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AFTER-EFFECTS OF REPETITIVE STIMULATION AT LOW FREQUENCY ON FAST-CONTRACTING MOTOR UNITS OF CAT MUSCLE

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SUMMARY

1. Twitch and tetanic contractions of single motor units of the cat peroneus tertius muscle were examined after application of a test allowing their identification as either fast fatigable (f.f.) or fast fatigue-resistant (f.r.) or fast intermediate (f.i.) or slow units as established by Burke, Levine, Tsairis & Zajac (1973).

2. The test was found to leave two kinds of after-effects in f.f., f.r. and f.i. units whereas it did not affect slow units. The first after-effect was an early and brief potentiation of twitch tension occurring in all f.r. and f.i. units and in most f.f. units.

3. The second after-effect, termed 'delayed fatigue', was a prolonged depression of tension output, that developed slowly following the early potentiation in all f.f. and f.i. units and more than half of the f.r. units. One hour after the test, unfused tetanic contractions elicited by 20–40/sec stimulation were deeply depressed in motor units that had been left without stimulation since the end of the test. Recovery took place in 3–5 hr.

4. Motor units affected by delayed fatigue could nevertheless be made to develop nearly normal tension by gradual build-up upon prolonged stimulation at 30–40/sec.

5. Maximal tetanic contractions elicited by 200/sec stimulation were much less depressed during delayed fatigue than unfused tetanic contractions.

6. These observations suggest that contractile mechanisms were not impaired by delayed fatigue. Since absence of change in muscle action potential indicated that excitation of muscle fibres was not affected either, delayed fatigue might be due to a temporary failure of excitation–contraction coupling.

INTRODUCTION

Repetitive muscle contraction may cause a type of fatigue manifested by a reduction of tension output, with no apparent alteration of muscle action potentials or change in contractile capacity. Studies made *in vitro* on single muscle fibres indicate that the site of this fatigue is at some step of excitation–contraction coupling (Eberstein & Sandow, 1963; Grabowski, Lobsiger & Lüttgau, 1972). Similarly, in man, muscular fatigue of long duration following intense exercise has been attributed to a deficiency of activation processes (Edwards, Hill, Jones & Merton, 1977). In rat motor units, the prolonged depression of twitch tension observed after a period of low-frequency stimulation might also result from 'fatigue' of excitation–contraction coupling (Kugelberg & Lindergren, 1979).

The present paper reports observations suggesting that a comparable fatigue occurs in fast-contracting motor units of cat muscle as an after-effect of a test used to identify their physiological type as either fast fatigable (type f.f.) or fast fatigue-resistant (type f.r.) or fast intermediate (type f.i.). During the test, as originally established by Burke, Levine, Tsairis & Zajac (1973), trains of stimulation at 40/sec lasting 330 msec are repeated every second for 2 min and the fatigue index is defined as the ratio of tension developed during the last tetanus to that developed during the first. Type f.f. units have fatigue indices below 0·25 and type f.r. units have indices above 0·75, whereas those of type f.i. units range between 0·25 and 0·75 (Burke *et al.* 1973). Most muscles are heterogeneous, containing these three types of motor units in various proportions, as well as a fourth type, made of slow-contracting motor units which are uniformly resistant to fatigue (see the recent review by Burke, 1981).

In the course of previous investigations, for which it was necessary to identify the type of motor units under study (Jami, Murthy, Petit & Zytnicki, 1983*a*), we observed that measurement of fatigue indices posed a problem for the continuation of the experiment because the test left after-effects in fast-contracting motor units, whereas it did not affect slow units. In the present study we systematically examined these after-effects and found that they were of two kinds. First, an early transient potentiation of twitch tension occurred in all f.r. and in most f.f. units. In this latter case, the occurrence of potentiation was surprising since, towards the end of a fatigue test, most f.f. units appeared unable to develop any tension at all.

The second after-effect was a prolonged depression of twitch tension and of unfused tetanic contraction that developed slowly following the early potentiation and was therefore termed 'delayed fatigue'. It affected not only the f.f. but also some f.r. units, which again was unexpected since these units, by definition, had resisted the fatigue test.

Preliminary accounts of the results have been presented (Jami, Murthy, Petit & Zytnicki, 1981, 1983*b*).

METHODS

Adult cats (2–3·5 kg) were anaesthetized with an initial intraperitoneal dose of pentobarbitone sodium (Nembutal, Abbott Laboratories, 45 mg/kg); the level of anaesthesia was subsequently maintained as necessary by intravenous injections of the drug. Blood pressure was monitored and body temperature was maintained at 38 °C.

The muscle used was the peroneus tertius (also termed peroneus digiti quinti) a small heterogeneous muscle of the leg, consisting of about thirty motor units (Barker, Stacey & Adal, 1970) among which the four types are distributed as follows: 30 % f.f., 9 % f.i., 39 % f.r. and 22 % slow (Jami, Murthy, Petit & Zytnicki, 1982). In each experiment it was possible to examine several motor units of each type, applying an identical regime of stimulation to each unit.

Details of the methods for isolating the axons of single motor units, recording their nerve and muscle action potentials and measuring their isometric tension, have already been fully described (Jami *et al.* 1982). In outline, single motor axons were isolated by splitting ventral roots until stimulation of a filament elicited an all-or-none muscle action potential of the motor unit. Stimulation rates of 20–30/sec were used during this procedure, care being taken to reduce as far as possible the amount of stimulation applied to each motor unit. In addition, all the motor units prepared in an experiment (usually ten to twelve units, i.e. about one-third of the estimated total population of the muscle) were allowed at least 1 hr to recover from possible fatigue caused by repeated stimulation during the isolation procedure. The physiological characteristics of each unit were then recorded, namely twitch tension and contraction time, tension developed during unfused tetanic contraction elicited by stimulation at 40/sec, maximal tetanic tension developed during stimulation at 200/sec and fatigue index. These data allowed identification of motor unit types, as previously reported in details (Jami *et al.* 1982).

