol* of Pi released/*mg* protein/hr), aldolase/FDPase ratio, pyruvate (*µmol/g* dry wt) and lactate (*µmol/g* dry wt) in experimental muscles in comparison to control muscles.

Each value represents the mean of eight observations. Mean±S.D., +and — indicate percent increase and decrease over control. 'P' denotes the level of significance and 'NS' non-significance.

S. No.	*Component*	*Control*	*Experimental*	
			SMS	*PMS*
1	Aldolase	73.61±1.86	66.63±3.11	83.94± 2.44
			—9.48	+14.03
			P <0.001	P< 0.001
2	FDPase	1.68±0.41	1.54±0.17	4.21±0.23
			—8.33	+150. 6
			NS	P< 0.001
3	Aldolase/FDPase	41.35	38.08	+21.71
			—7.91	—47. 5
4	Pyruvate	4.64±0.42	3.47±0.11	5.68±0.35
			—25.21	+22.41
			P <0.001	P <0.001
5	Lactic acid	3.54±0.20	2.88±0. 2	4.27±0.16
			—18.64	20.62
			P <0.001	P< 0.001

phorylase (Phosphorylase a) and total phosphorylase (phosphorylase ab) respectively. The reaction mixture was incubated for 15 min for total phosphorylase and for 30 min for active phosphorylase. The incubation was arrested by the addition of 5 *ml* of 5N sulphuric acid. The inorganic phosphate (in sulphuric acid filtrates) was estimated by the method of Taussaky and Shorr (34). The enzyme activity was expressed as *µmole* Pi formed *mg* protein^{-1} hour^{-1}.

Aldolase (EC 4.1.2.13) was assayed by the colorimetric method of Bruns and Bergmeyer (4) in which the the triose phosphates formed were estimated with 2,4-dinitrophenyl hydrazine. The incubation mixture in a final volume of 3 *ml* contained 175 *µmols* of collidine hydrazine buffer (pH 7.4), 25 *µmols* of fructose-1, 6–diphosphate (FDP) and 1 *ml* of the enzyme. The mixture was incubated at 37°C for 15 *min* and the reaction was arrested by the addition of 3 *ml* cold 10% (W/V) tricholoroacetic acid. Aldolase activity was expressed as *µmols* FDP eleaved *mg* protein^{-1} hour^{-1}.

TABLE III : Activity levels of LDH, SDH and MDH (*µmols* formazan/*mg* protein/hr) in experimental muscles in comparison to control and blood total carbohydrates (*mmols* glucose/dl) and blood lactic acid (*mg/dl*) in experimental animals in relation to controls.

Each value represents mean of eight observations. Mean±S.D., + and — indicate percent increase and decrease over control.

S. No.	Component	Control	Experimental	
			SMS	PMS
1	LDH	0.148±0.009	0.111±0.012	0.172±0.008
			—25.0	+16.2
			P< 0.001	P< 0.001
2	SDH	0.118±0.007	0.091±0.01	0.125±0.008
			—22.88	+5.92
			P< 0.001	P< 0.05
3	MDH	0.055±0.005	0.043±0.003	0.06±0.003
			—21.81	+9.09
			P< 0.001	P< 0.01
4	MDH/SDH	0.466	0.472	0.48
			+1.28	+3. 0
5	Blood total carbohydrates	0.192±0.012	0.119±0.007	0.224±0.017
			—38.02	+16.67
			P< 0.001	P< 0.001
6	Blood lactic acid	14.99±2.29	64.96±7.54	26.79±3.15
			+333.64	+78.84
			P< 0.001	P< 0.001

The activity levels of lactate dehydrogenase — LDH (EC 1.1.1.27), succinate dehydrogenase – SDH (EC 1.3.99.1) and malate dehydrogenase – MDH (EC 1.1.1.37) were estimated by the method described earlier (28) and the activity levels were expressed as *µmol* formazan formed *mg* protein^{-1} hour^{-1}. Blood total carbohydrates were estimated by the method of Carroll *et al.* (5), and lactic acid was estimated by the method of Barker and Summerson (3).

RESULTS

The data presented in Tables I–III indicate the changes in carbohydrate metabolism of skeletal muscle during short–term (SMS) and prolonged (PMS) *in vivo* electrical stimulations.

(a) SMS muscle: Glycogen content decreased (–71.96%) significantly in response to the applied electrical stimulations. The activity levels of the enzymes involved

in glycogenolysis and glycolysis, phosphorylase (a, ab & b) and aldolase were significantly decreased. Glucose content was depleted while FDPase showed non–significant change from control. Aldolase/FDPase ratio was slightly lower in SMS than the control. Lactic and pyruvic acid levels in SMS decreased along with depleted LDH activity. Activity levels of SDH and MDH were decreased in these muscles. Total carbohydrate level of the blood decreased, while lactic acid accumulated in response to electrical stimulations.

