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Effect of Prolonged Electrical Stimulations on the Biochemical Constituents and Exercise Potential of the Amphibian Muscle

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Gastrocnemius muscles of *Rana hexadactyla* (Lesson) were subjected to repeated *in vivo* electrical stimulations and the effect of prolonged muscular electrical stimulations on the biochemical constituents and exercise potential of the muscle was studied. The muscle exposed to prolonged electrical stimulations (PSM) had elevated contractile potential with delayed onset of fatigue in comparison to unstimulated muscle (USM). The extent of depletion in dry matter and accumulation of water were more in USM than PSM during the course of exercise. The total carbohydrate content in PSM was depleted only to half the extent to that of USM during the same period of exercise and PSM exhibited higher level of depletion in the lipid content than that of USM. Exercise-induced proteolysis, absent in PSM could be observed in USM. Advantage of the application of prolonged *in vivo* electrical stimulations to improve the working potential of the muscle are discussed.

Key Words: Electric stimulations, Amphibian muscle, Biochemical constituents, Excercise potential

Introduction

Among all metabolic loads, exercise or work performance is considered to be important one (Angeli et al. 1979). The glycogen-content of the muscle tissue decrease more or less linearly with the duration of exercise and it was considered to be a limiting factor for work performance (Hultman & Bergstrom 1973). A higher intramuscular lipid component and higher activities of enzymes involved in oxidations of fatty acids enable the trained muscle to utilize more of lipid substrate and to spare glycogen. There is practically no attempt to elucidate the impact of prolonged *in vivo* muscular electrical stimulations on the exercise potential and modulations in the biochemical constituents of the muscles during exercise. The present study was undertaken to demonstrate the impact

of electrical stimulations on the capacity of the work performance of the muscle.

Material and Methods

The male specimens of *Rana hexadactyla* (Lesson) 30±2 g were divided into two groups of 50 each. The first group of animals was maintained as controls. The right gastrocnemii of the second group of animals were subjected to *in vivo* electrical stimulations through two platinum electrodes placed on the skin (5V, 2 c/sec, biphasic pulses for 30 min. a day) for 10 successive days and termed as prolonged muscular stimulated muscles (PSM) (Reddanna et al. 1980, Narasimha Moorthy et al. 1981a, b, c). The gastrocnemii of normal (USM) and prolonged stimulated (PSM) were removed into the frog-Ringer and the single muscle twitch and fatigue recordings were taken with Kymograph (Narasimha Moorthy et al. 1981). Water content, wet weight and dry weight of these muscles were estimated gravimetrically. Total carbohydrates (Caroll et al. 1956), soluble and structural proteins (Lowry et al. 1951), and total lipids (Folch et al. 1957) were estimated,

The muscles of the normal frogs (USM) and prolonged stimulated frogs (PSM) were subjected to different periods (2–20 min.) of exercise with *in vivo* electrical stimulations and temporal sequence of analysis of organic constituents such as total carbohydrates, proteins and lipids were undertaken.

Results and Discussion

The data are presented in tables 1 and 2 and figures 1–3. The analysis of single muscle twitch indicates contractile kinetic potential of the muscle. Hence single muscle twitch was recorded for unstimulated (USM) and prolonged stimulated (PSM) muscles (figure 1). The amplitude

(a) SINGLE TWITCH OF UNSTIMULATED MUSCLE (USM)

(a) SINGLE TWITCH OF PROLONGED STIMULATED MUSCLE (PSM)

Figure 1

of PSM was higher than USM, suggesting improved contractile potential of the PSM. Similarly PSM recorded delayed onset of fatigue in comparison to USM (figure 2). These observations demonstrate that a muscle treated with chronic electrical stimulations for longer periods develops improved contractile efficiency. This observation finds its support from the previous studies (Reddanna & Govindappa 1980, Narasimha Moorthy et al. 1981a, b, c) where the authors have conclusively demonstrated that a muscle can be trained through *in vivo* electrical stimulations. Hence the PSM of the present study can be considered as a trained muscle as suggested by these investigators. The PSM had considerably lesser water content with significant elevation in the dry matter (table 1). Since both soluble and structural protein fractions of PSM were significantly elevated over that of USM, it can be

