

# Chronic long-term electrostimulation creates a unique metabolic enzyme profile in rabbit fast-twitch muscle

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Long-term low-frequency stimulation (up to 120 days) of rabbit fast-twitch tibialis anterior muscle led, in a first-order-like time course, to changes in enzyme activities of energy metabolism which became stable with ongoing stimulation after 50 days. The glycolytic enzymes decreased to 30–40% of their normal values, but remained 2–3-fold higher than in heart or soleus muscle. The LDH isozyme pattern ultimately resembled that of the slow-twitch soleus muscle. Citrate synthase activity increased 3.7-fold which brought this enzyme to a value 45% above that of heart. These results indicate that chronic stimulation does not simply convert the fast-twitch muscle into a soleus-like slow-twitch muscle, but creates a tissue of unique metabolic properties.

Fast-twitch muscle; Chronic electrostimulation; Enzyme activity; Energy metabolism; (Rabbit, Heart)

## 1. INTRODUCTION

Increased contractile activity, as induced by low-frequency stimulation, evokes a pronounced rearrangement of the enzyme activity and isozyme pattern of energy metabolism [1–8]. This ultimately leads to changes which are believed to be causally related to an improved resistance to fatigue [9,10]. These effects have recently become of interest with regard to the use of chronically stimulated skeletal muscle as a myocardial substitute (e.g. [10–12]). However, the question has never been addressed as to whether chronic stimulation is capable of transforming skeletal muscle metabolically into a heart-like tissue. This has prompted us to perform the present study in which we have used extremely long stimulation periods to investigate both the time course and the extent of changes in the activity pattern of selected enzymes of anaerobic and aerobic metabolic pathways. The stimulation protocol

chosen was sufficiently long to attain maximal changes such that comparisons between the transformed fast-twitch muscle, normal slow-twitch soleus muscle and the heart could be meaningfully assessed.

## 2. MATERIALS AND METHODS

### 2.1. Animals; chronic stimulation

Male adult White New Zealand rabbits (3–4 kg) were used. Electrode implantation and chronic low-frequency (10 Hz) stimulation (12 h daily, i.e. 1 h on, 1 h off) of the left lateral peroneal nerve have been described previously [1,7]. Stimulation times, expressed in days, and number of animals (in parentheses) were: 4 ( $n = 5$ ), 8 ( $n = 5$ ), 15 ( $n = 5$ ), 23 ( $n = 5$ ), 28 ( $n = 2$ ), 35 ( $n = 3$ ), 50 ( $n = 2$ ), 72 ( $n = 2$ ), 82 ( $n = 3$ ), 90 ( $n = 5$ ), 99 ( $n = 2$ ), and 120 ( $n = 3$ ). Animals were killed at the respective time points and both contralateral unstimulated and stimulated tibialis anterior (TA) muscles were excised and frozen in liquid N<sub>2</sub>. Soleus muscles and heart ventricles were also collected from some animals ( $n = 5$ ) and treated in the same way.

### 2.2. Tissue extraction and enzyme assays

Muscle pieces were pulverized under liquid N<sub>2</sub> and 15–20 mg muscle powder were suspended 1:40 (w/v) in 0.1 M KH<sub>2</sub>PO<sub>4</sub>/Na<sub>2</sub>PO<sub>4</sub> buffer (pH 7.2) containing 2 mM EDTA. This suspension was sonicated  $8 \times 10$  s with intense cooling and then stirred on ice for 15 min. The supernatant fraction obtained after short-term centrifugation at  $15000 \times g$  was used for the

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photometric determination of total cellular enzyme activities at 30°C. Enzyme activities were calculated as U/g wet wt. The assay mixtures for citrate synthase (CS), glyceraldehyde-phosphate dehydrogenase (GAPDH), lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) were as described [13]. LDH isozymes were separated in 12.5% polyacrylamide gels, stained for activity, quantified densitometrically, and evaluated for the percent distribution of H- and M-subunits [14].

### 3. RESULTS

Chronic low-frequency stimulation induced pronounced decreases in the activity levels of GAPDH and LDH concomitant with large increases in CS and MDH (fig.1). Both decreases and increases in enzyme activities followed similar first-order-like time courses reaching maximal deviations after about 50 days of stimulation. Half-maximal deviations were attained for CS and MDH after 15–20 days and for GAPDH and LDH after approx. 12 days.