The after-effects of the fatigue test were investigated on single twitches, on responses to stimulation at 40/sec and on responses to stimulation at 200/sec producing the maximal tetanic tension. Fast-contracting motor units of peroneus tertius usually displayed unfused contractions when stimulated at 40/sec, while the tension of slow units appeared fused. The tension responses of f.f., f.i. and f.r. units to stimulation at 40/sec showed variable shapes (see responses recorded before the fatigue test in Figs. 1, 2, 4 and 7–9). In addition, the responses of a given unit to successive stimulation did not always have identical shapes. However, after about 3 sec of stimulation the level of tension appeared roughly constant for a non-fatigued unit (see Fig. 2 in Jami *et al.* 1982). For this reason we systematically measured the tension of the examined motor units at the end of 3 sec periods of stimulation at 40/sec applied before the fatigue test and at different times after the test. Values found in measurements made after the test were expressed as percentages of control values measured before the test.

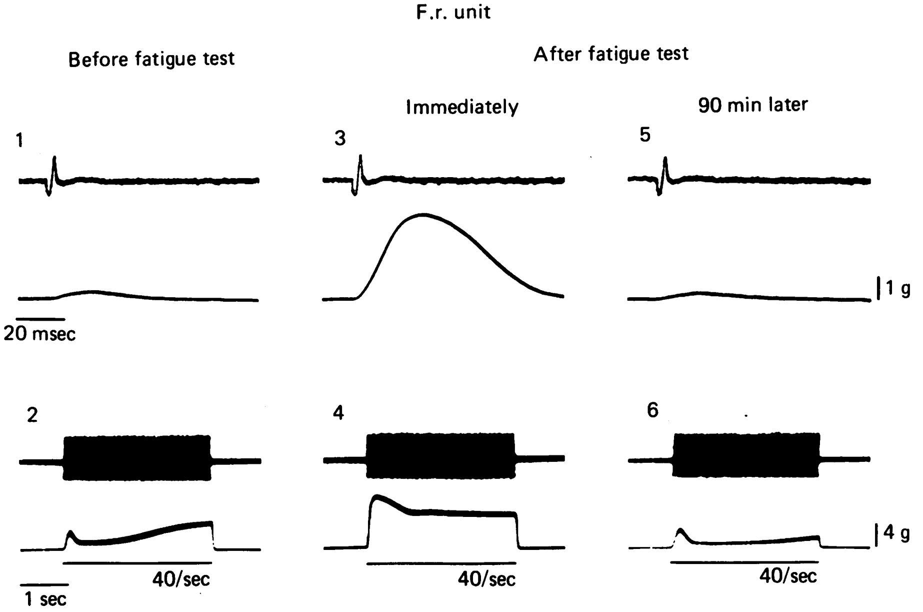

Fig. 1. Early potentiation and delayed depression of tension in an f.r. motor unit after a fatigue test. 1, 3, 5, twitch responses to single shock. 2, 4, 6, responses to stimulation at 40/sec lasting 3 sec. Simultaneous records of muscle action potentials (upper traces) and isometric tension (lower traces).

RESULTS

All the examined f.r. units and most f.f. units displayed early potentiation after a fatigue test, whereas delayed fatigue affected all f.f. units and only some f.r. units. Motor units of the f.i. type behaved similarly to either f.f. or f.r. units. Among slow units, very few showed slight potentiation of tension consecutive to the test and none showed signs of delayed fatigue.

Figs. 1 and 2 illustrate examples of the early potentiation and delayed depression of tension responses in an f.r. and an f.f. unit. It may be seen that size and shape of muscle action potentials of both units remained constant throughout. Within 1 min after the fatigue test, the twitch tension of the f.r. unit (Fig. 1) exhibited a 10-fold increase (record 3) while in the response to 40/sec stimulation (record 4), the initial peak and the subsequent plateau were both higher than before the test (record 2). The unit was then left without any stimulation for 90 min and, when tested again after this interval, the twitch was smaller (record 5) than before the fatigue test (record 1) and the tension at the end of a 3 sec period of 40/sec stimulation (record 6) was less than 50 % of control (record 2).

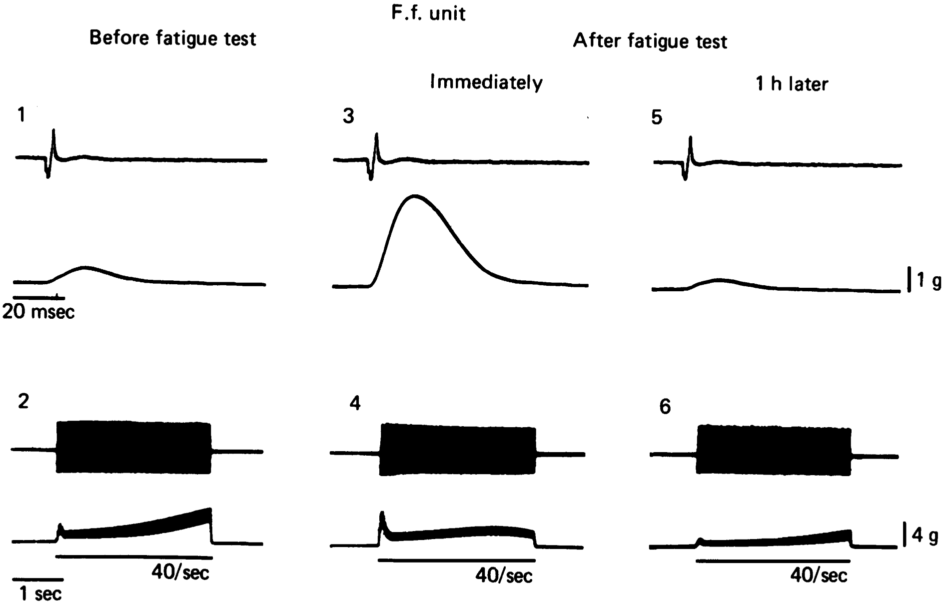

Fig. 2. Early potentiation and delayed depression of tension in an f.f. motor unit after a fatigue test. Same arrangements as in Fig. 1. Both units are from the same experiment.

