Journal of Physiology (1993), **460**, *pp.* 573–580
With 3 figures
Printed in Great Britain

ASYNCHRONOUS INCREASES IN OXIDATIVE CAPACITY AND RESISTANCE TO FATIGUE OF ELECTROSTIMULATED MUSCLES OF RAT AND RABBIT

BY JEAN-AIMÉ SIMONEAU*, MARGIT KAUFMANN AND DIRK PETTE
From the Fakultät für Biologie, Universität Konstanz, D-7750 Konstanz, FRG

(*Received 27 November 1991*)

SUMMARY

1. The present study investigates to what extent increases in resistance to fatigue and aerobic oxidative capacity of energy metabolism are correlated in fast-twitch tibialis anterior muscles of rat and rabbit subjected to chronic low-frequency stimulation.

2. Changes in the aerobic oxidative capacity of the stimulated muscles were judged from increases in citrate synthase activity, representing the constant-proportion enzyme group of the citric acid cycle.

3. Resistance to fatigue reached maximal values in both rat and rabbit tibialis anterior muscles after stimulation periods of 14 days, whereas citrate synthase activity continued to increase with longer stimulation periods.

4. Different time courses of the changes in resistance to fatigue and citrate synthase activity were observed not only with prolonged stimulation periods but also during the first week, when pronounced increases in resistance to fatigue were accompanied by only moderate elevations in citrate synthase activity.

5. The dissociation between the changes of the two parameters studied suggests that factors other than elevated aerobic oxidative capacity contribute to enhanced resistance to fatigue.

INTRODUCTION

It is generally assumed that the ability for sustained contractile activity of skeletal muscle correlates with a high capacity of aerobic oxidative metabolism. This notion was established by comparing fatigue properties of different motor units displaying a large spectrum of histochemically assessed mitochondrial enzyme activities (Edström & Kugelberg, 1968; Burke, Levine & Zajac, 1971). It was confirmed by microphotometric (Kugelberg & Lindegren, 1979) and microbiochemical (Nemeth, Pette & Vrbová, 1981) enzyme activity measurements on single fibres from motor units differing in their fatigue properties. Also, marked increases in resistance to fatigue induced by chronic low-frequency stimulation of fast-twitch muscles have been shown to be accompanied by elevations in enzyme activities of aerobic

* Present address: Physical Activity Sciences Laboratory, Laval University, Ste-Foy, Québec, Canada G1K 7P4.

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oxidative metabolism (Peckham, Mortimer & van der Meulen, 1973; Pette, Ramirez, Müller, Simon, Exner & Hildebrand, 1975; Salmons & Sréter, 1976; Hudlická, Brown, Cotter, Smith & Vrbová, 1977).

These observations suggest that a relationship exists between a muscle's ability to sustain contractile activity and the aerobic oxidative capacity of its energy metabolism. However, Hudlická *et al.* (1977) observed increases in resistance to fatigue 4 days after the onset of low-frequency stimulation of rabbit muscle, although significant increases in enzyme activities of aerobic oxidative metabolism occurred later (Pette, Smith, Staudte & Vrbová, 1973). Kernell, Donselaar & Eerbeek (1987), performing microphotometric evaluations of succinate dehydrogenase histochemistry in fibres of chronically stimulated fast-twitch muscles of the cat, found that increases in this mitochondrial enzyme activity were not tightly linked to the degree of contractile endurance.

A quantitative study of the temporal patterns of chronic stimulation-induced increases in resistance to fatigue and aerobic oxidative capacity has, to our knowledge, not been performed. In the present study, fast-twitch tibialis anterior (TA) muscles of rabbit and rat were chronically stimulated for different time periods at low-frequency to investigate possible relationships between changes in resistance to fatigue and aerobic oxidative capacity. Resistance to fatigue was measured using the test of Burke *et al.* (1971). Total activity of citrate synthase was chosen as a marker of aerobic oxidative metabolism. This enzyme has previously been shown to represent the constant-proportion group of mitochondrial enzymes of the citric acid cycle and the respiratory chain (Pette, 1966). Furthermore, citrate synthase activity increases in parallel with mitochondrial volume density, as well as with other mitochondrial enzymes of the citric acid cycle in chronically stimulated rabbit muscle (Reichmann, Hoppeler, Mathieu-Costello, von Bergen & Pette, 1985). Finally, comparative electron microscopic morphometry and biochemical studies have shown a correlation between citrate synthase activity and volume density of mitochondria in equine muscle fibres (Hoppeler, 1990).