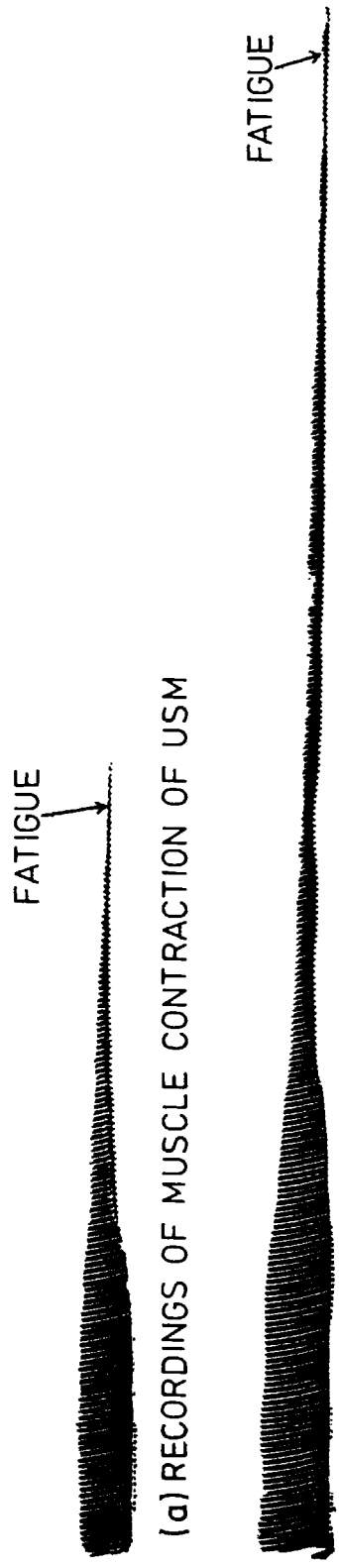
FATIGUE
(a) RECORDINGS OF MUSCLE CONTRACTION OF USM
FATIGUE
(b) RECORDINGS OF MUSCLE CONTRACTION OF PSM

Figure 2

Table 1 *Biochemical constituents in unstimulated (USM) and prolonged stimulated (PSM) muscles*

Sl. No.	Parameter	Unstimulated muscle (USM)		Prolonged stimulated muscle (PSM)
1.	Water content (mg/g. wt.)	0.76* ±0.006	−1.97 $P < 0.001$	0.745 ±0.003
2.	Dry matter	0.24 ±0.006	+ 6.25 $P < 0.001$	0.255 ±0.003
3.	Total carbohydrates (mg/g. dry wt.)	40.98 ±2.69	+ 4.56 N.S.	42.85 ±1.66
4.	Total lipids (mg/g. dry wt.)	129.35 ±5.69	− 15.94 $P < 0.001$	108.73 ±8.52
5.	Soluble proteins (mg/g. dry wt.)	158.32 ±4.51	+ 19.82 $P < 0.001$	189.7 ±9.69
6.	Structural proteins (mg/g. dry wt.)	500.4 ±22.49	+ 11.87 $P < 0.01$	559.82 ±34.51

* Values are mean of 8 observations ± S.D.; 'p' denotes the level of significance: + and − indicate the percent increase and decrease respectively

suggested that a muscle on repeated electrical stimulations acquires higher protein biosynthetic mechanisms and/or decreased tissue proteolysis. Hence the protein metabolism of PSM appears to be switched over to higher anabolic phase. Since the total lipid content of PSM was significantly lower than that of USM, lipid oriented metabolic pattern can be envisaged in PSM. Red muscles were known to utilize lipids for the metabolic activities (Hillman et al. 1979) and hence it can be suggested that the muscle on repeated days of electrical stimulations appears to convert itself into the red muscle type of metabolic pattern with more oxidative metabolism. In view of such a difference in metabolic pattern, the PSM might have had delayed onset of fatigue during the course of exercise. Hence it will be worthwhile to analyse the changes in the biochemical components of PSM during the course of exercise for understanding the reasons for delayed onset fatigue.