The f.f. unit of Fig. 2 developed 6 times more twitch tension after the end of the test than before (compare records 1 and 3) although its tension output had decreased by 95 % during the fatigue test (not illustrated). After the test the response to 40/sec stimulation of this unit had a larger initial peak than the pre-fatigue response but, following the sag, tension increased only slightly and showed a decline by the end of the 3 sec period of stimulation (compare records 2 and 4). One hour later both responses (records 5 and 6) were smaller than controls although the unit had been left 'to rest' in the interval.

Reduction of tension output from motor units that have not been stimulated for 60–90 min raises the question of possible deterioration in the condition of the preparation. But after a further delay of 3–5 hr, all the fatigued units tended to recover a normal tension response (see Figs. 5 and 10 below), which would not be expected in a deteriorated preparation. Moreover, the sample of motor units examined in any given experiment always included slow units and some f.r. units that did not show delayed fatigue although they were submitted to the same regime of stimulation as f.r., f.i. and f.f. units in which delayed fatigue did develop. Finally, in five experiments, motor units of all types were repeatedly tested at fixed intervals of 1 hr during 3–5 hr and depression was never seen to occur before application of the fatigue test. Altogether these observations support the view that delayed fatigue was a genuine after-effect of the test and not a sign of failure in the preparation.

Early potentiation

Enhancement of twitch tension after a fatigue test was studied in twenty-six f.r. and twenty-eight f.f. units from six experiments. In f.r. units, the ratio of twitch tension measured shortly after the test to that of control responses measured before had a mean value of 4·9 ± 3·8 (range 1·1–15·3). Among the twenty-eight f.f. units, twenty-two displayed early potentiation of twitch tension, in two units there was no change in twitch tension after the test and in four others there was a reduction. In the total f.f. sample, the ratio of twitch tension measured after the test to that measured before had a mean value of 2 ± 1·3 (range 0·3–6·4), i.e. less than half the value found for f.r. units. Lengthening of contraction and half-relaxation times regularly accompanied tension potentiation in both types of units.

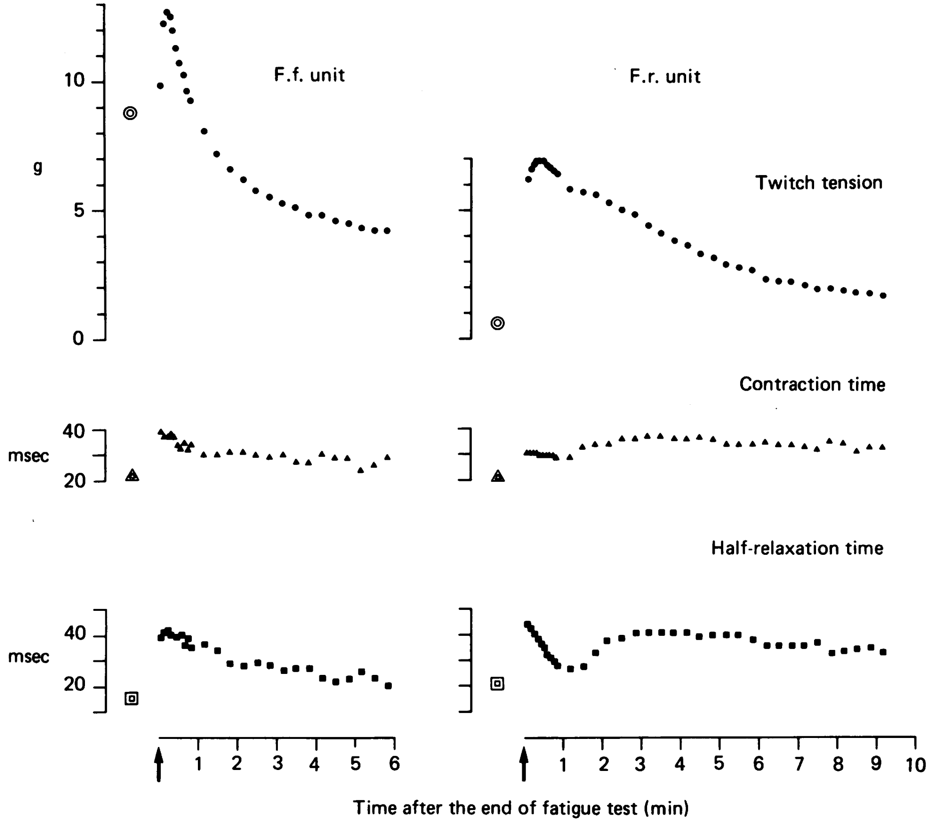

Fig. 3. Time course of early potentiation. Two motor units from the same experiment. Open symbols are twitch parameters before the fatigue test. Arrows on time scale mark the end of fatigue test. Stimulation applied every 5 sec during the first minute and every 20 sec thereafter.

