EFFECTS OF LOW FREQUENCY ELECTRICAL STIMULATION ON ENZYME AND ISOZYME PATTERNS OF DYSTROPHIC MOUSE MUSCLE

Heinz REICHMANN, Dirk PETTE and Gerta VRBOVÁ*

Fakultät für Biologie, Universität Konstanz, 7750 Konstanz, FRG and **Dept. of Anatomy and Embryology, University College London, England*

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1. Introduction

Fast-contracting glycolytic muscle fibres are affected more than slow-contracting oxidative ones in some types of muscular dystrophy [1]. Fast-contracting muscle fibres can be transformed into slow-contracting ones by long term indirect electrical stimulation at low frequencies [2]. Therefore, electrical stimulation might be a means to increase the resistance of muscle fibres to the dystrophic process. This hypothesis was tested by stimulating in vivo fast twitch muscles in mice of a strain with hereditary muscular dystrophy. Effects of stimulation were examined by determining maximum tetanic tension, by measurements of selected enzymes in energy metabolism and by analysing isozyme patterns of lactate dehydrogenase (LDH). In agreement with [3], electro-stimulation proved to be beneficial and increased tetanic tension. These changes of the stimulated dystrophic muscles were paralelled by an increase in enzymes of anaerobic and aerobic catabolism and by a shift in the LDH isozyme pattern.

2. Materials and methods

Teflon-coated stainless steel electrodes were implanted either side of the lateral popliteal nerve in one hind leg of adult dystrophic mice (strain C57 BL dy^{2J}/dy^{2J}). The wires were externalized at the back of the animal and small hooks were attached to each bared end. The operation was performed under ether anaesthesia, using sterile precautions. Stimulation was started in <1 h after the operation. Animals were stimulated at 8 Hz for 30 min and left to rest for at least 30 min. Stimulation was repeated 6 times a day. In the final experiment the animals were anaesthetized with chloralhydrate (1 ml/100 g body wt of 4% solution). Contractile properties were determined as in [3]. For biochemical analyses, tibialis anterior (TA) and extensor digitorum longus (EDL) muscles were frozen in liquid nitrogen and stored at −80°C until further processing. Extraction of enzymes was performed by homogenizing the muscle 1:19 (w/v) in 0.1 M phosphate (pH 7.2) containing 2 mM EDTA. Additions were made to aliquots of the homogenate resulting in either 1 mM fructose 1,6-bisphosphate for stabilisation of phosphofructokinase or 1% Triton X-100 for complete extraction of hexokinase. Enzyme activities were determined as in [4–6] in the clear supernatants after 20 min high speed centrifugation at 130 000 × *g* in a Beckman airfuge. For measurement of phosphorylase, an aliquot of the homogenate was centrifuged at only 20 000 × *g* for 10 min. Capilliary electrophoresis for LDH isozymes was performed in 7.2% polyacrylamide gels.

3. Results and discussion

Enzyme activities in tibialis anterior (TA) muscles of adult normal and dystrophic mice are given in table 1. The results are in agreement with [7]. Glucose 6-phosphate dehydrogenase (EC 1.1.1.49) is 2.5-fold elevated in the dystrophic muscle by all the other enzymes studied are decreased. Phosphorylase (EC 2.4.1.1), aldolase (EC 4.1.2.13) and lactate dehydrogenase (EC 1.1.1.27) are reduced to 40–45% of their activity levels in normal muscle, and phosphofructokinase (EC 2.7.1.11), pyruvate kinase

Table 1
Activity levels of selected enzymes in tibialis anterior muscles (TA) of adult normal and dystrophic mice

Enzyme	A (n = 8) TA dystrophic	B (n = 4) TA normal	A/B
Phosphorylase	8.4 ± 3.9	22.4 ± 4.3	0.38
Hexokinase	3.5 ± 0.69	3.1 ± 1.0	1.14
Phosphofructokinase	13.4 ± 5.0	22.3 ± 4.4	0.60
Aldolase (Fru-1,6-P_2)	42.5 ± 12	94.9 ± 15	0.45
Pyruvate kinase	167 ± 30	230 ± 44	0.73
Lactate dehydrogenase	282 ± 88	616 ± 58	0.46
Glc-6-P dehydrogenase	0.37 ± 0.1	0.15 ± 0.05	2.47
Citrate synthetase	22.6 ± 6.5	33.0 ± 5.2	0.68
3-OH-acyl-CoA dehydrogenase	12.6 ± 4.2	14.4 ± 0.9	0.88
Creatine kinase	233 ± 76	379 ± 16	0.61
Glutamate oxaloacetate transaminase	100 ± 27	120 ± 19	0.83

Activities (U/g muscle) are given as means ± SD

(EC 2.7.1.40) and creatine kinase (EC 2.7.3.2) activities amount to 60–70% of the normal. Slight decreases are also seen in the activities of citrate synthetase (EC 4.1.3.7) and 3-hydroxy-acyl-CoA dehydrogenase (EC 1.1.1.35). These changes are compatible with the hypothesis that dystrophy affects predominantly the population of the 'glycolytic' type II fibres [1].

