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RESPONSE OF SUCCINATE DEHYDROGENASE ACTIVITY IN FIBRES OF RABBIT TIBIALIS ANTERIOR MUSCLE TO CHRONIC NERVE STIMULATION

BY D. PETTE AND K. R. TYLER

From the Faculty of Biology, University of Konstanz, D-7750 Konstanz, F.R.G. and the Department of Physiology, The Medical School, University of Birmingham, Birmingham B15 2TJ

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SUMMARY

1. Succinate dehydrogenase activity (SDH) was estimated kinetically in individual muscle fibres from the rabbit tibialis anterior, in cryostat sections using computer-linked microphotometry to record initial reaction velocities. These were correlated with fibre type based on myofibrillar actomyosin ATPase staining.

2. Analysis of type IIA and IIB fibre populations in control muscles demonstrated wide variations in SDH activity between fibres of identical myosin ATPase type, with a considerable overlap in oxidative activities of the IIA and IIB populations.

3. Muscles chronically stimulated via the peroneal nerve, using two different frequency patterns, showed increases in SDH activity which were primarily located in the type IIB fibres. This increase was observed both in muscles stimulated continuously at 10 Hz, and when similar numbers of stimuli were applied in brief trains at higher frequency.

4. An earlier onset and more rapid rate of increase of SDH activity was seen with 10 Hz stimulation than with higher frequency, though the levels after 14 days of either pattern of stimulation were not significantly different.

INTRODUCTION

Most mammalian skeletal muscles consist of a heterogeneous population of muscle fibres, which can be classified into a number of subpopulations on the basis of histochemical reactions. Of the more frequently used systems, that of Peter, Barnard, Edgerton, Gillespie & Stempel (1972) is based upon a combination of histochemical staining for myofibrillar actomyosin ATPase (myosin ATPase) and for enzymes involved in aerobic and glycolytic metabolism. Under this scheme, muscle fibres would be classified as fast or slow, and oxidative or glycolytic in type. An alternative scheme proposed by Brooke & Kaiser (1970) relies on the differential staining of myosin ATPase after acid pre-incubation. While it is often assumed that the groups within each of these classifications are largely interchangeable (*vide* Close, 1972), it

Correspondence should be addressed to: Dr K. R. Tyler, Department of Physiology, The Medical School, The University of Birmingham, Birmingham B15 2TJ.

has been demonstrated that this is not so within the leg muscles of the rat, where considerable variety of oxidative activity occurs within the fast-twitch II A and II B fibre subgroups (Nemeth, Hofer & Pette, 1979; Nemeth & Pette, 1980, 1981*a*, *b*).

We were interested in the distribution of oxidative enzyme activity within different fibre types in the fast leg muscles of the rabbit, and in the changes occurring after chronic electrical stimulation. During long-term stimulation of these fast muscles at frequencies normally found in the nerves to slow muscles (10 Hz), increases in several enzyme activities of aerobic oxidative metabolism have been reported (Pette, Smith, Staudte & Vrbová, 1973; Heilig & Pette, 1980) with accompanying increases in capillary density within these muscles (Cotter, Hudlická, Pette, Staudte & Vrbová, 1973; Brown, Cotter, Hudlická & Vrbová, 1976). Qualitatively similar increases in the activity of succinate dehydrogenase (SDH) occur when these muscles are stimulated for 14 days with equal numbers of stimuli applied in brief trains at higher frequency (40 Hz). Using conventional histochemical methods, the time course of these increases appeared similar in each treatment (Hudlická & Tyler, 1980). With the 40 Hz stimulation, however, capillary density increases were seen to occur later than with lower frequency stimulation, although prolonged periods of stimulation at either frequency produced almost complete conversion of muscle fibres to an oxidative type, and equivalent increases in capillary density (Hudlická & Tyler, 1980). The reason for the discrepancy of these findings is not clear.

These previous results have been based upon qualitative histochemistry. The kinetic microphotometric assay of enzyme activity within individual fibres (Pette, Wasmund & Wimmer, 1979; Pette & Wimmer, 1979; Pette, 1981; Nemeth & Pette, 1981*b*; Reichmann & Pette, 1982) offers a number of distinct improvements. The method relies on a kinetic estimation of reaction velocity, rather than end-point determination of product levels: the velocity is assessed early in the reaction before significant end-product inhibition of enzyme activity occurs. It provides quantitative measurements with high resolution and repeatability (Pette, Wimmer & Nemeth, 1980; Reichmann & Pette, 1982) of the reaction velocity (equivalent to enzyme activity) within a number of cross-sectioned muscle fibres in cryostat sections. By measurement of activity within the same reference fibre in each of the estimations it is possible to map relative SDH activity in large numbers of fibres within a single muscle sample, and serial cross-sections stained for myosin ATPase (Brooke & Kaiser, 1970) allow classification of each of the quantitated fibres into the relevant subpopulations (Types I, II A, II B).

