MUSCULAR TRAINING THROUGH LOCALIZED *IN VIVO* ELECTRICAL STIMULATIONS

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Summary : *In vivo* electrical stimulations were applied to the gastrocnemius muscles of intact frogs for prolonged periods which lead to improved muscular efficiency and delayed onset of fatigue. The muscular strength improvement was correlated to increased tissue contractile proteins and decreased collagen content. Elevated levels of muscular fuels, mitochondrial content, oxidative metabolism in the stimulated muscles were suggested to be responsible for the delayed onset of fatigue. In view of these characteristics regarding the improvement at physical and metabolic levels, the muscles exposed to chronic electrical stimulations were termed as trained muscles. The applicability of electrical stimulations to induce the training effects into the muscles in atrophic and dystrophic conditions to avert the muscle wastings was suggested.

Key words: electrical stimulations, muscle strength, fatigue, contractile proteins, trained muscles

INTRODUCTION

Prolonged and exhaustive whole body exercises lead to trained conditions of the skeletal muscles improving contractile machinery and muscular fuels (1, 17 28). Electrical stimulations have been widely employed to induce muscular exercises (14, 16, 29). In our earlier work (31, 32) electrical stimulations have beeen employed for inducing exercises to the muscles for short-term and prolonged periods. However, no conclusive attempt has been made to induce training into the muscles through the electrical stimulations. Hence the present work has been undertaken to induce training conditions into the muscle through *in vivo* eiectrical stimulations.

MATERIALS AND METHODS

Frogs, *Rana hexadactyla* (Lesson) were employed for the present investigation. Special chamber has been devised and limbs of the animals were fixed with soft clips. The right gastrocnemii of intact and conscious animals were stimulated using electronic stimulator (INCO/CSIO Research Stimulator - Ambala) as described earlier (32), for 30 min daily

for 10 days. On day 11 the stimulated muscles were isolated and termed as PSM (prolonged stimulated muscles). The muscles from normal animals were taken as controls.

Single stimulus of 5 V, AC was found to elicit optimal response and hence this threshold potential was employed in the present investigation. The twitch properties and associated contractile kinetics were analysed by the kymographic method as adopted by Venkateswarlu and Sasira Babu (34). The muscle contractions were recorded on a paper pasted to a kymographic drum. The speed of the drum was calibrated by determining the mean time taken for 10 revolutions. The amplitude of contraction, total twitch duration, half contraction time (HCT) and half relaxation time (HRT) were calculated from the calibrated speed of the drum. Average values of 12 twitch analyses were determined. The time taken for fatigue in control and stimulated muscles was determined kymographically. 5V voltage, 120 c/min frequency, 100 ms duration were employed for recording fatigue.

Control and stimulated frogs were double pithed, gastrocnemii were isolated from them and taken for biochemical assays. Tissue somatic indices and dry matter of the tissue were calculated by gravimetric method. Tissue protein fractionation was carried out by the method of Barany *et al.* (2) and the protein content in them was estimated by the method of Lowry *et al.* (22). Collagen content was estimated gravimetrically. Muscle mitochondria were isolated by the method described by Ernster and Nordernbrand (10) using Chappel, Perry medium (6). The homogenate was first centrifuged at 800 g for 20 minutes and supernatant was collected. This supernatant was centrifuged again at 15000 g for 30 min and packed sedimented mitochondrial pellet was eluted into 20 *ml* of sucrose medium. Appropriately diluted enzyme concentrations after due standardization were employed for all the assays. Glycogen was estimated by the method of Carroll *et al.* (5). Phospholipids by Bieri and Prival (4), Triglycerides and cholesterol by Natelson (26), free ammonia by Bergmeyer (3) and glutamine by Colowick and Kaplan (9) were determined. Succinate dehydrogenase – SDH (E.C. 1.3.99.1), glutamate dehydrogenase – GDH (E.C. 1.4.1.3). Malate dehydrogenase – MDH (E.C. 1.1.1.37) were estimated by the method of Nachlas *et al.* (25) as described by Reddanna and Govindappa (31). The isocitrate dehydrogenase – ICDH (E.C. 1.1.1.41) activity was determined by the method of Kornberg and Pricer (21). The cytochrome 'C' oxidase activity (E.C. 1.9.3.1) was estimated by using 'New colorimetric method' described by Oda *et al.* (27).

RESULTS

The data presented in Tables I to III indicate the extent of changes in physical performance, protein fractions, metabolic substrate levels and mitochondrial constituents of control and PSM.

The total twitch duration of stimulated muscle increased considerably over the control muscle (Table I). Half contraction time (HCT) was significantly elevated over control

TABLE I : Single twitch analysis and fatigue period of control and prolonged stimulated muscles (PSM). Values represent mean of 12 observations. Mean ± S.D. and + and — indicate percent increase and decrease over control respectively.

