EFFECT OF *IN VIVO* ELECTRICAL STIMULATION ON THE CARBOHYDRATE METABOLISM OF CONTROL AND DENERVATION ATROPHIED MUSCLE OF DOG, *CANIS DOMESTICUS*

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Summary : The standardized programme of electrical stimulation was applied to the control and denervation atrophied muscle of dog, *Canis domesticus* and the pattern of changes in the carbohydrate metabolism was analysed in the control (C), denervated control (DC), control stimulated (CS) and denervated stimulated (DS) gastrocnemius muscles. The programme of electrical stimulation of the control muscle has elevated glycogenolysis, glycolysis and increased the level of operation of TCA cycle with decreased mobilization of carbohydrates into hexose monophosphate pathway, indicating the setting in of trained condition. Sciatectomy, on the other-hand, lowered the level of operation of glycogenolysis and decreased the utilization of carbohydrates through hexose-mono and di-phosphate pathways and TCA cycle. The programme of electrical stimulation applied to the denervated muscle has restored the utilization of carbohydrates through hexose mono and diphosphate pathways and oxidative metabolism indicating the applicability of this programme of electrical stimulation in the treatment of muscular atrophy.

Key words : Electrical stimulation, glycolysis, gastrocnemius muscle, denervation atrophy, glycogenolysis, TCA cycle

INTRODUCTION

Electrical stimulation has been widely employed to induce exercise in the muscles (10, 11, 16). Repeated muscle exercise induced through electrical stimulation was shown to improve muscular performance along with setting in of training conditions in the frog (20, 15). Sciatectomy, disuse and atrophy of the muscle was widely known to lead to dystrophy and muscle wasting leading to impaired structural and functional organization (6, 8). Our earlier studies on the impact of electrical stimulation on the protein metabolism of atrophic muscles have indicated improved structural organization of atrophic muscles (5, 12). In the present study the impact of such programme of electrical

stimulation on the carbohydrate metabolism of normal and atrophied muscle of dog, *Canis domesticus* was analysed.

MATERIAL AND METHODS

The proposed study was undertaken utilizing twelve healthy male mongrel dogs, *Canis domesticus* aged 3-4 years weighing 11-16 kgs. The dogs were initially dewormed and maintained under identical experimental conditions. The dogs were randomly divided into four groups of three animals each, i.e.,

Group I : Normal sham-operated dogs - Control (C).

Group II : Electrical stimulation for normal animals for 15 days - Control stimulated (CS).

Group III : Sciatic denervation - Denervated control (DC).

Group IV : Electrical stimulation for denervated animals for 15 days Denervated stimulated (DS).

Sciatic denervation : The dogs were placed in right lateral recumbency and anaesthetized by administration of thiopentane sodium, intravenously at a dose rate of 5 *mg/kg* body weight. The dogs were then connected to Boyle anaesthetic apparatus to maintain surgical plane of anaesthesia using ether, nitrous oxide and oxygen mixture.

The left hip joint area was prepared for aseptic surgery. A 3 cm long cutaneous incision was made in line with and posterior to the summit of the trochanter major. The sciatic nerve was isolated in the vicinity of the hip joint, where it turns distal. Sciatic neurectomy was performed by severing 2 cm of nerve. The overlying muscles were opposed with No. 1 chromic catgut. The skin incision was closed using No. 2 black braided silk. The surgical wound was dressed with Tr. Benzoin. No antibiotics were administered to the dogs following denervation.

Electrical stimulation : A special chamber has been designed to restrain the animals during electrical stimulation. The gastrocnemius muscle of intact conscious control and denervated (after 15 days of sciatectomy) animals was stimulated for 30 minutes daily for fifteen days using an Electrical Stimulator (INCO/CSIO-Research Stimulator - Ambala, India). Biphasic pulses of 10V ; 100 ms duration and 2 cps were applied to stimulate the muscles.