This method was applied to measurement of SDH activity within individual fibres of the rabbit tibialis anterior, with two main objectives. The first was to investigate the correspondence between the myosin ATPase subpopulations, particularly types II A and II B, and the oxidative enzyme activities of fibres within these groups. The second was to study the time course of changes in aerobic oxidative capacity of different fibre types during electrical stimulation, and to provide new data on the fibre populations most affected by each pattern of stimulation.

A preliminary report of some of these results has already been published (Pette & Tyler, 1981).

METHODS

Experiments were carried out on adult male rabbits (2·5–3·5 kg body wt.) of New Zealand Red and White outbred strains. Unilateral implantation of coiled multistranded stainless-steel electrodes for chronic stimulation was performed under halothane anaesthesia and aseptic conditions as previously described (Brown *et al.* 1976). The group of fast muscles innervated by the lateral popliteal (peroneal) nerve (tibialis anterior, t.a.; extensor digitorum longus and peroneal muscles) were stimulated for 8 hr day, over periods from 2–14 days using portable stimulators (Tyler & Wright, 1980). Square-wave stimuli, 0·3 msec in duration, were applied either continuously at 10 Hz or in three 5 sec trains of 40 Hz stimuli per min. These patterns provided equal numbers of impulses/hr and output voltages of the stimulators were adjusted to produce maximal contractions on palpation. Previous experiments (Hudlická, Tyler & Aitman, 1980) have demonstrated that such stimulating voltages proved very close to those producing maximal isometric twitch tension, and indicated that all motor units were activated in these conscious animals.

At the end of each period of stimulation the animals (two per time point) were killed by cervical dislocation, and the tibialis anterior muscles were excised and cut longitudinally into four strips. These were rapidly frozen in a slightly stretched position, using melting isopentane (−160 °C). Composite blocks, consisting of two muscle strips (one stimulated, and its contralateral control) were prepared, and serial transverse sections (10 μm) were cut at −25 °C on a cryostat. Tissue sections were alternatively stained for myosin ATPase after pre-incubation at pH 4·6 (modified from Brooke & Kaiser, 1970; *vide infra*) or prepared for microphotometric assays of succinate dehydrogenase (SDH).

The ATPase method was performed as follows: freshly cut sections were pre-incubated for 6 min at room temperature in a 100 mM-sodium acetate/acetic acid buffer, pH 4·60, containing 100 mM-KCl. After a brief rinse in distilled water, sections were incubated for 30 min at 37 °C in a solution containing 3 mM-ATP, 30 mM-$CaCl_2$ and 55 mM-NaCl in a 50 mM-glycine/NaOH buffer at pH 9·4. Sections were rinsed twice in distilled water and incubated for 3 min in 85 mM-$CoCl_2$. After three rinses (30 sec each) in distilled water, sections were incubated for 1 min in 1% $(NH_4)_2S$ to reveal the reaction product. After thorough rinsing with distilled water, slides were dehydrated, cleared and mounted. The staining reactions of these slides were used to classify muscle fibres as types I, IIA or IIB.

For microphotometry, an aqueous reaction medium was used containing 5 mM-EDTA, 1 mM-KCN, 0·2 mM-phenazine methosulphate, 50 mM-sodium succinate, 1·5 mM-Nitroblue Tetrazolium in a 100-mM-phosphate buffer (pH 7·6). Microphotometric SDH activity determinations were made using the Leitz MPV2 microscope/computer microphotometric system (Pette *et al.* 1979). This system allowed kinetic measurement of reaction velocity at room temperature, during the first 3 min of reaction, in up to eleven selected muscle fibres/section. A measuring field of 15 μm diameter was chosen, and the co-ordinates describing the positions of the centres of the selected fibres were stored in the computer. After addition of the reaction medium, the section was moved to each of these co-ordinates in turn, measuring extinction at 548 nm at each for 0·2 sec. The process was repeated over a total of fifteen complete cycles, taking 2–3 min to complete, providing data for calculation of rate of change of extinction (ΔE_{548}/sec) at each co-ordinate. Relative enzyme activities were calculated from these data using the Leitz LINREG program (Pette *et al.* 1979), as maximal initial reaction velocities. Each fibre measured in this fashion was typed according to the myosin ATPase reaction produced in the corresponding serial section.