(b) PMS muscle: The extent of depletion in the glycogen content (–35·8%) of the muscle in PMS was far lesser to that of SMS muscle (–71·96%). The activity levels of phosphorylase ('ab' & 'b') and aldolase were significantly increased. The glucose content was significantly lesser than the control. FDPase activity was drastically elevated while Aldolase/FDPase ratio was highly depleted. The levels of lactic and pyruvic acids in the muscle and total carbohydrates in the blood of the PMS animals were increased. The percent accumulation of lactic acid in the blood of PMS animals was lesser than SMS animals. The activity levels of the dehydrogenases studied viz., LDH, SDH and MDH were considerably elevated.

DISCUSSION

(a) SMS muscle : Depleted level of glycogen in the muscle of SMS animals suggests the onset of glycogenolysis towards energy release for the muscular working in response to applied electrical stimulations. Inspite of the presence of sufficient energy stores as evidenced by the residual glycogen content, the muscle was unable to respond to the applied stimulations and attained fatigue state (visual observation) indicating that fatigue was not due to lack of substrates. Highly depleted level of activity of phosphorylase 'a', regulatory enzyme of glycogenolysis, indicates a check which was imposed on further breakdown of glycogen. Total carbohydrate level in the blood was lowered, suggesting the setting–in of hypoglycemic condition as a result of induced muscular work. Decrease in the activity levels of phosphorylase b & ab indicates overall decrease in the levels of total phosphorylase itself in the muscles of SMS animals. Reported increase in the permeability of the muscle membrane during electrical stimulations and exercise resulting in efflux of several enzymes (1, 24) and ions (15), suggest the possible efflux of enzyme into the blood. Similarly, decrease in the activity of aldolase, suggests an over–all decrease in the levels of operation of glycolysis in the muscles of SMS animals. Hence the regulation on glycogenolysis and glycolysis by phosphorylase and aldolase respectively, might be responsible for preventing complete degradation of glycogen. Consequent upon such a decrease in phosphorylase activity, the free glucose content was depleted. Inspite of such a large depletion in the glycogen content in the muscle, the levels of lactic and pyruvic acids were below the controls indicating either their probable leakage into

blood or mobilization into citric acid cycle. Decreased LDH activity in the muscle suggests the lesser mobilization of lactic acid into TCA cycle. The observed elevation in the level of blood lactic acid of these animals reveal the leakage of lactic acid from the working muscle into the blood. Similar decrease in the lactic acid was reported in the muscles of intact animals stimulated over a period of 30 mins (14). The observed elevation in blood lactic acid in the SMS animals was in consonance with earlier reports (13, 14, 20, 22). As a result of decreased mobilization of lactic acid into TCA cycle, the activity levels of SDH and MDH were decreased indicating an overall suppression in the oxidative metabolism in response to the applied electrical stimulations.

(b) *PMS muscles :* In contrast, the muscles of PMS animals showed contractions during the entire period of stimulations and thus delayed the onset of fatigue (visual observations). Further these animals utilized 50% lesser glycogen in comparison with those of SMS animals. Moreover, the total carbohydrate level in the blood was elevated suggesting the prevalence of hyperglycemic condition in PMS animals. Elevated activity level of phosphorylase 'ab' suggests the active *de novo* synthesis of the enzyme itself. In comparison to SMS muscles, PMS muscles showed elevation in the phosphorylase 'ab' activity which might be due to arrested efflux of the enzyme into the blood. Increased activity levels of phosphorylase and aldolase suggest the stepped-up glycogenolytic and glycolytic pathways in the PMS muscles. Free glucose content was highly depleted suggesting its active utilization into the glycolysis. Similarly, significant elevation in the levels of lactic and pyruvic acids in the PMS muscles along with lesser rise in blood lactic acid confirms enhanced glycolysis with decreased efflux of organic acids into the blood.

The presence of higher residual glycogen in the PMS muscles than those of SMS, inspite of elevated glycogenolysis was quite interesting. Such a situation suggests active glycogen synthesis in the muscle. Elevated activity level of NAD-LDH suggests increased mobilization of lactic acid into citric acid cycle. Further, our previous works revealed elevated GDH activity in the PMS muscles (26) suggesting increased oxidative deamination of amino acids. As a result of increased mobilization of amino acids and lactic acid into the cycle, SDH and MDH activity levels were elevated indicating the overall elevation in oxidative pathway of the muscle. This increased oxidative phase of metabolism might be responsible for providing the energy for prolonged muscular work and thus delaying the onset of fatigue (visual observation). Further, elevated MDH/SDH and increased aminotransferases in the PMS muscles (26) which forms an index of gluconeogenesis, suggests the operation of gluconeogenesis. Drastic elevation in the level of FDPase activity in the muscle was suggestive of active glycogen synthesis. Aldolase/

FDPase ratio was highly depleted indicating higher level of mobilization of FDP towards glycogen synthesis than into glycolysis. Reported increase in the activity level of glycogen synthetase in the trained muscles (35,36) indicates the occurrence of similar events in PMS muscles resulting in elevated glycogenesis. The glycogenesis and glycogenolysis might be in dynamic equilibrium with each other orienting more towards glycogen synthesis in the PMS muscles.

Thus the muscle adaptability in PMS animals oriented towards increased muscular efficiency and glycogen sparing process have developed out of modulating the enzymes involved in glycogenolysis, glycolysis, oxidative metabolism and gluconeogenesis.

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