On sixteenth day the dogs were sacrificed using thiopentane sodium. The gastrocnemius muscle was collected and the homogenates were prepared for the estimation of biochemical parameters.

The glycogen content was estimated by the method of Carroll *et al.* (3). Phosphorylase activity (a., b & ab) was assayed by the method of Cori, Illingworth and Killer (4). The glucose content was estimated by Mendal *et al.* (14). Aldolase activity was assayed by the colorimetric method of Bruns and Bergmeyer (2) in which the triose phosphates formed were estimated with 2, 4-dinitro-phenyl hydrazine. Lactic acid in the tissue was estimated by the method of Barker and Summerson (1). Pyruvate was estimated by the method of Friedman and Hangen (7). The activity levels of SDH, MDH, GDH and LDH were estimated by the method described earlier (19). The activity level of G-6-PDH was determined by the method of Bruns and Bergmeyer (2).

RESULTS

The data presented in the Tables I to IV reveal the impact of repeated electrical stimulations on carbohydrate metabolism of control and deneravation atrophied muscle of dog, *Canis domesticus.*

TABLE I : Levels of glycogen, phosphorylase 'a', 'b' & 'ab' activities, glucose, aldolase and lactate in gastrocnemii of control (C) and control stimulated (CS) dogs. Each value represents the mean of 6 observations. Mean ± S.D.; + and — indicate percent increase and decrease over the control muscle. 'P' denotes level of significance and 'N.S.' nonsignificance.

S. No.	*Component*	*Control muscle (C)*		*Control muscle stimulated (CS)*
1.	Glycogen (*mg/g* wet wt)	3.98 ±0.14	—2.01 N.S.	3.9 ±0.23
2.	Phosphorylase 'a' (μmoles pi formed/*mg* protein/h)	±0.72 ±0.04	+133.33 P<0.001	1.68 ±0.13
3.	Phosphorylase 'b' (*μmoles* pi formed/*mg* protein/h)	1.1 ±0.07	—45.45 P<0.001	0.60 ±0.04
4.	Phosphorylase 'ab' (*μmoles* of pi formed/*mg* protein/h)	1.8 ±0.12	+26.66 P<0.001	2.28 ±0.14
5.	Glucose (*mg/g* wet wt)	1.24 ±0.16	+15.32 P<0.05	1.42 ±0.14
6.	Aldolase (*μmoles* of FDP cleaved/*mg* protein/h)	1.45 ±0.08	+55.17 P<0.001	2.25 ±0.16
7.	Lactate (*mg/g* wet wt)	4.03 ±0.36	+37.72 P<0.001	5.55 ±0.43

TABLE II : Levels of LDH, Pyruvate, GDH, SDH, MDH and G-6-PDH in the gastrocnemii of control (C) and control stimulated (CS) dogs. Each value represents the mean of 6 observations. Mean ± S.D.; + and — indicate percent increase and decrease over the control muscle. 'P' denotes level of significance.

S. No.	Component	Control muscle (C)		Control muscle stimulated (CS)
1.	LDH	0.07		0.117
	(*μmoles* of formazan formed/*mg* protein/h)	±0.007	+ 67.14	±0.01
			P<0.001	
2.	Pyruvate	2.64		2.47
	(*mg/g* wet wt)	±0.11	— 6.44	±0.12
			P<0.05	
3.	GDH	0.085		0.1405
	(*μmoles* of formazan formed/*mg* protein/h)	±0.007	+ 65.29	±0.005
			P<0.001	
4.	SDH	1.088		1.307
	(*μmoles* of formazan formed/*mg* protein/h)	±0.1	+ 20.13	±0.06
			P<0.001	
5.	MDH	0.086		0.103
	(*μmoles* of formazan formed/*mg* protein/h)	±0.01	+19.767	±0.008
			P<0.01	
6.	G-6-PDH	1.889		1.027
	(*μmoles* of formazan formed/*mg* protein/h)	±0.1	— 45.63	±0.1
			P<0.001	