In order to measure further fibres within serial sections from the same block, a procedure was adopted which allowed comparison of activities between sections, correcting for uncontrollable variations in section thickness, temperature and processing conditions. A previous microphotometric study (Pette, Wimmer & Nemeth, 1980) on distribution of SDH along muscle fibres has shown extremely even distribution (S.D. = 4·6% of the mean) of activity throughout the length of single fibres. In this study, one of the large type IIB fibres from the control muscle in each block, which showed similarly low SDH activity, was chosen as a reference fibre from which to calculate relative activities. This fibre was identified in each of the serial sections and its SDH activity assayed. Its activity was set to 1 and all other fibres (control and stimulated) were evaluated relative to it. This procedure allowed measurement of relative SDH activities of approximately sixty fibres from each of the control and stimulated muscles from each animal.

Although this procedure does allow comparison of fibres in different sections from the same block, differences in absolute activity of the reference fibres from different blocks necessitate further normalization between animals. This was achieved by arbitrarily setting the mean activity in control type IIB fibres to a value of 1, and referring all activities to this value.

RESULTS

Control muscle

A total of 415 type II fibres were assayed for SDH activity. Their relative activities ranged from 0·45 to 4·16 – a range of almost a decade. Of these fibres, 151 were classified as type IIA and 264 as type IIB. Type I fibres represented a negligible fraction and were therefore not considered. The range and distribution of SDH activities measured in type IIA and IIB populations is shown in Fig. 1.

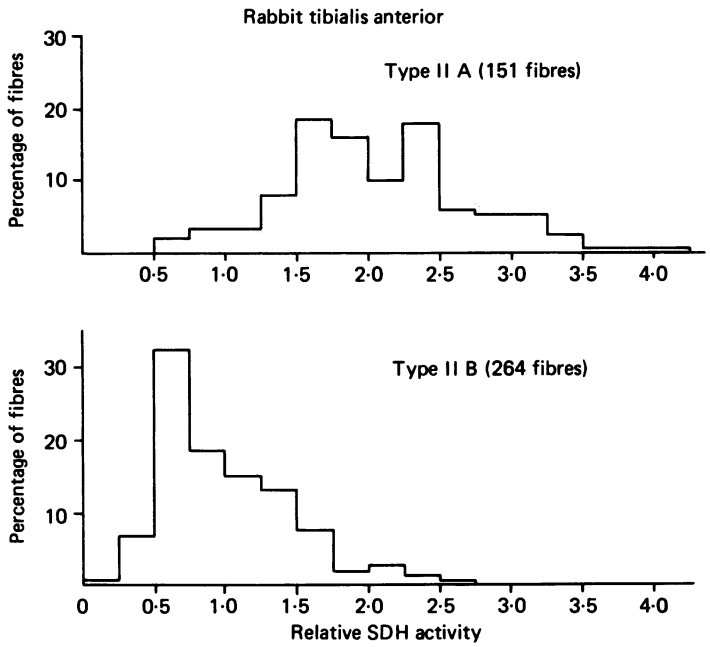

Fig. 1. Frequency distributions of relative SDH activity (mean for IIB fibres is set at 1·0) in type IIA and type IIB fibres of the rabbit tibialis anterior muscle. The vertical axis represents the percentage of the total number of fibres of each type which fall into each group. Both of these distributions are measured in normal control muscles: the wide range of enzyme activities within each group is obvious.

The majority of myosin type IIA fibres have activities of SDH which are relatively high ($\bar{x}$ = 2·3), compared with those seen in the type IIB population (mean defined as 1·00). While these mean values would seem consistent with the view that myosin type IIA fibres correspond to the fast-oxidative-glycolytic (f.o.g.) type, and the myosin type IIB fibres to the fast-glycolytic (f.g.) type (Spurway, 1980, 1981), it is also apparent from Fig. 1 that the actual relative activities of SDH in individual fibres of each type overlap considerably.