On exercise both USM and PSM accumulated water (table 2, figure 3) from 10th min onwards suggesting the possibility of water imbibition due to increased blood flow and altered membrane permeability properties as reported by Oritsland et al. (1977). Since the accumulation of water in USM was almost double that of PSM after the completion of exercise, operation of regulatory mechanisms towards water accumulation in PSM can be envisaged. The dry matter was depleted during the course of exercise in both USM and PSM. However, the higher percent depletion of dry matter in the

Table 2 *Changes in the levels of water content, dry matter, total carbohydrates, total lipids, soluble and structural proteins* (mg/g wt) *in unstimulated (USM) and prolonged stimulated (PSM) muscles during the temporal sequence of exercise*

Parameter	Duration of exercise in min											
	0		2		5		10		15		20	
	USM	PSM	USM	PSM	USM	PSM	USM	PSM	USM	PSM	USM	PSM
Water content (mg/g wt)	0.76† ±0.006	0.745 ±0.003	0.761 ±0.03	0.748 ±0.06	0.765 ±0.00	0.75 ±0.008	0.775* ±0.007	0.76* ±0.009	0.79* ±0.012	0.767* ±0.007	0.799* ±0.035	0.769* ±0.007
Dry matter (mg/g wt)	0.24 ±0.006	0.255 ±0.003	0.239 ±0.003	0.252 ±0.006	0.235 ±0.006	0.25 ±0.008	0.224* ±0.007	0.24* ±0.009	0.21* ±0.012	0.233* ±0.007	0.201* ±0.025	0.231* ±0.007
Total carbohydrates (mg/g wt)	40.98 ±2.69	42.85 ±1.66	31.42* ±1.45	38.86* ±1.28	24.13* ±1.97	36.42* ±2.54	16.38* ±2.41	33.47* ±2.19	12.67* ±1.06	31.1* ±1.76	11.37* ±1.26	33.13* ±1.77
Total lipids (mg/g wt)	129.35 ±5.69	108.73 ±8.52	128.61 ±8.35	108.49 ±10.26	127.98 ±7.44	105.55 ±6.4	123.18** ±2.88	104.3 ±5.79	122.55*** ±3.0	96.41* ±4.28	119.05*** ±5.43	96.3** ±2.56
Soluble proteins (mg/g wt)	158.32 ±4.51	189.7 ±9.69	162.58 ±6.95	193.82 ±9.46	170.11** ±9.1	199.95** ±10.64	178.27** ±11.2	202.21* ±13.33	180.84* ±9.41	198.39** ±14.75	202.94* ±6.54	193.2 ±7.06
Structural proteins (mg/g wt)	500.4 ±22.49	559.82 ±34.51	506.05 ±12.33	557.4 ±18.73	481.0 ±25.06	543.21 ±24.71	478.14**** ±21.9	562.99 ±15.6	466.98** ±20.79	572.44 ±7.87	474.44**** ±22.32	567.53 ±38.6

† Values are mean of 8 observations ± S.D.; + and — indicate percent increase and decrease respectively