METHODS

Animals, chronic stimulation, resistance to fatigue

Experiments were carried out on male adult Wistar rats (300–400 g body weight) and male adult New Zealand White rabbits (3–4 kg body weight). Electrode implantation and chronic low-frequency stimulation (10 Hz, 0·3 ms pulse width) of the common peroneal nerve of the left hindlimb were performed as previously described (Simoneau & Pette, 1988). Chronic stimulation (10 h daily) lasted for different periods of time, i.e. 3, 5, 7, 10, 14, 21, 28, and 35 days for rat and 4, 7, 14, 21 and 35 days for rabbit. Three to six animals were investigated for each time point. Stimulation was stopped 14–16 h before the animals were anaesthetized and prepared for the measurement of fatigue resistance. Rats and rabbits were anaesthetized with sodium pentobarbitone (rats: 55 mg/kg injected I.P., supplemented as necessary; rabbits: 45 mg/kg injected I.V., additional doses as required). Both hindlimbs were secured to the table by drills put into the distal part of the femur and tibia. The tibialis anterior (TA) muscles were surgically isolated, their distal tendons were dissected free and attached to strain gauges (Pette *et al.* 1975). The strain gauges were connected to a microprocessor for evaluation of tension output. Skin pouches were formed around the muscles and filled with prewarmed (37 °C) paraffin oil to maintain proper temperature. The TA muscles were stretched to the length at which they generated maximum isometric twitch tension, and resistance to fatigue was measured according to Burke *et al.* (1971). Using bipolar electrodes, the TA muscles were stimulated by the proximal stump of the sectioned

peroneal nerve with square wave pulses of 0·3 ms duration and supramaximal intensity. The fatigue test consisted of 40 Hz stimulation bursts of 330 ms duration repeated every second for up to 6 min. The isometric tension generated after 2 min was evaluated as a percentage of the maximum tension output and used as an index of fatigue. After completion of the physiological measurements, animals were killed by injecting an additional dose of sodium pentobarbitone and the TA muscles of both stimulated and contralateral legs were excised, weighed, and stored in liquid N_2.

Measurement of citrate synthase

Frozen muscle was pulverized under liquid N_2 and the powder was suspended in a 19-fold (w/v) volume of 0·1 M phosphate buffer (pH 7·2) containing 2 mM EDTA. This suspension was sonicated 6×10 s interspaced by 30 s pauses with intense cooling. The homogenate was centrifuged at $20000 \times g$ and the pellet was re-extracted with fresh buffer and centrifuged. Total cellular citrate synthase (CS) activity was measured photometrically at 30 °C in the combined supernatant fractions (Simoneau & Pette, 1988). Enzyme activities were expressed as U/g wet weight. Enzyme activities and fatigue index are given as means ± S.E.M.

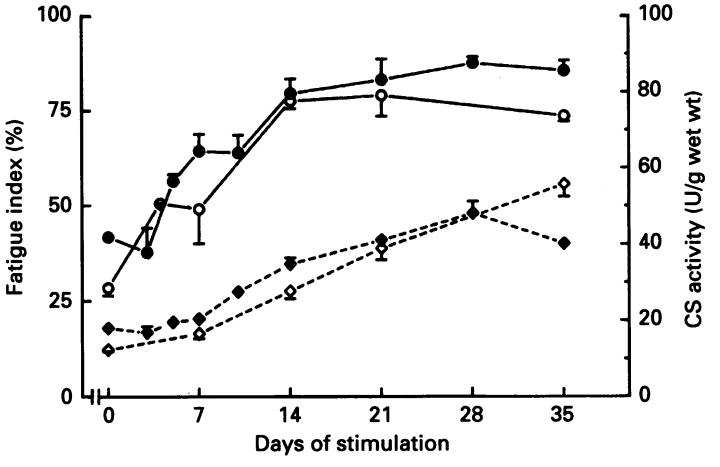

Fig. 1. Time course of increases in fatigue index and citrate synthase activity in low-frequency stimulated tibialis anterior muscles of rat (closed symbols) and rabbit (open symbols). The fatigue index (continuous line) is defined as percentage of maximum tetanic tension measured after 2 min of repetitive tetanic contractions (1/s, each lasting 330 ms). Citrate synthase (CS, dashed line) is expressed as U/g wet weight. Values are means ± S.E.M. (fatigue index, $n = 3$–6 animals per time point; citrate synthase, $n = 16$ and 38 for control muscles of rabbit and rat, respectively, and $n = 3$ per time point for stimulated muscles).

RESULTS

A rapid decrease in tension output was observed in the unstimulated control muscles of rabbit and rat during repetitive tetanic contractions with a 33% duty cycle. After 2 min, isometric tension had decreased to approximately 30 and 40% of the initial maximum values in the control TA muscles of rabbit and rat, respectively. Conversely, muscles subjected to low-frequency stimulation for longer than 14 days exhibited almost no decrease in tension output even after up to 6 min of repetitive tetanic contractions.