Stimulated muscles

The changes in relative activities of SDH in type II A and type II B fibres of stimulated muscles are shown in Figs. 2 and 3. With both patterns of stimulation, marked changes in relative SDH activity were apparent over the whole period of stimulation (14 days). In each case, transitory increases of SDH activity were observed during this period in the type II A fibres, although the activity after 14 days stimulation was only marginally greater than in control II A fibres. In type II B fibres,

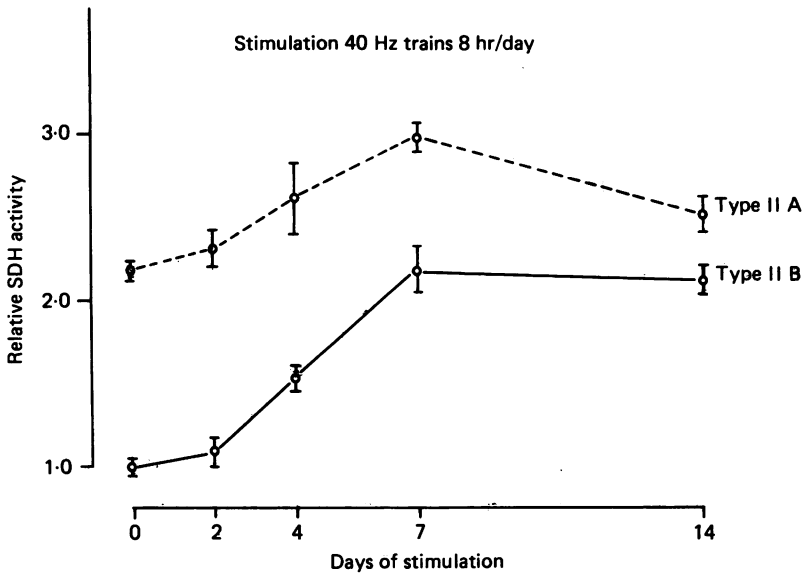

Fig. 2. Changes in the relative activity of SDH occurring in types II A and II B fibres of the rabbit tibialis anterior during the first 14 days of 10 Hz electrical stimulation. Values at 0 days are from control muscles. Each point represents the mean and standard error calculated from approximately seventy-five individual fibres.

however, much greater increases in relative SDH activity were observed, and they resulted in activities after 14 days of stimulation which were not significantly different from the type II A fibres of control muscles (Student's t test) and similar with both types of stimulation in II B fibres.

Differences in the time course of these increases are apparent between the two patterns of stimulation. Continuous activation with 10 Hz stimuli appears to produce an earlier onset of increase in SDH activity, and more rapid increase, than stimulation with equal numbers of impulses applied in brief trains at 40 Hz. Indeed, with 10 Hz stimulation a plateau was observed by 7 days of stimulation, with apparently no further increase. In contrast, the muscles stimulated at 40 Hz showed SDH increases in type II B fibres which continued throughout the period of stimulation.

DISCUSSION

The above microphotometric analyses show a wide variation of SDH activity in type II A and type II B fibres and are consistent with previous reports of extensive metabolic heterogeneity in the fibre populations of several mammalian muscles (Spamer & Pette, 1977, 1979, 1980; Lowry, Kimmey, Felder, Chi, Kaiser, Passonneau, Kirk & Lowry, 1978; Lowry, Lowry, Chi, Hintz & Felder, 1980; Hintz, Lowry, Kaiser, McKee & Lowry, 1980; Nemeth, Pette & Vrbová, 1981). While the means and modal

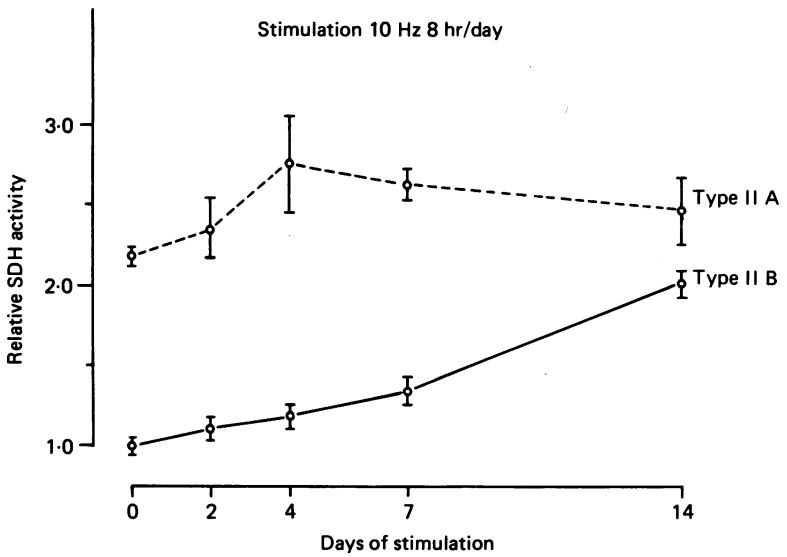

Fig. 3. Changes in the relative activity of SDH occurring in types II A and II B fibres of the rabbit tibialis anterior during the first 14 days of 40 Hz electrical stimulation. Values are plotted as the mean and standard error, each derived from approximately seventy-five individual fibres.

values for II A and II B fibres are distinctly different (Fig. 1), both of these groups contain high and low oxidative fibres. It seems, therefore, that while the majority of fibres in the II A group have high oxidative activities, and the majority of those in the II B group have lower oxidative activities (and hence the apparent correspondence of classification), there is a considerable overlap. This overlap is much less pronounced in the rabbit than in hind limb muscles of a variety of mammals studied so far (Nemeth & Pette, 1981*b*; Reichmann & Pette, 1982).

