PATTERN OF SKELETAL MUSCLE CHEMICAL COMPOSITION DURING *IN VIVO* ELECTRICAL STIMULATIONS

P. REDDANNA, C.V. NARASIMHA MOORTHY AND S. GOVINDAPPA

Molecular Physiology Unit, Department of Zoology,
Sri Venkateswara University, Tirupati - 517 502

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Summary : Electrical stimulations of low voltage, frequency and short duration of time are suitable for amphibian muscle to induce localized muscular exercise in an intact animal. The pattern of changes in the muscle chemical components on chronic exposure to repeated electical stimulations suggested the onset of regulation on muscle metabolism leading to carbohydrate sparing process and muscular hypertrophy. The applicability of this procedure to prevent the muscle wasting in atrophic and dystrophic muscles is suggested.

Key words : electrical stimulations gastrocnemii carbohydrate sparing process

INTRODUCTION

There is considerable controversy in the field of electrotherapy (6,10,14). Proper understanding of the therapeutic procedure requires precise knowledge on changes in the normal muscle metabolism during electrical stimulations. The metabolic changes induced in the muscle tissue under the influence of electrical stimulations depend on the type of muscle stimulated and the type of stimulations applied (9). The information available on the effect of chronic exposure to electrical stimulations in *in vivo* condition on the muscle components is meagre. Hence an attempt has been made to investigate the changes in the normal muscle components during variable work intensities and to understand the muscle adaptability to chronic exposure to electrical stimulations.

MATERIALS AND METHODS

Frogs, *Rana hexadactyla* (Lesson) were employed for the present investigation. A special chamber was designed for inducing muscular stimulations in *in vivo* conditions. The frog was fixed in the chamber by clamping the cushioned clips to the fore and hind limbs. The right gastrocnemius muscle was directly stimulated by placing two plantinum electrodes on the skin at a distance of 1 cm apart to localize the stimlations, as adopted by several workers (3,5,7, 22). Biphasic, rectangular electrical stimulations were applied

through electronic stimulator (INCO/CSIO Research Stimulator - Ambala), since they were known to minimise electrode polarization causing least tissue injury (21) The duration of each impulse was 100 ms. The muscles were stimulated at variable voltage (5V, 10V, 15V, 20V and 30V), frequencies (60c/min, 120 c/min, 240 c/min, 300 c/min and 600 c/min) and periods of stimulation (5 min, 10 min, 15 min, 20 min, 30 min, 45 min and 60 min) to determine the suitable intensity, speed and duration of stimulation for subjecting the amphibian muscle to exercise. From these studies 5V, 120 c/min and 30 min duration were selected to induce repeated electrical stimulations daily for 10 days.

Immediately after the electrical stimulations, the muscles were isolated from double pithed frogs, with least injury and chilled in freezing mixture. The stimulated muscles were termed experimental muscles, while those from normal animals as control muscles.

The water content, wet weight and dry weight of control and experimental muscles were estimated gravimetrically. Glycogen and total carbohydrates were estimated by the method of Carroll *et al.* (4) and total proteins by the method of Lowry *et al.* (15). Ten frogs have been employed for each parameter.

RESULTS

The data presented in Tables I to IV indicate the impact of *in vivo* muscular stimulations in terms of voltage, frequency, period of stimulation and days of stimulation on the chemical composition of the skeletal muscle.

The water content and dry weight of the tissue showed significant elevation and depletion respectively, at all the voltages studied, the maximum change being at 5V (Table I). Glycogen content decreased consistently with increasing voltage, the drop being more conspicuous when it was expressed in terms of unit wet weight. The total protein content showed significant decrease at all the voltages when expressed in terms of unit wet weight and only at higher voltages when expressed per unit dry weight.

The muscle tissue accumulated considerable water content with significant decrement in dry weight at all the frequencies employed (Table II). At frequency of 300 c/min, maximum dry weight decrease and water accumulation were witnessed. Total protein content showed non-significant changes at all the frequencies. Glycogen content showed consistent drop with increase in the frequency.

The muscle tissue had increased water content and decreased dry weight at all the periods of stimulation (Table III). The glycogen content was consistently depleted with

TABLE I : Effect of voltage on the levels of glycogen, total proteins, dry weight and water content in the gastrocnemius muscle of frog. Each value represent the mean of ten observations. Mean ± SD. + and — indicate percent increase and decrease over control.