Control vs control stimulated muscles (*Tables I and II*) : The differences in the mean level of glycogen in the muscles of control and control stimulated were observed to be nonsignificant. Mean muscle glucose and lactic acid contents were found to be significantly higher in the control stimulated dogs. The activity levels of phosphorylase 'a' and 'ab' were significantly elevated, while a significant decline was observed in phosphorylase 'b' activity. Similarly aldolase activity level was significantly elevated with a decrease in the pyruvic acid content. The activity levlels of LDH, GDH, SDH and MDH were significantly elevated in CS muscle with a significant decrease in G-6-PDH activity.

Control vs denervated muscles (*Tables III and IV*) : Significant increase in

the glycogen and glucose contents were recorded in sciatectomized gastrocnemius muscle. The mean activity levles of phosphorylase 'a' and 'ab' and aldolase were significantly depleted along with increased lactate and pyruvate contents. The activity levels of lactate, glutamate, succinate, malate and glucose 6-phosphate dehydrogenases were depleted significantly in the denervated muscles.

Control vs denervated stimulated muscles (Tables III and IV) : Glycogen content of the denervated stimulated muscle attained the mean level observed in control muscle. The activity level of phosphorylase 'a' of DS muscle was elevated significantly over that of control muscle, with a decrease in that of 'b' and no significant change in 'ab'. The contents of glucose, lactate and pyruvate were observed to increase significantly in DS muscle. The activity levels of aldolase and lactate dehydrogenase were more or less same, both in the control muscle and DS muscle. Glutamate and malate dehydrogenases were elevated significantly with a decrease in the activities of succinate and glucose-6-phosphate dehyrogenases.

TABLE III : Levels of glycogen, phosphorylase 'a', 'b' & 'ab' activities, glucose, aldolase and lactate in the gastrocnemii of denervated control (DC) and denervated stimulated (DS) dogs. Each value represents the mean of 6 individual observations. Mean ± S.D.; + and — indicate the percent increase and decrease over the control (C) and denervated control (DC) muscle. 'P' denotes the level of significance and 'NS' nonsignificance.

S. No.	*Component*	*Denervated Muscle (DC)*	*DS (Vs) DC*	*Denervated muscle stimulated (DS)*	*DC (Vs) C*	*DS (Vs) C*
1.	Glycogen (*mg/g* wet wt)	4.77 ±0.17	—15.77 P<0.001	4.02 ±0.17	+19.84 P<0.001	+1.006 NS
2.	Phosphorylase 'a' (*µmol* pi/*mg* protein/h)	0.325 ±0.02	+221.84 P<0.001	1.046 ± 0.12	—54.86 P<0.001	+45.27 P<0.001
3.	Phosphorylase 'b' (*µmol* pi/*mg* protein/h)	1.135 ±0.13	—44.14 P<00.001	0.634 ± 0.04	+3.182 NS	—42.36 P<0.001
4.	Phosphorylase 'ab' (*µmol* pi/*mg* protein/h)	1.46 ±0.12	+15.07 P<0.01	1.68 ± 0.13	—18.89 P<0.001	—6.66 NS
5.	Glucose (*mg/g* wet wt)	1.73 ±0.13	—14.45 P< 0.01	1.48 ±0.14	+39.51 P<0.001	+19.35 P<0.02
6.	Aldolase (*µmoles* of FDP cleaved/*mg* protein/h)	1.206 ±0.17	+28.19 P<0.21	1.546 ±0.18	—16.83 P< 0.001	+6.62 NS
7.	Lactate (*mg/g* wet wt)	6.14 ±0.52	—22.96 P<0.01	4.73 ±0.32	+52.35 P<0.001	+17.37 P.<0.01

TABLE IV : Levels of LDH, pyruvate, GDH, SDH, MDH and G-6-PDH in the gastrocnemii of control (C), denervated control (DC) and denervated stimulated (DS) dogs. Each value represents the mean of 6 observations. Mean±S.D.; +and—indicate percent increase and decrease over the control (C) and denervated control (DC) muscles. 'P' denotes the level of significance and 'NS' nonsignificance.