Previous studies (Nemeth *et al.* 1981) have demonstrated that muscle fibres belonging to a single motor unit have virtually identical enzyme activities. It is possible that the variation of SDH activity may be related to the recruitment of different motor units and hence over-all activity. Thus, those motor units which are normally only recruited for very brief periods might be expected to adapt toward a predominantly anaerobic metabolism, while those activated for more prolonged periods would base their activity on a predominantly aerobic metabolism.

The independence of the oxidative enzyme activity within individual fibres from

their myosin ATPase reaction is emphasized by the results from the stimulated muscles. While the proportions of fibres in the myosin groups II A and II B do not appear to change during the period of stimulation used in this study, there are marked changes in the aerobic oxidative capacities. With both patterns of stimulation, the effect appears most pronounced within the type II B fibre population, whose enzyme activity approaches that seen in normal type II A fibres by the end of the stimulation period (14 days). However, these fibres display unaltered properties of their histochemically determined myofibrillar ATPase. Much more prolonged periods of stimulation have been reported to alter the myosin characteristics of the fibres (Sréter, Gergely, Salmons & Romanul, 1973; Pette, Müller, Leisner & Vrbová, 1976; Hudlická, Tyler, Srihari, Heilig & Pette, 1982). Metabolic properties and characteristics of the contractile system follow different time courses during the induced fibre transformation.

The mutability of enzyme activity measured in these fibres after varied periods of electrical stimulation emphasizes their adaptability to meet the changed metabolic requirements imposed by stimulation. The effects of both patterns of stimulation were most pronounced in the type II B fibre population. This population includes the majority of the low oxidative fibres which are normally recruited for relatively brief periods during heavy exercise. The capillary supply of these fibres has been shown to be less than half that to the more highly oxidative fibres within similar muscles (Gray & Renkin, 1978). The stimulation regimes used during this study, however, activate all motor units of the muscle for prolonged periods – considerably in excess of the activity imposed even during heavy exercise. Previous studies (Hudlická & Tyler, 1980; Hudlická, Tyler & Aitman, 1980) have demonstrated similar increases in capillary density after 14 days of either stimulation regime. It was also reported, however, that onset of capillary density increase occurred later in muscles stimulated at 40 Hz than in those stimulated at 10 Hz. It is interesting to note in connexion with this finding, the different time courses of SDH increase in type II B fibres with these two stimulation patterns. Previous studies using qualitative enzyme histochemistry have failed to resolve differences at this time between the changing SDH activities. It seems that, when the changes are examined more closely using the present technique, there is in fact agreement between the increases in SDH activity and in capillary density within these muscles.

The two stimulation patterns applied in this study provide identical numbers of stimuli per hour – only the time-distribution is different. Why is the increase in SDH activity more gradual and later in onset when the stimuli are applied in 5 sec trains at higher frequency than when continuously applied? Observation of rabbits during the period of stimulation often reveals the resulting contraction of the muscle to be at least partially isotonic in nature, with extension of the digits and elevation of the paw. During 10 Hz stimulation, single isotonic twitches are seen, while 40 Hz produces a partially fused tetanic contraction with slight relaxation between individual stimuli. Since external work done by the muscle is the product of contractile force and the distance moved, it is possible that, although the maximum force is higher, the work output within 40 Hz contractions might be lower than that during 10 Hz twitches, with correspondingly less requirement for energy from catabolism.

It is interesting to speculate on the connexion between increases seen in oxidative enzymes and stimulation of capillary growth around the IIB fibres. If these fibres are forced into activity for prolonged periods by stimulation they may well become ischaemic. Accumulation of metabolites, or local hypoxia (Hudlická & Schroeder, 1978) may act as the stimulus for capillary growth initiation and for triggering the increase in oxidative activity. Activity of oxidative enzymes has previously been shown to increase in muscles with decreased blood supply and low oxygen partial pressure (Holm, Björntorp & Scherstén, 1972; Bylund-Fellenius, Walker, Elander, Holm, Holm & Scherstén, 1981).

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