Component	*Control*	*5 V*	*10 V*	*15 V*	*20 V*	*30 V*
Glycogen	3.25	0.86	0.70	0.58	0.53	0.55
(*mg/g* wet wt)	±0.19	±0.02	±0.02	±0.02	±0.02	±0.02
		—73.4	—78.3	—82.1	—83.6	—83.12
		P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
Glycogen	14.38	4.82	3.89	3.21	2.75	2.89
(*mg/g* dry wt)	±0.82	±0.11	±0.14	±0.09	±0.09	±0.1
		—66.48	—72.91	—77.67	—80.90	—79.92
		P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
Total proteins	182	145	143	140	141	137
(*mg/g* wet wt)	±11.64	±13.53	±15.42	±18.22	±14.1	±14.34
		—20.33	—21.43	—23.08	—22.53	—24.72
		P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
Total porteins	805	808.8	790.07	772.1	726.43	720.48
(*mg/g* dry wt)	±51.9	±75.47	±85.19	±100.48	±72.64	±75.42
		±0.47	—1.85	—4.09	—9.76	—10.5
		NS	NS	NS	NS	P<0.001
Dry weight	228.0	179.3	181.0	181.3	194.1	190.1
(*mg/g* wet wt)	±15.49	±10.17	—11.8	±9.94	±7.85	±15.21
		—20.66	—19.91	—19.78	—14.11	—15.88
		P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
Water content	774.0	820.7	819.0	818.7	805.9	809.9
(*mg/g* wet wt)	±29.25	±32.2	±27.8	±25.6	±28.1	±34.1
		+6.03	+5.81	+5.78	+4.12	+4.63
		P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

increasing period of stimulation. The total protein content showed non-significant change in the initial periods with a later significant decrease.

When the muscle was subjected to chronic exercises for 10 days, serial changes were seen progressing from marked depletion to considerable sparing of metabolites from day 1 to day 10. The total carbohydrate content showed maximum depletion (—70.54%)

TABLE II : Effect of frequency on the levels of glycogen, total proteins, dry weight and water content in the gastrocnemius muscle of frog. Each value represents the mean of ten observations. Mean ± S.D. + and — indicate percent increase and decrease over control.

S. No.	Component	Control	60c/min	120c/min	180c/min	240c/min	300c/min	600c/min
1	Glycogen	14.717	4.814	4.1	4.186	4.164	3.78	3.039
	(*mg/g* dry wt)	±2.14	±0.555	±0.525	±1.05	±0.143	±0.526	±0.375
			—67.29	—72.07	—71.56	—71.71	—74.31	—79.35
			P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
2	Total proteins	890.53	940.22	858.19	850.64	858.9	862.11	892.31
	(*mg/g* dry wt)	±88.53	±63.61	±54.29	±57.23	±80.3	±73.31	±56.38
			+5.58	—3.63	—4.48	—3.55	—3.19	+0.2
			NS	NS	NS	NS	NS	NS
3	Dry weight	231.4	176.6	171.3	164.6	153.7	142.7	154.7
	(*mg/g* wet wt)	±9.47	±10.16	±7.85	±12.49	±11.84	±7.8	±9.35
			—17.24	—19.73	—22.87	—27.98	—33.13	—27.51
			P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
4	Water content	786.6	823.4	828.7	835.4	846.3	857.3	845.6
	(*mg/g* wet wt)	±22.4	±27.48	±31.2	±22.8	±34.8	±38.5	±27.85
			+4.68	+5.35	+6.20	+7.59	+8.99	+7.50
			P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

TABLE III : Effect of period of stimulation on the levels of glycogen, total poteins dry weight and water content in the gasrocnemius muscle of frog. Each value represents the mean of ten observations. Mean ± S.D., + and — indicate percent increase and decrease over control.

S.No.		Control	5 min	10 min	15 min	20 min	30 min	45 min	60 min
1	Total protein	874.13	861.14	841.3	795.69	810.6	852.4	816.03	751.7
	(*mg/g* dry wt)	±57.12	±43.73	±28.72	±47.73	±51.23	±45.99	±53.98	±43.26
			—1.49	—3.75	—8.91	—7.27	—2.48	—6.45	—14.8
			NS	NS	P<0.01	P<0.01	NS	P<0.02	P<0.001
2	Glycogen	13.12	8.145	7.51	6.91	5.46	4.62	4.03	3.34
	(*mg/g* dry wt)	±0.77	±0.591	±0.536	±0.437	±0.72	±0.536	±0.503	±0.464
			—37.92	—42.76	—47.37	—58.38	—64.81	—69.3	—74.54
			P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
3	Dry weight	224.8	206.3	205	190.9	188.6	175.4	178.8	191.8
	(*mg/g* wet wt)	±11.47	±7.08	±10.98	±9.90	±13.8	±10.49	±14.49	±15.42
			—8.23	—8.80	—15.08	—16.10	—22.97	—20.46	—14.68
			P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
4	Water content	775.2	794.4	795.0	803	811.4	824.6	821.2	808.2
	(*mg/g* wet wt)	±24.42	±18.29	±25.47	±31.09	±22.7	±32.28	±26.6	±41.5
			+2.48	+2.55	+3.6	+4.67	+6.37	+5.93	+4.26
			P<0.02	P<0.05	P<0.001	P<0.001	P<0.001	P<0.001	P<0.01

TABLE IV : Temporal changes on the levels of total carbohydrates, total proteins, dry weight and water content in the gastrocnemius muscle of frog during repeated stimulations. Each value represents the mean of ten observations. Mean ± S.D. + and — indicates percent increase and decrease over control.