The time course of decay of early potentiation revealed further differences between f.f. and f.r. units. It was explored by recording single twitches every 5 sec during the first minute after the end of fatigue test and every 20 sec in the following 5–10 min. Fig. 3 shows that in both types of unit twitch tension continued to increase in the responses to the first 3–4 stimuli after the test. Twitch tension of f.f. units then diminished rapidly, so that after 2–3 min it was already well below the pre-fatigue control value. During this rapid drop in tension the contraction and half-relaxation times shortened more slowly, recovering their initial values within 6–8 min. In f.r. units, potentiation had a slower decline, and restoration of initial twitch tension usually took place within 10–15 min. Contraction and half-relaxation times of these units were increased at the end of the fatigue test, and after an initial rapid decrease they lengthened again (Fig. 3) and recovered their pre-fatigue value with a longer delay.

Early potentiation of the tension response to 40/sec stimulation (Fig. 1, record 4) was observed in thirty-two of thirty-four f.r. units examined in seven experiments. Its time course was roughly similar to that of twitch potentiation. No particular relation prevailed between resistance to fatigue (as expressed by the fatigue index) and potentiation. Neither was there any correlation between early potentiation and delayed fatigue.

Since early potentiation decayed very quickly in f.f. units, the response of these units to 40/sec stimulation recorded shortly (i.e. 30 sec–3 min) after the fatigue test rarely showed persistence of potentiation during the tetanus. Of thirty-one f.f. units examined in seven experiments, only four displayed early potentiation of response during the whole of a 40/sec tetanus, while three gave responses similar to that shown in Fig. 2 (record 4). All the others appeared unable to sustain even a reduced contraction for 3 sec, as their tension output was seen to collapse before the end of stimulation (see two examples in Figs. 4 and 7), although their muscle action potential remained normal.

Time course of delayed fatigue

The development of delayed fatigue was explored by repeated stimulation applied at intervals of 10–15 min starting 30 sec after the end of the test. Fig. 4 shows that f.f. and f.r. units followed different time courses: in f.f. units the depression of tension seemed to progress slowly and the minimum responses was usually observed 1 hr after the test, whereas in f.r. units most of the fall occurred within 10–20 min and only small fluctuations of tension were seen later on.

Recovery took several hours. Fig. 5 illustrates an experiment in which motor units were examined at 1 hr intervals during the 2 hr before and 3–4 hr after the fatigue test (marked by arrows in Fig. 5). Before the test, motor units of all types showed in their responses fluctuations of tension that did not exceed 10 % of control. They were not considered significant and consequently a depression of tension inferior to 10 % observed after the test was not taken as a sign of delayed fatigue.

Occasionally an f.f. unit might display up to 20 % increase of tension before the test (see one example in Fig. 5), probably due to recovery from fatigue unintentionally produced during isolation of the unit. A motor unit could receive more or less stimulation, depending on whether or not the isolation of its axon required repeated splitting of ventral root filaments, entailing repeated stimulation of each filament. Although the rate was kept at 20–30/sec, some of the most fatigable units were quite sensitive to such stimulation (Kugelberg & Lindergren, 1979). On an *a priori* estimate, it was considered sufficient to allow 1 hr of rest for each motor unit after isolation of its axon (see Methods), but in several instances this time proved too short for a full recovery.

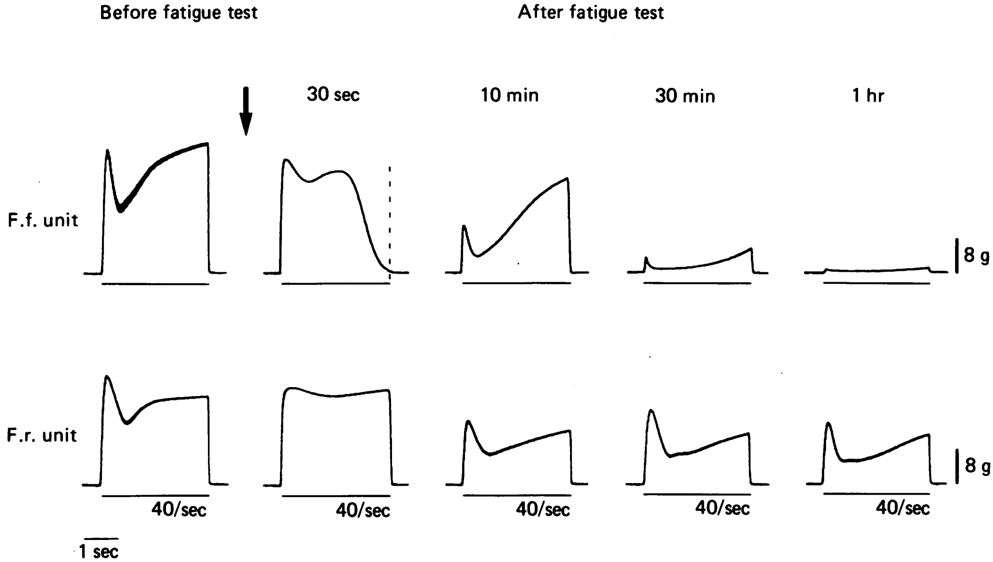

Fig. 4. Evolution of delayed fatigue in f.f. and f.r. motor units. Records of isometric tension. Muscle action potentials not shown since they did not vary. The vertical arrow represents the fatigue test. Time elapsed after the end of test is indicated for each record. On the f.f. unit response recorded after 30 sec the vertical dashed line marks the end of stimulation. Both units were from the same experiment. Fatigue indices, 0·06 and 0·84 for f.f. and f.r. units respectively.