S. No.	*Component*	*Denervated muscle (DC)*	*DS (Vs) DC*	*Denervated muscle stimulated(DS)*	*DC (Vs) C*	*DS (Vs) C*
1.	LDH (μ*moles* of formazan formed/*mg* protein/h)	0.053 ±0.004	+28.3 P<0.001	0.068 ±0.005	—24.28 P<0.001	—2.86 NS
2.	Pyruvate (μ*moles/g* wet wt)	3.11 ±0.14	—5.79 P<0.05	2.93 ±0.13	+17.42 P<0.001	+10.98 P<0.01
3.	GDH (μ*moles* of formazan formed/*mg* protein/h)	0.064 ±0.004	+73.44 P<0.001	0.111 ±0.006	—24.7 P<0.001	+30.58 P<0.001
4.	SDH (μ*moles* of formazan formed/*mg* protein/h)	0.565 ±0.03	+42.48 P<0.001	0.805 ±0.006	—59.29 P<0.001	—26.01 P<0.001
5.	MDH (μ*moles* of formazan formed/*mg* protein/h)	0.066 ±0.004	+43.94 P<0.001	0.095 ±0.005	—23.25 P<0.001	+10.46 NS
6.	G-6-PDH (μ*moles* of formazan formed/*mg* protein)	1.053 ±0.1	+24.97 P<0.001	1.316 ±0.06	—44.25 P<0.001	—30.33 P<0.001
7.	MDH/SDH	0.116	+1.69	0.118	+46.87	+33.05

Denervated control (DC) vs denervated muscle stimulated (DS) (Tables III and IV) :

The DS muscle in relation to DC muscle showed significant decrease in the glycogen and glucose contents. Phosphorylase 'a' and 'ab' activity levels were elevated significantly with a decrease in that of 'b' activity level. The mean activity of aldolase was elevated significantly with an associated decrease in lactate and pyruvate contents. The activity levels of all dehydrogenases studied were significantly elevated in the DS muscle over those of DC muscle.

DISCUSSION

Electrical stimulation of standardized voltage, frequency, duration and period of stimulation for a period of 15 days was applied to the control (CS) muscles and the pattern of carbohydrate metabolism was studied in it. The control stimulated muscle can be compared with that of trained muscle reported earlier (20, 15). Such training

programme of electrical stimulation was applied to the denervation atrophied muscle to find out its impact on the atrophy.

Control vs control stimulated muscles : The increase in the mean activity of phosphorylase 'a' and 'ab' in the CS muscle suggests increase in the *de novo* synthesis of the enzyme which might have contributed to increased glycogenolysis leading to depletion in the tissue glycogen content (18). Elevated aldolase activity and increased lactate content suggest the steppedup glycogenolysis in the muscle tissue subjected to electrical stimulation. These observations were in agreement with the reported elevation of glycolysis of the gastrocnemius muscle of frog trained through electrical stimulation (21). Increased activity level of NAD dependent lactate dehydrogenase along with decreased pyruvate content suggests the lactate oxidations and subsequent mobilization of pyruvate into the citric acid cycle. Elevated activity level of NAD dependent glutamate dehydrogenase reveals the increased oxidative deamination and possible induction of keto-acids into the citric acid cycle as intermediary substrates. The enzymes of citric acid cycle (SDH and MDH) might have been elevated as a result of this increased mobilization of lactate, pyruvate and deaminated amino acids. This increase in citric acid cycle operation indicates the increased oxidative potential of the CS muscle. Similar increase in the oxidative potential was reported in the muscles, trained through whole body exercises (17, 13) and electrical stimulation (15,21).