A comparison of the maximally attained deviations with the activity levels of the investigated enzymes in slow-twitch soleus muscle and heart ventricle is provided in table 1. It is evident that GAPDH and LDH did not decrease to the levels found in soleus muscle or in heart. Their steady-state values in long-term stimulated TA muscles were still approx. 2–3-fold higher than in heart and soleus muscles. Conversely, steady-state values of both CS and MDH in long-term stimulated TA far exceeded those found in normal soleus muscle, i.e. 2.2-fold for MDH and 3.6-fold for CS. In the case of CS, the activity in the stimulated muscle even exceeded that in heart muscle by 44%. However, MDH attained only about 65% of its value in heart (table 1).

Normal fast-twitch TA contained predominantly (90%) the M<sub>4</sub> isoform of LDH (LDH-5). Over 90% of the LDH was composed of the H<sub>4</sub> LDH isoform (LDH-1) in heart muscle. Slow-twitch soleus mus-

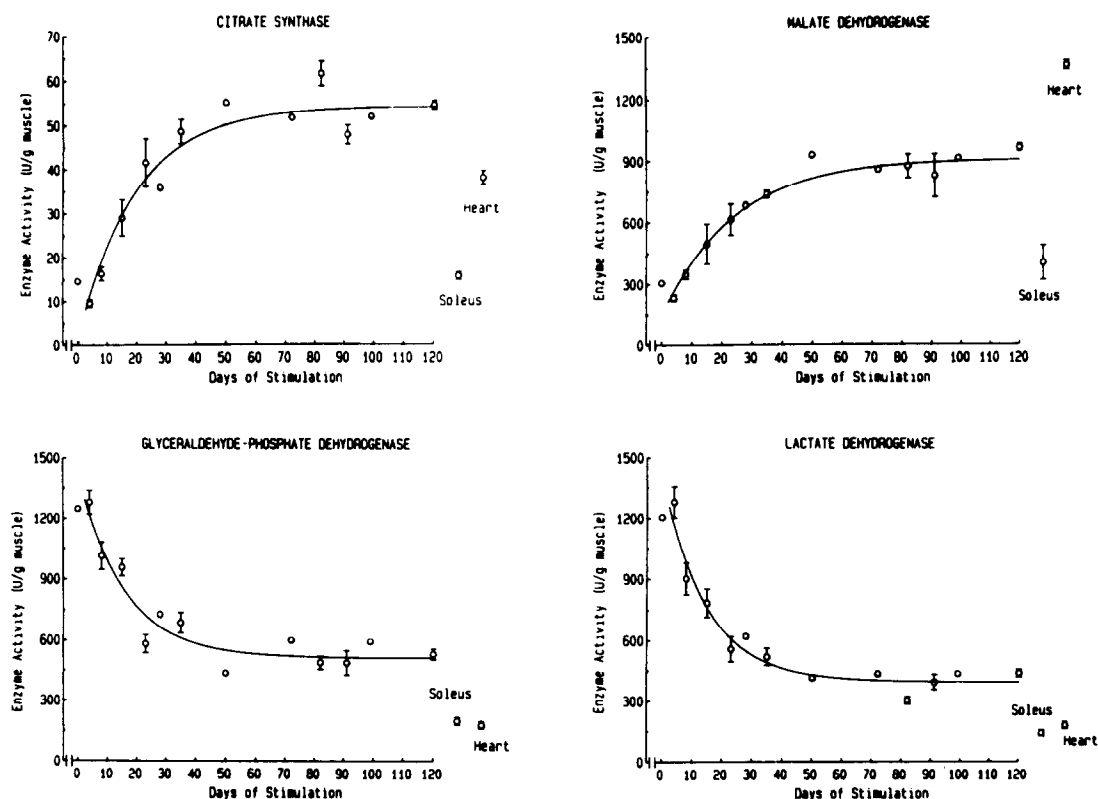


Fig.1. Time course of low-frequency stimulation-induced changes in the activity levels of reference enzymes of aerobic and anaerobic metabolic pathways in fast-twitch rabbit tibialis anterior muscle. For comparison, enzyme activities determined in slow-twitch soleus and cardiac muscle have also been plotted. Values are means  $\pm$  SE with the number of animals for each point ranging between 2 and 5.