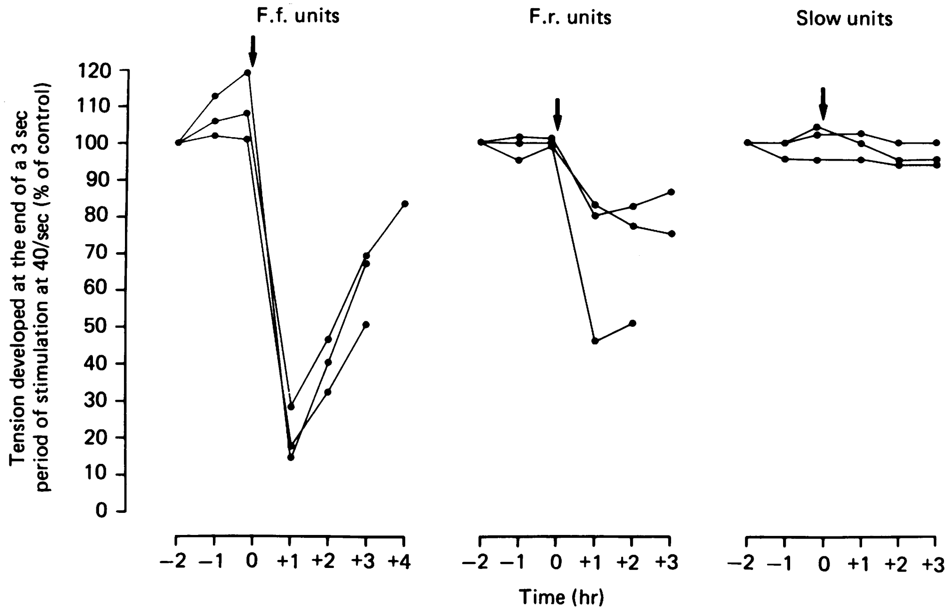

Fig. 5. Absence of tension depression previous to fatigue test and time course of recovery from delayed fatigue. Nine motor units from the same experiment. Control responses (time −2 hr) were recorded 1 hr after isolation of each motor unit. Arrows mark application of fatigue test after the response recorded at time zero.

Most f.f. and f.r. units recovered 70–90 % of their control tension within 3–4 hr (Fig. 5, see also Fig. 10). The time course of recovery was independent of the degree of depression and often slower for f.r. than for f.f. units.

As the stability of slow unit responses was found to be a good test of the condition of the preparation, a few slow units were systematically examined in each experiment, and it was verified at regular intervals that their tension output did not vary by more than 10 % (Fig. 5).

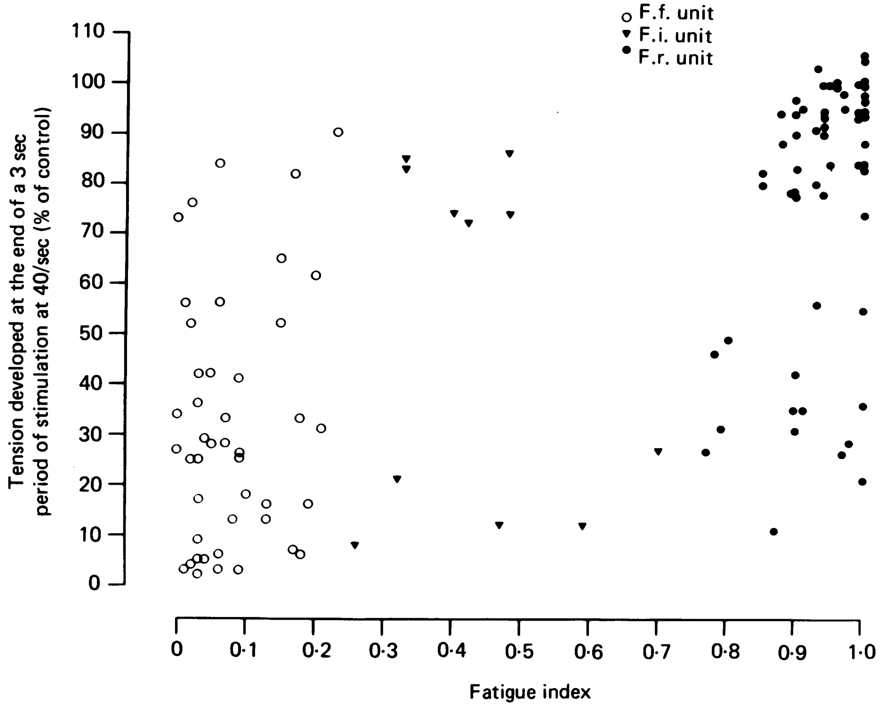

Fig. 6. Absence of relation between fatigue index and delayed fatigue. Control responses were recorded before application of the fatigue test. The percentage of remaining tension in responses recorded 60–90 min after the test provides an estimate of the degree of delayed fatigue.

Absence of correlation between fatigue index and delayed fatigue

Fig. 6 shows the percentages of remaining tension measured at the end of 3 sec periods of 40/sec stimulation applied 60–90 min after the fatigue test for 115 fast-contracting motor units in fourteen experiments. For each unit the percentage of remaining tension was plotted against fatigue index. The sample included forty-four f.f., thirteen f.i., and fifty-eight f.r. units, and also thirty-one slow units that have been omitted from the plot. Motor units in which delayed fatigue caused the deepest depression of tension were mostly f.f. units with low fatigue indices whereas motor units insensitive to delayed fatigue were found among f.r. units with high indices. Apart from this general trend, no clear correlation emerged between the resistance exhibited by a motor unit during the fatigue test and the degree of delayed fatigue (Fig. 6).

In the f.f. group there was a wide range of remaining tensions (2–90 %) with three quarters of the sample below 50 % and a mean value of 32 ± 25 %. In the f.r. group delayed fatigue affected more than half of the examined units, fifteen in the 70–90 % range and fifteen in the 11–58 % range. The mean value of remaining tension in the total f.r. sample was 77 ± 26 %.