S. No.	Component	Control muscle	PSM	% difference of PSM over control
1	Total twitch duration (msec)	150.69 ±3.74	162.15 ±12.44	+7.6 P<0.05
2	Half contraction time (HCT) (msec)	26.04 ±1.80	33.33 ±3.99	+28.0 P<.001
3	Half relaxation time (HRT) (msec)	49.31 ±1.77	47.74 ±4.12	—3.17 NS
4	Amplitude (mm)	20.58 ±1.39	33.0 ±2.81	+60.3 P<0.001
5	HCT/HRT	0.53	0.7	+32.2
6	Amplitude/HCT (mm/msec)	0.79	0.99	+25.3
7	Amplitude/HRT (mm/msec)	0.42	0.69	+65.47
8	Total fatigue time (mins)	2.63 ±0.24	5.61 ±0.36	+112.29 P<0.001

while half relaxation time (HRT) showed non-significant change. The amplitude of the twitch was highly elevated in stimulated muscle. HCT/HRT ratio was elevated while Amplitude/HCT and Amplitude/HRT were depleted. The period of onset of fatigue was significantly prolonged in stimulated muscle over the control. The tissue somatic index (TSI) and dry matter of the stimulated muscle were elevated, while collagen content was depleted (Table II). Sarcoplasmic proteins (SP) were elevated in stimulated muscle. The contents of contractile proteins viz., actin and myosin were considerably increased in PSM over the control. The glycogen content was elevated in stimulated muscle over the control. Phospholipid content was elevated and triglyceride content was depleted. The levels of cholesterol and free ammonia decreased while glutamine content was elevated. Mitochondrial protein content was elevated significantly (Table III). The mitochondrial enzyme activity levels viz,, ICDH, cytochrome 'C' oxidase, SDH, GDH and MDH were

elevated in stimulated muscle over the control. MDH/SDH ratio was increased in the stimulated muscle over the control value.

TABLE II : Tissue somatic indices (TSI) and dry matter, sarcoplasmic protein, actin, myosin, collagen, glycogen, phospholipids, triglycerides, cholesterol, glutamine and free ammonia in control and prolonged stimuelated muscles (PSM). Values are mean of 8 observations. Mean ± S.D., + and — indicate percent increase and decrease over control respectively.

S. No.	*Component*	*Control muscle*	*PSM*	*% difference of PSM over control*
1	TSI	2.18 ±0.01	2.7 ±0.01	+23.8 P<0.001
2	Dry matter (*mg/g* wet wt)	0.24 ±0.005	2.25 ±0.003	+6.25 P<0.001
3	Sarcoplasmic protein (SP) -ibid-	41.57 ±1.76	47.83 ±1.89	+15.07 P<0.001
4	Actin -ibid-	27.39 ±1.36	31.81 ±1.08	+16.14 P<0.001
5	Myosin -ibid-	52.31 ±1.43	58.2 ±1.31	+11.03 P<0.001
6	Collagen -ibid-	20.0 ±2.24	17.62 ±2.74	—11.87 P<0.001
7	Glycogen -ibid-	4.33 ±0.05	4.89 ±0.05	+12.97 P<0.001
8	Phospholipids -ibid-	14.25 ±2.36	17.92 ±1.09	+25.73 P<0.001
9	Triglycerides -ibid-	0.95 ±0.06	0.68 ±0.06	—28.44 P<0.001
10	Cholesterol -ibid-	0.8 ±0.03	0.57 ±0.04	—28.75 P<0.001
11	Glutamine (*µmoles/g* wt)	20.05 ±0.87	22.34 ±0.38	+11.43 P<0.001
12	Free ammonia (*µmoles/g* wt)	3.76 ±0.32	3.21 ±0.36	—14.68 P<0.001

TABLE III : The mitochondrial protein content and the activity levels of cytochrome C oxidase, isocitrate dehydrogenase, SDH, GDH and MDH in mitochondrial fractions of control and prolonged stimulated muscles (PSM). Values are mean of 8 observations. Mean±S.D., + and — indicate percent increase and decrease over control respectively.