Decreased glucose-6-phosphate dehydrogenase activity level suggests the reduced level of mobilization of carbohydrates through hexose monophosphate shunt in the control stimulated muscles.

Control vs denervated control muscles : Accumulation of glycogen and glucose in the muscles of neurectomized animals might be due to the retardation of glycogenolysis and glycolysis. The inhibition of glycogenolysis and glycolysis can be substantiated from the observed decrease of phosphorylase and aldolase activities. Accumulation of mean lactate and pyruvate contents inspite of decreased glycogenolysis and glycolysis, might be attributed to a conspicuous decrease in lactate oxidations as observed in the activity of LDH. The decrease in the TCA cycle activity could also be attributed to the failure of introduction of the intermediary products like pyruvate and amino acids into the citric acid cycle because of the lowered LDH and GDH activities respectively observed in the present study. Similar decrease in the glycogenolysis, glycolysis and oxidative metabolism was reported in denervation atrophied muscles in various animals (22). The observed decrease in the mean activity of Glucose-6-phosphate dehydrogenase suggests the lowered mobilization of carbohydrates into hexose monophosphate shunt in the DC muscles.

Control vs denervated stimulated muscles : The restored level of glycogen in the DS muscle to that of control muscle indicates either decreased glycogenolysis or increased glycogenesis. Elevated activity level uof phosphorylase 'a', on the otherhand, suggests increased glycogenolysis. Hence, restored level of muscle glycogen inspite of elevated glycogenolysis must be due to increased glycogenesis. Restored level of aldolase and NAD-LDH from the decreased activity levels observed in DC muscle indicates increased glycolysis and subsequent mobilization of lactate towards citric acid cycle, since NAD-LDH in muscle is indicative of mobilization of lactate into TCA cycle (9). Increased activity level of GDH in the DS muscle suggests the increased oxidative deamination of amino acids and addition of intermediary substrates into TCA cycle. Inspite of increased mobilization of citric acid cycle intermediaries, SDH activity was significantly depleted with restored activity level of MDH in the DS muscle. This differential changes in the activity levels of SDH and MDH with a raise in the MDH/SDH ratio indicates the operation of glyconeogenesis (9). Prevalence of such a mechanism in the DS muscle might lead on to increased contents of glycogen and glucose, inspite of elevated glycogenolysis and glycolysis. Lowered activity level of G-6-PDH in the DS muscle over the control muscle indicates the incomplete restoration of the enzyme in the DS muscle.

Denervated vs denervated stimulated muscles : Electrical stimulation to the denervated muscle resulted in the restoration of glycogenolysis and glycolysis from the low levels observed in denervated-controls. This can be substantiated from the observed decrease in the mean levels of glycogen and glucose contents and by the increased activity levels of phosphorylase and adolase. Electrical stimulation applied to the denervated muscles restored the quantitative introduction of lactate and pyruvate into TCA cycle. This conclusion appears to be logical because of the observed decrease of lactate and pyruvate in the DS muscle over the DC muscles. The observed elevation in the mean levels of LDH, SDH and MDH also substantiates the above hypothesis. Elevated GDH activity indicates the increased level of introduction of keto acids also into the citric acid cycle. As a result of this, the TCA cycle turnover might have been increased indicating the increased oxidative metabolism of DS muscle.

Increased mean activity level of G-6-PDH suggests the elevated level of utilization of carbohydrates through HMP shunt also in the DS muscle, oriented either towards lipogenesis or towards nucleic acid synthesis, probably to counteract the atrophic effects of sciatectomy.

In conclusion, it can be stated that electrical stimulation given to a degenerating muscle, restored the utilization of carbohydrates through hexose mono-and di-phosphate pathways and oxidative metabolism. In view of improved carbohydrate utilization

towards energy release in CS and DS muscles, this programme of electrical stimulation can be utilized for the treatment of atrophic muscles.

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