The development of enhanced resistance to fatigue followed similar time courses in low-frequency stimulated muscles of rabbit and rat (Fig. 1). By 14 days, the fatigue index had increased in rabbit and rat to 78±2 and 80±4% (means±S.E.M.), respectively. As judged from the fatigue test used, low-frequency stimulation for

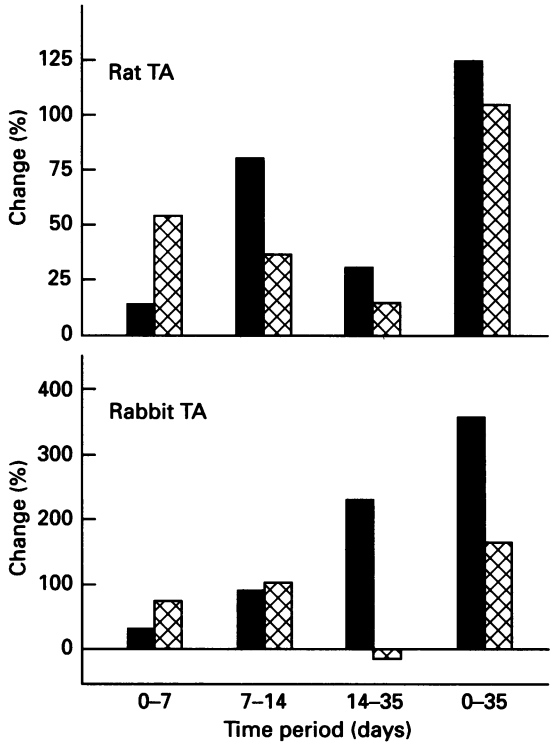

Fig. 2. Percentage increases of citrate synthase (CS, filled bars) activity and fatigue index (FI, cross-hatched bars) in tibialis anterior (TA) muscles of rat and rabbit during different time periods of chronic low-frequency stimulation. The percentage increases shown in the columns refer to the absolute levels reached during the preceding time period. The last two columns show the percentage increases in CS and FI after 35 days of low-frequency stimulation as referred to the values of the unstimulated muscles.

longer time periods did not lead to further significant improvements in resistance to fatigue.

Confirming results from a previous study with the same stimulation protocol (Simoneau & Pette, 1988), citrate synthase activity increased severalfold in both rat and rabbit TA muscles (Fig. 1). It amounted to 18±1 U/g in unstimulated rat TA muscle and reached its maximum (48±3 U/g) after 28 days of stimulation. At 35 days, citrate synthase activity was lower and amounted to 40±1 U/g. In unstimulated rabbit TA, citrate synthase activity was 12±1 U/g and it reached a 4·7-fold higher value (56±3 U/g) in the 35-day stimulated muscle.

A comparison of the stimulation-induced increases in resistance to fatigue and citrate synthase activity indicated that the two parameters did not change in parallel throughout the whole stimulation period (Fig. 1). Asynchronous changes of the two

parameters were especially obvious in the rabbit where resistance to fatigue reached its maximum after 14 days, while citrate synthase activity continued to rise with prolonged stimulation. The discrepancy between rise in citrate synthase and increase in resistance to fatigue was further substantiated by comparing percentage changes

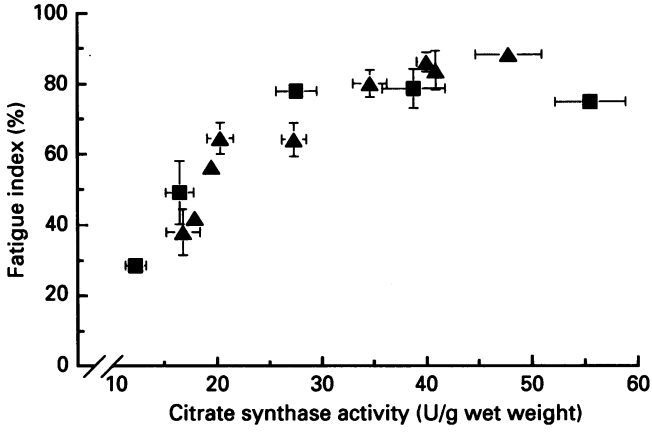

Fig. 3. Relation between increases in citrate synthase activity and fatigue index within the same low-frequency stimulated TA muscles of rat (▲) and rabbit (■). Values are given as means ± S.E.M. (n = 3–6 animals for each point).