Enhancement of tension during prolonged stimulation

For 10–15 min after the fatigue test, most f.f. units appeared unable to sustain a contraction for 3 sec and prolongation of stimulation did not result in recovery of tension. Afterwards, when delayed fatigue had developed, prolonged stimulation could elicit a gradual increase of tension (Fig. 7) such that, if stimulation was continued long enough, tension nearly reached the pre-fatigue level (Figs. 8 and 9). Subsequent decline of tension (Fig. 8) was not necessarily due to delayed fatigue since, in non-fatigued f.f. units stimulated at 30–40/sec, we repeatedly observed that tension could not be maintained beyond 6–10 sec (Fig. 9). Enhancement of tension during prolonged stimulation also occurred in f.r. units affected by delayed fatigue, and the increase in tension was usually faster in f.r. than in f.f. units (Fig. 7).

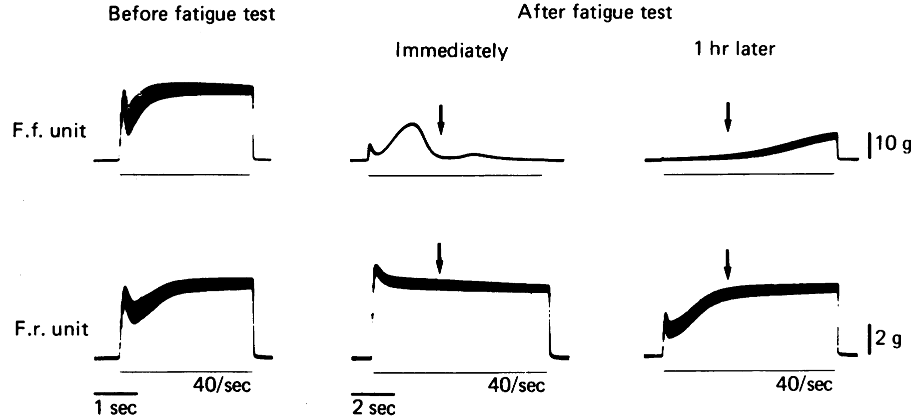

Fig. 7. Responses to prolonged stimulation applied immediately after fatigue test and 1 hr later. Both motor units are from the same experiment. Records of isometric tension. Muscle action potentials not shown since they did not change. Duration of stimulation before the test 3 sec, after the test 8 sec. Arrows point to the tension developed after 3 sec of stimulation. Fatigue indices, 0·08 and 0·87 for f.f. and f.r. units respectively.

Fatigued motor units in which tension was enhanced during prolonged stimulation did not have sufficient lengthening of contraction or relaxation times to account for the build-up of tension. In fact the contraction and half-relaxation times of their twitches (measured before application of prolonged stimulation) were often shorter during delayed fatigue than before the test.

In order to look for possible changes of compliance, indicating modifications in the mechanical properties of the muscle, length–tension relations were examined in whole muscles, before and after a fatigue test applied through stimulation of the nerve. Small fluctuations were observed in muscle stiffness but they were too slight to account for either delayed fatigue or enhancement of tension during prolonged stimulation (J. Petit & D. Zytnicki, unpublished observations).

Gradual enhancement of contraction during prolonged stimulation could also take place in non-isometric conditions when the muscle was allowed to shorten, as shown by Fig. 9. This observation ruled out the possibility that increase of tension during prolonged stimulation might be due to progressive stretching of series elastic components.

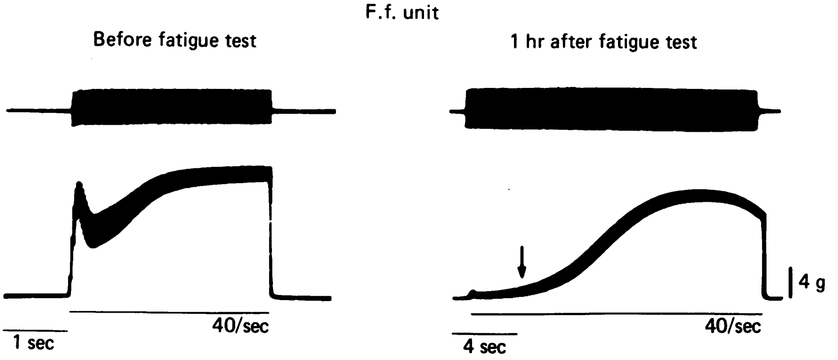

Fig. 8. Enhancement of tension in response to prolonged stimulation applied 1 hr after fatigue test. Muscle action potentials (upper trace) recorded simultaneously with isometric tension (lower trace). Duration of stimulation before the test 3 sec, after the test 18 sec. Arrow points to tension developed after 3 sec of stimulation. Fatigue index of this unit, 0·17.

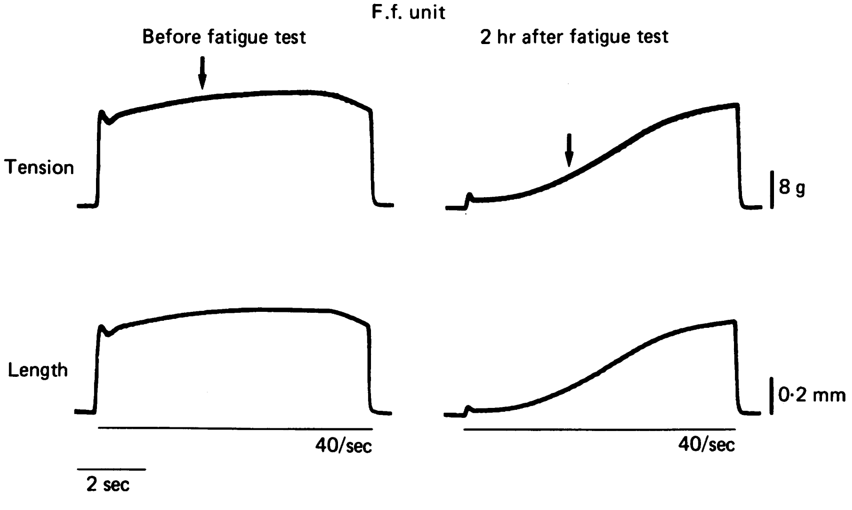

Fig. 9. Enhancement of contraction during prolonged stimulation in an f.f. motor unit. Tension is only indicative since recorded in non-isometric conditions. Length recorded with a differential transformer bearing the force transducer (constructed in the laboratory by one of us, J.P.). Upward deflexion represents shortening. Duration of stimulation 8 sec. Arrows point to tension developed after 3 sec.