Chronic electrical stimulation appeared to reverse to some extent the alterations in the enzyme activity pattern. Table 2 summarizes changes in contractile properties (tetanic tension) and in enzyme activities of stimulated dystrophic muscles in 5 animals. Results are expressed as ratios between stimulated and contralateral unstimulated muscles. In order to estimate changes which might have resulted directly or indirectly from the operation (e.g., traumatic nerve lesion, inactivity), an unstimulated EDL muscle of an animal with implanted electrodes was analysed. The slight variations between the muscle of the operated and the contralateral leg are negligible and are within

Table 2
Influence of electrostimulation on tetanic tension and on enzyme activities in dystrophic mouse extensor digitorum longus (EDL) and tibialis anterior (TA) muscles

Muscle:	EDL	TA	EDL	EDL	TA	TA
Days stimulation:	0	9	9	12	19	28
Tetanic tension ratio (stimulated/unstimulated):	–	1.0	1.4	2.2	1.6	2.4
Enzyme	Activity ratios (stimulated/unstimulated)					
Phosphorylase	0.99	0.97	1.77	1.73	2.07	2.48
Hexokinase	1.01	1.19	1.02	1.42	1.20	4.41
Phosphofructokinase	1.02	1.20	1.40	1.78	1.36	1.70
Aldolase (Fru-1,6-P_2)	0.98	0.97	1.13	1.25	1.84	2.05
Pyruvate kinase	1.08	1.22	1.10	1.16	1.42	1.95
Lactate dehydrogenase	0.91	0.75	1.24	1.44	1.26	2.41
Glc-6-P dehydrogenase	0.82	1.41	1.40	0.87	1.19	1.91
Citrate synthetase	1.10	0.72	1.03	1.54	1.09	2.73
3-OH-acyl-CoA dehydrogenase	1.05	0.69	1.02	1.62	1.13	2.27
Creatine kinase	0.97	0.72	0.93	1.30	1.56	2.50
Glutamate oxaloacetate transaminase	1.07	0.77	0.98	1.64	1.12	2.54

Results are expressed as values relative to controls by referring data in stimulated to those of the contralateral unstimulated muscle in each animal

the range of the experimental error.

Except for the 9 days stimulated tibialis muscle, pronounced changes in enzyme activities are seen in table 2 for the stimulated dystrophic muscles. As compared to the contralateral muscles, stimulation induced increases in the enzymes of glycogen and glucose catabolism (phosphorylase, hexokinase (EC 2.7.1.1), phosphofructokinase, aldolase, pyruvate kinase, lactate dehydrogenase) as well as in citrate synthetase, 3-hydroxy-acyl-CoA dehydrogenase, creatine kinase and glutamate oxaloacetate transaminase (EC 2.6.1.1). The increases are more pronounced after longer stimulation periods. After 28 days, the longest period studied, enzyme activities increased 2–4-fold. As is evident from the changes in tetanic tension, the increases in enzyme activities appear to be correlated. Thus, muscles with higher increases in tetanic tension show also greater increases in enzyme activities (table 2).

The finding that stimulation of dystrophic muscle increased activity levels of glycogenolytic and glycolytic enzymes, is unexpected considering the effect of chronic stimulation at low frequencies on fast muscles [8,9]. Chronic stimulation (10 Hz) induced decreases in glycolytic enzymes as well as in creatine kinase and increases in the mitochondrial enzymes of aerobic substrate oxidation in fast-twitch rabbit muscles. These changes were interpreted as an 'anaerobic to aerobic' transition of the metabolic type [8,9]. In contrast, the increases in enzyme activities of anaerobic glucose metabolism as well as of creatine kinase in stimulated dystrophic muscles suggest a rearrangement of the enzyme pattern towards that of a normal fast muscle. In addition, stimulation also induced increases in mitochondrial enzymes similar to those observed in previous studies on stimulated rabbit muscle [8,9]. After 28 days of stimulation (table 2), citrate synthetase and 3-hydroxy-acyl-CoA dehydrogenase but also hexokinase reached activity levels which exceed their values in normal muscles.

The suggestion that low frequency electrical stimulation induces a process of recovery in the dystrophic muscle is supported by changes in the isozyme pattern of lactate dehydrogenase. Distributions of LDH isozymes in 4 stimulated dystrophic and unstimulated contralateral muscles are shown in table 3. In agreement with [10], unstimulated dystrophic muscles display an isozyme pattern with decreased LDH-5 and increased LDH-4, LDH-3 and LDH-2 as compared to normal mouse muscle (table 3, bottom line). In each of the muscles studied, stimulation induced increases in LDH-5 and decreased the other isozymes.

These changes differ from those of normal fast-twitch rabbit muscle in which chronic stimulation decreased LDH-5 and increased LDH-4 and LDH-3 [8,9]. The inverse changes brought about by stimulation of diseased mouse muscles (table 3) thus indicate a transition of the LDH isozyme distribution towards the pattern characteristic of normal fast twitch mouse muscle. These results indicate that:

(i) Low frequency stimulation has beneficial effects on diseased muscle;
(ii) The effects of stimulation on diseased muscle differ from those on normal mouse.

Table 3
Influence of electrostimulation on total activity and isozymes of lactate dehydrogenase in dystrophic extensor digitorum longus (EDL) and tibialis anterior (TA) mouse muscles

Muscle	Days stim.	Activity (U/g muscle)	LDH-5 (%)	LDH-4 (%)	LDH-3 (%)	LDH-2 (%)	LDH-1 (%)
TA-dystrophic	0	201	65 ± 4.2	24 ± 0.6	8 ± 1.8	3 ± 1.7	0
	9	357	82 ± 2.8	15 ± 0.7	6 ± 2.0	1	0
TA-dystrophic	0	407	70	26	5	0	0
	15	473	85 ± 3.2	14 ± 0.7	2	0	0
EDL-dystrophic	0	235	44 ± 0.8	35 ± 2.8	17 ± 1.6	5 ± 1	0
	15	235	56	31	12	1	0
TA-dystrophic	0	179	68	26	4	2	0
	19	226	79	18	3	1	0
TA control	–		90 ± 1.7	10 ± 1.1	0	0	0

Isozymes were separated by polyacrylamide gel electrophoresis in capillaries, stained for activity and evaluated microphotometrically. The values represent means either from 2 or means ± SD from 3 electrophoreses

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