S.No.	Control	1 day	3 days	4 days	6 days	8 days	10 days
1 Total carbohydrates (mg/g dry wt)	61.47 ±2.32	18.11 ±1.07 —70.54 P<0.001	23.47 ±1.18 —61.82 P<0.001	41.17 ±1.93 —33.02 P<0.001	49.81 ±2.32 —18.97 P<0.001	45.77 ±2.53 —25.54 P<0.001	48.83 ±1.54 —20.56 P<0.001
2 Total protein (mg/g dry wt)	844 ±53.22	821 ±64.09 —2.72 NS	914 ±76.43 +8.29 P<0.02	935 ±77.52 +10.78 P<0.01	907 ±44 53 +7.46 P<0.01	941 ±61.97 +11.49 P<0.001	903 ±40.27 +6.99 P<0.001
3 Dry weight (mg/g wet wt)	221.7 ±10.7	174.5 ±7.8 —21.29 P<0.001	177.9 ±9.9 —19.75 P<0.001	174.4 ±12.2 —21.33 P<0.001	188.6 ±9.2 —14.93 P<0.001	193.8 ±14.2 —12.58 P<0.001	216.0 ±15.5 —2.57 NS
4 Water content (mg/g wet wt)	778.3 13.9	825.5 ±14.4 +6.06 P<0.001	822.1 ±17.8 +5.63 P<0.001	825.6 ±9.9 +6.6 P<0.001	811.3 ±24.5 +4.25 P<0.001	803.2 ±19.8 +3.58 P<0.001	783.9 ±17.3 +0.72 NS

after one day of stimulation. Increasing number of days of stimulation resulted in decreased depletion leading to a sparing of about 55% of glycogen for the same period of exercise in 10 days stimulated muscle (Table IV). The total protein content had a non-significant change on one day stimulation with a significant elevation on 10 days of stimulations. The pattern of changes in water content and dry matter was opposite in one day stimulated and 10 days stimulated muscles.

DISCUSSION

The muscle water content increased with simultaneous decrease in dry matter in response to electrical stimulations. Water accumulation in the tissue might be due to elevated blood flow in the muscles (1,10,12,18), altered muscle membrane permeability (11, 16) and decreased plasma water content (17, 24), as reported in whole animal exercises.

In view of increased involvement of water in muscle metabolism under stimulated condition, it might lead on to erroneous conclusions, if the values of organic components were to be expressed in terms of unit wet weight as observed in the present study. Hence all the values have been represented in unit dry weight.

The glycogen depletion increased with increasing voltage, suggesting higher levels of glycogenolysis with increasing work intensities. However, the rate of glycogen depletion was maximum at lowest voltage employed. The total protein content revealed the possibility of protein degradation at higher voltages. These observations were in consonance with the earlier investigations where increased proteolysis was reported (13, 19) at higher work intensities. Hence it can be suggested that at lower voltages, the muscle was dependent on carbohydrates and on proteins at higher voltages. Similarly glycogen content showed a consistent drop with increasing frequencies. However, the rate of depletion was more at lower frequencies suggesting that the tissue chemical components other than glycogen were mobilized at higher frequencies. The pattern of changes in the total protein content revealed lack of impact of frequency on muscular protein which was in consonance with earlier report (25).

The pattern of changes in the total protein content of the muscle in relation to period of stimulation revealed the degradation of muscle proteins only during prolonged periods of work performance The glycogen content recorded rapid decline upto 30 mins with a later decline in the rate of depletion, suggesting higher level of utilization of glycogen during short duration of muscular contractions. Elevated level of relative contribution of blood borne components in relation to intramuscular substrates with increase in the duration of work (13) might be the probable reason for lesser rate of utilization of glycogen at later periods of work. Hence lower voltage and frequencies and short duration of stimulations were suitable for the amphibian muscle to induce localized muscular exercise in an intact animal.

The impact of exercise on the muscle components was found to be different during chronic exposure to electrical stimulations. The water content of the muscle increased maximally on one day stimulation leading to nonsignificant change on 10 days of stimulations. This observation suggests the possible onset of regulation on the inflow of water into the muscle tissue during successive days of stimulations. Similarly the pattern of changes in dry matter during the temproal sequence of stimulations reveal the possible onset of resistance on degradations and/or activation of synthetic phase of metabolism. The total protein content showed nonsignificant change on one day stimulation and significant elevation on further days of stimulation. The extent of depletion of total carbohydrates

decreased with increasing days of stimulations for the performance of the same work leading to a sparing of 55% of carbohydrates. These observations revealed the gradual onset of efficient metabolic machinery in the 10 days stimulated muscle tissue, which can be comparable to the changes in the training programmes reported in whole animal exercise (2,8,20,23).

In view of these results, the localized *in vivo* electrical stimulations can be exploited as a therapeutic procedure to prevent the muscle wasting in atrophic and dystrophic conditions of the muscles.

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