S. No.	*Component*	*Control muscle*	*PSM*	*% difference of PSM over control*
1	Mitochondrial protein content (*mg/g* wet wt)	21.84 ±2.1	26.51 ±2.36	+21.37 P<0.001
2	Cytochrome 'C oxidase (*µg* of diformazan formed/*mg* protein/hr)	4447.72 ±583.78	7961.13 ±430.1	+79.01 P<0.001
3	Isocitrate dehydrogenase (*µmoles* of formazan formed/*mg* protein/hr)	2.10 ±0.25	2.83 ±0.23	+34.44 P<0.001
4	SDH -ibid-	2.06 ±0.18	2.3 ±0.15	+11.38 P<0.01
5	GDH -ibid-	1.21 ±0.09	1.37 ±0.088	+13.22 P<0.001
6	MDH -ibid-	0.56 ±0.01	0.73 ±0.06	+31.25 P<0.001
7	MDH/SDH	0.27	0.32	+17.7

DISCUSSION

Elevation in TSI of PSM over control was suggestive of the possible onset of hypertrophy in the stimulated muscle. The muscular hypertrophy has been widely reported in the trained animals, through whole animal exercises (13, 19). Since the possibility of muscular hypertrophy has been envisaged in the muscles of present study after repeated stimulations, it was felt desirable to investigate the characteristics of the muscle in terms of physical performance and biochemical efficiency. Increased twitch duration of the stimulated muscle over the control suggests that the stimulated muscle could retain contractile

tension for longer periods than the normal muscle. Since the depletion in twitch duration in malathion treated muscles was correlated towards inhibition of contractile kinetics of muscular tissue (30), elevation of the same in the present study might suggest improved contractile kinetics of the PSM. The increased twitch duration might be due to elevation either in contractile phase or in relaxation phase. HCT was highly elevated in PSM over control, while HRT showed non-significant change leading to elevated HCT/HRT. In view of these obseravations it was likely that PSM was capable of building higher contractile efficiency. The amplitude which indicates the strength of the muscle was increased in the PSM over the control. Amplitude/HCT and Amplitude/HRT which denotes the speed of contractile and relaxation phases respectively, were elevated over the control suggesting that the entire speed of contractile machinery was geared-up in PSM. From these results it can be inferred that PSM might have improved the contractile protein constituency and energy mechanisms, since both contractile (passive process) and relaxation phases (active process) were activated Probably in view of these improvements, the period of onset of fatigue was delayed which was indicative of functioning of PSM for prolonged periods in comparison to that of control. Since the trained muscles of whole animal exercises developed strength improvement (7, 8). efficient contractile capacity, and delayed onset of fatigue (8, 18), PSM of the present study seem to be similar to those of trained muscles.

Elevated dry matter in PSM over the control was suggestive of the active anabolic processes in the muscle leading to an increase in TSI. The levels of actin and myosin were elevated supporting the possibility of higher contractile capabilities in PSM. Since free ammonia content decreased with simultaneous rise in glutamine content in PSM, it can be suggested that the stimulated muscle developed improved ammonia detoxificatory mechanisms. Elevated glutamine levels suggest accumulation of basic environment in the tissue which might buffer metabolic acids produced during the exercise of the muscle. In contrast, the collagen content of PSM was significantly depleted over the control. Since increased proportion of collagen was directly linked towards decreased functional capacity of the muscle (12), its decrease in PSM might be responsible for the improved physical performance as revealed through twitch analysis. The trained muscles of whole animal exercises had elevated contractile protein constituency (13, 28) with depleted collagen content (12) and hence PSM can be comparable to trained muscles. Glycogen content was highly elevated in the PSM over the control. Since the carbohydrate reserves have been widely reported to be the immediate source of energy to the muscular contraction, building-up of carbohydrate reserves in PSM was suggestive of higher energy potential. Since phospholipids were involved in improving energy releasing mechanisms through Ca^{2+} ion transport and elevated ATPase activity (23, 24), elevated phospholipids in PSM was suggestive of stepped-up energy releasing mechanisms in

them. The levels of triglycerides and cholesterol have been depleted in PSM over the control which might have been a prerequisite for the efficient and prolonged muscular contraction.

The elevation in mitochondrial protein content was suggestive of increased mitochondrial fraction in PSM which might be an adaptive feature in muscle metabolism towards the oxidative phase. Similar elevation in mitochondrial protein, number and size have been witnessed in trained muscles of whole animal exercises (11, 15). The activity levels of cytochrome 'C' oxidase was highly elevated supporting such a possibility of increased oxidative metabolism of stimulated muscle tissue in comparison to the control. In the light of increased oxidative phase of muscle metabolism and increased oxidative deamination of amino acids as represented by GDH activity, elevated operation of citric acid cycle can be envisaged in PSM. The activity levels of isocitrate dehydrogenase, SDH & MDH which were the markers of TCA cycle and mitochondrial activity were elevated suggesting that the TCA cycle was operating at a higher level in the PSM. The trained muscles of various training programmes also had elevated oxidative metabolism (18, 20, 33).

The observation of the present study clearly elucidated that the stimulated muscle can be comparable with the trained muscles at structural and dynamic levels of organizations. Hence it can be suggested that the localized muscular exercise through *in vivo* electrical stimulations can be successfully utilized for inducing training programme into the muscles. Since the muscle strength was improved, the utilitarian value of *in vivo* electrical stimulations in averting the muscle wastings during atrophies and dystrophies can be envisaged.

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