Table 1

Comparison between enzyme activities (U/g muscle) in normal fast-twitch and long-term stimulated tibialis anterior, as well as normal soleus and heart muscles of the rabbit

Muscle	Enzyme activity (U/g)			
	GAPDH	LDH	MDH	CS
Normal TA	1247 ± 38	1205 ± 42	307 ± 15	14.6 ± 0.8
Stimulated TA	516 ± 23	398 ± 17	887 ± 35	53.3 ± 1.5
Soleus	233 ± 38	164 ± 25	403 ± 35	14.8 ± 1.2
Heart	180 ± 16	179 ± 7	1367 ± 11	36.9 ± 1.5

Data represent means ± SE ( $n = 42$  for normal TA,  $n = 17$  for long-term stimulated TA, i.e. beyond 50 days,  $n = 5$  for soleus and heart)

cle contained an approximately equal mixture of all LDH isoforms (fig.2). Chronic stimulation in TA brought about a gradual and sequential transition from the  $M_4$  to the heterotetrameric LDH isoforms. This was particularly evident during the first 50 days of stimulation when sequential increases in  $M_3H$  LDH, followed by the  $M_2H_2$ ,  $MH_3$ , and  $H_4$  isoforms were observed (fig.2). These data were used to calculate the percent distribution of the M- and H-LDH subunits. Normal TA contained 95% M-LDH subunit and only 5% H-LDH subunit. The opposite pattern was found in heart. Normal slow-twitch soleus muscle contained approx. 50% of both M- and H-LDH subunits. Chronic stimulation of TA muscle led to a pro-

gressive exchange of the M- with the H-subunit, but even with stimulation periods up to 120 days this exchange did not significantly extend beyond the H/M ratio found in soleus muscle.

#### 4. DISCUSSION

As judged from the activity pattern of the investigated reference enzymes of energy metabolism, our results indicate that the metabolic character of long-term stimulated fast-twitch muscle may remain below, reach or even exceed the properties typical of cardiac muscle. The decreases in glycolytic enzyme activities do not attain the very low levels found in heart, nor do these enzyme activities reach levels characteristic of the slow-twitch soleus muscle. However, the changes in the LDH isozymes are of sufficient magnitude to resemble the pattern found in soleus muscle. The sequential transitions from LDH-5 to LDH-1 during the first 50 days of stimulation are striking. They are due to a progressive exchange of the M- with the H-subunit, and provide evidence for the random association of an M- and H-subunit-based LDH holoenzyme assembly.

In contrast, the stimulation-induced changes in activities of MDH and CS largely exceed those of soleus and, in the case of CS, even that of cardiac muscle. The finding that CS activity rises in the stimulated TA to levels higher than in cardiac muscle could indicate an increase in mitochondrial volume density beyond that in cardiac muscle.

Taken together, these data indicate that chronic, low-frequency stimulation of rabbit fast-twitch muscle induces changes which make the stimulated muscle not only resemble, but even exceed selected metabolic properties typical of heart. This is relevant with respect to the aerobic-oxidative potential which is commonly believed to be related to sustained performance and to a high resistance to fatigue. Even taking into account that the present study focused on only a few selected enzymes, the present data clearly document that chronic low-frequency stimulation under the chosen conditions does not simply bring about a metabolic conversion of the fast-twitch TA into a soleus-like slow-twitch muscle. Illustrating the remarkable plasticity of muscle, chronic low-frequency stimulation elicits specific metabolic properties in response to the imposed functional demand. Therefore, the

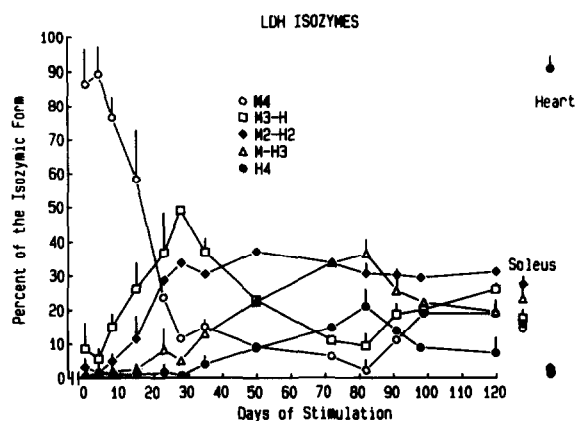


Fig. 2. Time course of low-frequency stimulation-induced transitions in lactate dehydrogenase isozyme distribution in rabbit tibialis anterior muscle. The changes were electrophoretically assessed and the percent distribution for each isoform determined by densitometric evaluation. Values are means ± SE for the same animals as those represented in fig.1.

transformed muscle represents a unique tissue reflecting an unusual state of expression especially endowed for sustained activity.

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