of the two parameters at different time points (Fig. 2). Pronounced increases in the fatigue index occurred in rat (+54%) and rabbit (+75%) during the first week of stimulation, when citrate synthase activity increased only moderately (rat: +14%; rabbit: +34%). However, a substantial rise in citrate synthase activity was observed during the second week in both rat (80%) and rabbit (90%). In the rat, this change was accompanied by a moderate increase in the fatigue index of only 36%, whereas in the rabbit the fatigue index was 100% elevated. No significant increases in fatigue index were recorded during the third week, although citrate synthase activity continued to rise in both rat and rabbit (Fig. 2). Increases in citrate synthase activity without concomitant changes in the fatigue index led to a dissociation of the two parameters in long-term stimulated muscles. Thus, contrary to an apparent correlation between citrate synthase activity and the fatigue index at low enzyme activity levels, the two parameters were unrelated at citrate synthase activities exceeding values of 30–35 U/g (Fig. 3).

DISCUSSION

Comparative studies on functionally and metabolically different motor units (Edström & Kugelberg, 1968; Burke *et al.* 1971; Kugelberg & Lindegren, 1979; Nemeth *et al.* 1981) have suggested a correlation between the aerobic oxidative capacity of energy metabolism and the ability to withstand fatigue during sustained contractile activity. The present study shows that increases in citrate synthase activity, a commonly used marker for aerobic metabolic capacity, and resistance to

fatigue do not follow identical time courses. Furthermore, at least for the rabbit it is evident that pronounced increases in citrate synthase activity after long-term stimulation are not accompanied by increases in resistance to fatigue.

A dissociation between increases in aerobic oxidative capacity and resistance to fatigue is evident in short-term stimulated muscles. It is unlikely that the improved ability to withstand fatigue at this time is fully explained by the relatively small increases in aerobic oxidative capacity. In the case of rabbit muscle, the early increase in resistance to fatigue with only moderate increases in citrate synthase activity could be partially explained by the degeneration of a portion of the fast-twitch glycolytic (FG) fibres. Previous studies have demonstrated that 10–20% of the FG fibres undergo degeneration during the first week of chronic low-frequency stimulation (Maier, Gambke & Pette, 1986; Maier & Pette, 1987; Maier, Gorza, Schiaffino & Pette, 1988). The partial removal of these fast-fatiguable fibres could, therefore, result in a relative improvement of the fatigue index. Indeed, measurements of maximum tetanic tension performed on the same muscles investigated in the present study, revealed a transient decrease in tetanic isometric tension during the first week for rabbit TA, but not for rat TA muscles (Simoneau, Kaufmann, Härtner & Pette, 1989).

Other factors may contribute to enhanced resistance to fatigue during the early phase of low-frequency stimulation. Increases in capillary density (Brown, Cotter, Hudlická & Vrbová, 1976; Hudlická *et al.* 1977; Hudlická, Dodd, Renkin & Gray, 1982; Dawson & Hudlická, 1989) may be important to match the augmented fuel demand at a time when the capacity for terminal substrate oxidation is still at a low level in fast-twitch muscle and its energy metabolism is mainly based on anaerobic carbohydrate catabolism. More than 10-fold increases in the cellular content of hexokinase isozyme II during the first days of stimulation (Pette *et al.* 1973; Weber & Pette, 1990) and elevations in the amount of sarcolemma-associated GLUT-4 glucose transporter (S. Hofmann & D. Pette, unpublished results), serve to enhance uptake and usage of glucose. Increased glucose supply combined with an enhanced uptake and phosphorylation of glucose could also explain the overshoot in glycogen after its rapid initial depletion in short-term stimulated rabbit muscle (Maier & Pette, 1987; Green, Düsterhöft, Dux & Pette, 1990, 1992).

These early adaptive responses are followed by pronounced increases in aerobic oxidative capacity. Elevations in the content of cytosolic fatty acid-binding protein (Kaufmann, Simoneau, Veerkamp & Pette, 1989) and increases in enzyme activities of fatty acid oxidation, as well as in carnitine palmitoyl-CoA transferase (Reichmann, Wasl, Simoneau & Pette, 1991), a key enzyme for intramitochondrial transport of long-chain acyl-CoA, enhance fatty acid utilization. Therefore, fatty acid catabolism seems to play an increasing role during the later phase of metabolic adaptation. Hence, the major increase in citrate synthase activity during the second and third week of low-frequency stimulation appears to relate to enhanced fatty acid oxidation.

In conclusion, the present study provides evidence that the increase in resistance to fatigue observed in chronically low-frequency stimulated muscle may not entirely be determined by elevations in the capacity of the citric acid cycle and related metabolic pathways of aerobic oxidative capacity.

This study was supported by the Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 156. J.-A. Simoneau was a post doctoral fellow of Fonds de la Recherche en Santé du Québec, Canada. The authors thank Mrs Elmi Leisner and Mrs Sara Krüger for excellent technical assistance.

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