* $P < 0.001$; ** $P < 0.01$; *** $P < 0.02$; **** $P < 0.05$

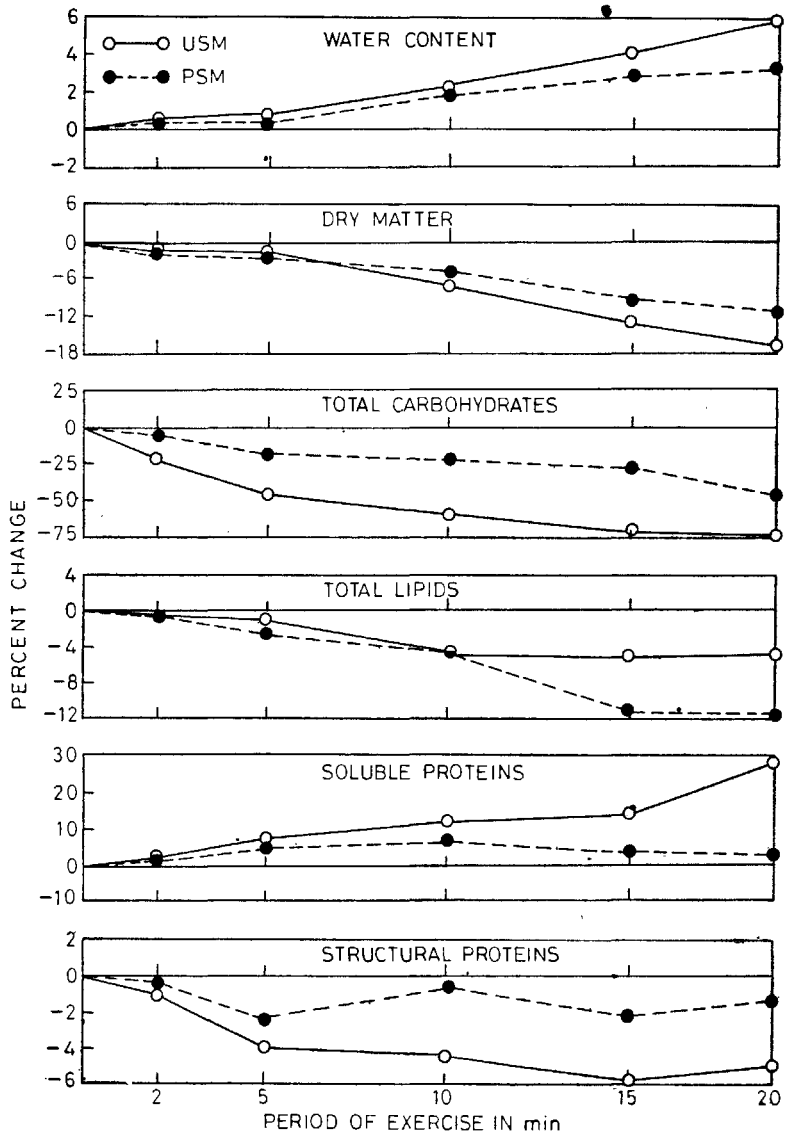

Figure 3

PSM indicates that PSM exerts regulation on the mobilization of organic reserves towards the energy release. This property of PSM is comparable to that of trained muscles of whole animal (Reddanna & Govindappa 1978a, b). Thus the muscle, on chronic exposure to electrical stimulations seems to be capable of regulating permeability properties. The total carbohydrate content in PSM was depleted to only half the extent to that of USM for the performance of the exercise. Hence, PSM has developed metabolic pattern oriented to spare the carbohydrates and this would have been responsible for delayed onset of fatigue, since carbohydrate content of the muscle forms the limiting factor towards the contractile

potential (Hultman et al. 1973). This is possible only by a shift in the utilization of organic fuels in the muscle metabolism. The PSM exhibited more depletion of lipids as compared to USM (figure 3) indicating that PSM was utilizing lipids in preference to carbohydrates leading to the sparing of carbohydrates in this muscle. Since the property of lipid utilization was the character of red muscle (Holloszy et al. 1977) and red muscles were known to be involved in prolonged and sustained activities (Newsome & Leduc 1975), the treatment of muscle with repeated electrical stimulations was resulting in the conversion of a normal muscle into an efficient type with orientation towards sustained activities. The soluble protein fraction of USM was consistently elevated over its respective control during the course of exercise with simultaneous depletion in the structural protein fraction (figure 3). Thus the performance of exercise by a muscle appears to involve changes in the solubility properties of proteins as suggested by Poortmans and Delisse (1977) which was a prerequisite for the proteolysis. Thus a normal muscle during the course of work performance involves the degradation of protein components, in particular, structural protein. But PSM recorded non-significant change in both soluble and structural protein fractions after the completion of exercise indicating least involvement in the proteolysis of the tissue during the course of work performance.

In general it can be concluded that a muscle on chronic exposure to *in vivo* electrical stimulations was developing efficient metabolic machinery which bestows improved contractile potential and the capacity for sustained activities.

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