In non-fatigued motor units, it was previously observed that a period of 3 sec stimulation at 40/sec could elicit a significant potentiation of twitch tension (see Fig. 5 in Jami *et al.* 1982). Similar observations were made in motor units affected by delayed fatigue. Although a period of 3 sec stimulation at 40/sec produced low tension responses from these units it was quite efficient in eliciting potentiation of their twitch response: after such stimulation, the twitch tension of deeply depressed units was seen to recover, or occasionally exceed, its pre-fatigue value. Also, fatigued motor units always developed more tension in the second than in the first response when a short period of stimulation was repeated at brief intervals.

Manifestations of delayed fatigue at different stimulation frequencies

It was not possible to test many different stimulation frequencies on a single motor unit because repeated stimulation would have reinforced fatigue and interfered with recovery. One limited sample (fifteen units) was tested with 20/sec and another one (fourteen units) with 60/sec stimulation. There was not much difference between the manifestations of delayed fatigue observed at a rate of 20/sec and those observed at 40/sec. But when stimulation at 60/sec was used the depression of tension was distinctly less than with 40/sec. In maximal tetanic contractions elicited by 200/sec stimulation, the depression of tension never went below 80 % of control even for the most fatigable units. Fig. 10 illustrates the parallel evolution of responses to 40/sec and 200/sec stimulation in four f.f. units, showing that tension was much less depressed in fused than in infused contraction.

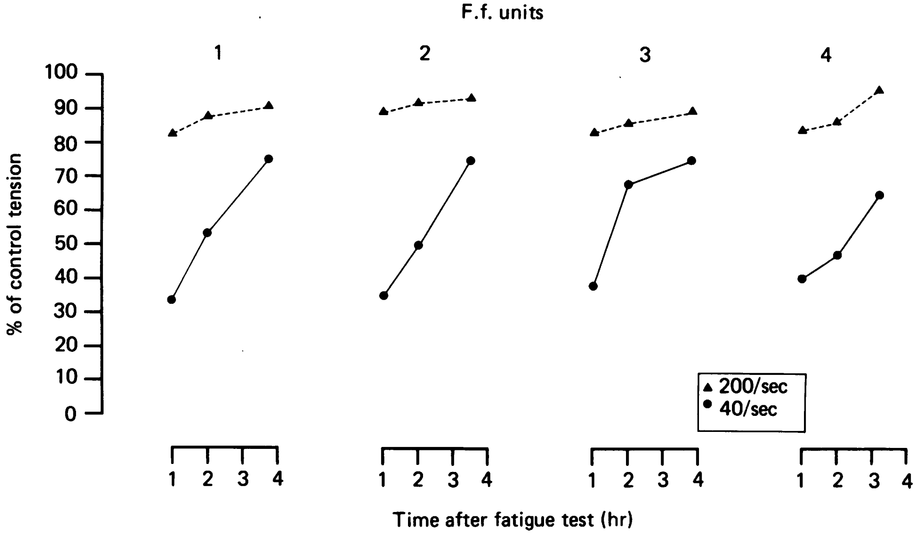

Fig. 10. Differences between manifestations of delayed fatigue in unfused contraction and in maximal tetanus. Four f.f. units from the same experiment. Stimulation at 40/sec was applied for 3 sec, followed by stimulation at 200/sec for 0·5 sec. Isometric tensions were measured at the end of each period of stimulation. Control responses were recorded before fatigue test as in Fig. 5.

The weak depression of maximal tetanic responses suggested that in motor units affected by delayed fatigue the contractile mechanism was still largely available for tension production.

DISCUSSION

The fatigue test produces three different effects on fast-contracting motor units. The first effect, on the tension developed during a 330 msec tetanus at 40/sec, is well known (Burke *et al.* 1973). It is visible during the test and allows assessment of resistance to fatigue in the different types of units. The two other effects occur after the test. One consists of an early and brief potentiation of contraction. This is followed by a delayed and long lasting depression of tension, developing in f.f. units and in some of the f.r. units, even in the absence of any stimulation subsequent to the test. None of these effects involved any change in muscle action potentials, indicating there is neither failure of neuromuscular transmission nor impairment in propagation of depolarization in muscle fibres.

Co-existence of early potentiation and fatigue

Since potentiation of twitch tension usually results from repetitive stimulation, it is easy to conceive that the regime of stimulation used in the fatigue test may cause such a potentiation in f.r. units (Proske & Waite, 1974). But even these resistant units displayed some early signs of fatigue since during the decay of potentiation their relaxation remained slower than normal, which is considered as a manifestation of fatigue (Edwards, Hill & Jones, 1975).

Clearly the fatigue test elicited less potentiation in f.f. than in f.r. units. On the contrary, a previous study showed that f.f. units were able to potentiate more and faster than f.r. units when submitted to identical regimes of non-fatiguing stimulation (Jami *et al.* 1982). The difference observed after the fatigue test, as well as the rapid decay of early potentiation in f.f. units, suggests that in these units the effects of fatigue interfered with potentiation (see Vergara, Rapoport & Nassar-Gentina, 1977; Nassar-Gentina, Passoneau, Vergara & Rapoport, 1978). Krarup (1981) recently reported that certain patterns of stimulation could produce fatigue concomitant with potentiation in the fast twitch extensor digitorum longus muscle of the rat. In another muscle of the rat, tibialis anterior, Kugelberg & Lindergren (1979) observed twitch potentiation in fatigue-sensitive motor units after a continuous period of stimulation that fatigued not only the tension response but the neuromuscular transmission as well.

Potentiation of twitch contraction is usually explained by an increased efficiency of excitation–contraction coupling, resulting in an increased activation of contractile proteins (see the review by Close, 1972). Occurrence of potentiation (which is a manifestation of enhanced activation) in f.f. units after a sequence of stimulation producing fatigue of contraction, appears paradoxical. The fact that repetitive stimulation enhances activation at the same time as it elicits fatigue of contraction seems to imply that this fatigue does not impinge on any of the successive steps of excitation–contraction coupling leading to activation. If this assumption were true, it would follow that the site of the fatigue observed in f.f. units during the test might be at a step beyond activation, i.e. that fatigue might directly affect the interaction of contractile proteins. Such a type of fatigue has never been demonstrated so far. An alternative explanation would imply that in an f.f. unit fatigue and enhancement of activation occur simultaneously during the test, each affecting a different step of excitation–contraction coupling.

Whatever may be the case, it is obvious that fatigue is predominant during the test since the tension output is seen to decrease. Then, within a very short delay after cessation of stimulation at the end of the test, some recovery should take place in order to allow manifestation of enhanced activation (i.e. potentiated twitch tension). But the rapid decay of potentiation indicates that recovery is only transient, and after disappearance of potentiation fatigue is again predominant.

Two types of fatigue produced by the test

The absence of correlation between fatigue index and delayed fatigue suggests that the latter is not merely the continuation of the fatigue observed during the test but might reflect some other event, consequent to the test, with a slower time course. This seems likely for f.r. units in which delayed fatigue developed although they remained insensitive to the test itself, such as the unit of Fig. 1, whose fatigue index was 1 (see other examples in Fig. 6).

Occurrence of delayed fatigue in some but not all f.r. units may be related to the wide range of resistance to fatigue that appears among these units when different patterns of stimulation are used as fatigue tests (Stephens, Gerlach, Reinking & Stuart, 1973; Proske & Waite, 1976). This wide range corresponds to the continuous spectrum of oxidative enzyme activity observed among muscle fibres in histochemical investigations (Burke *et al.* 1973; Kugelberg & Lindergren, 1979).

Three facts further support the view that delayed fatigue is different from the fatigue observed during the test in f.f. units, namely, (i) the interposition of early potentiation between the test and delayed fatigue (Fig. 2); (ii) the slow development of delayed fatigue after the end of the test (Fig. 4); (iii) the contrast between the inability of f.f. units to sustain a contraction immediately after the test (Figs. 4 and 7) and their ability to gradually increase their tension output upon prolonged stimulation applied 1 or 2 hr after the test (Figs. 8 and 9).

The metabolic correlates of delayed fatigue and the reason for its slow development are not known. In man, energy stores (in the form of phosphocreatine and ATP) are depleted after exercise but their recovery is almost complete in 60 min (Edwards *et al.* 1977). In fatigued frog muscle fibres, accumulation of H^+ (in the form of lactate) was proposed to account for inhibition of excitation–contraction coupling, but this inhibitory effect has not been directly tested (Nassar-Gentina *et al.* 1978).

Site of delayed fatigue

Delayed fatigue was much more marked during stimulation at low frequencies (20–40/sec) than during fused tetanic contraction (cf. Edwards *et al.* 1977 and also Fig. 11 in Kugelberg & Lindergren, 1979). However, upon repetitive stimulation at low frequency the tension developed by deeply depressed motor units gradually increased almost up to pre-fatigue level (Figs. 8 and 9). These observations suggest that contractile capability was not impaired in the affected muscle fibres. Then, if persistence of normal muscle action potentials is accepted as an indication that excitation propagates normally in muscle fibres, it follows that the most likely site for delayed fatigue would be at some step in excitation–contraction coupling. Dissociation between electromyogram and force output is well known to occur in man, upon attempts to sustain maximal voluntary contractions (Merton, 1954). It has also been observed during recovery after prolonged fatigue (Stephens & Taylor, 1972).

Assuming that in fatigued muscle the 'amount of activation' triggered by a single action potential is weaker than in non-fatigued muscle, repetition of excitation might build up some 'cumulative' activation which could gradually recover its pre-fatigue efficiency. Likewise, in motor units depressed by dantrolene sodium, a drug known to uncouple excitation from contraction, repetitive stimulation at 40/sec elicited a gradual recovery of tension back to its pre-drug level (see Fig. 7 in Jami *et al.* 1983*a*).

Another possibility would be that delayed fatigue occurs at the last step before excitation–contraction coupling, i.e. depolarization of the membrane of transverse tubules. It is not certain whether, in our experimental conditions, a block of transverse tubules depolarization would be reflected in changes of muscle action potentials.

The gradual enhancement of tension elicited in fatigued motor units by stimulation at low frequency might provide a simple mechanism of functional compensation for fatigue. In man, after an exhausting exercise the tension output of fatigued muscles, tested with low frequencies of stimulation applied during 1–2 sec, is reduced by 50 % for several hours (Edwards *et al.* 1977). However subjects experiencing such long lasting fatigue can nevertheless perform relatively efficient voluntary contractions. Since the normal firing rates of human motor units are 10–30/sec during voluntary contraction (see references in the recent review by Henneman & Mendell, 1981), gradual increase of the tension developed by fatigued motor units is likely to result from these firing rates, provided they were maintained for several seconds. Long-lasting fatigue might thus be overcome, at least partially, without the necessity of either recruiting a large number of motor units or increasing their firing rates.

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