ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Acta Neurobiol. Exp. 1996, 56: 881-887

Changes in fusion index during the fatigue test of fast motor units in the medial gastrocnemius muscle of the rat

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Abstract. Changes in the fusion of tetani were investigated in fatigue tests of 50 fast motor units of the rat medial gastrocnemius muscle. Fusion of the tetani was measured using the fusion index, being the ratio of the tension to which motor unit relaxed before the last component of tetanus to the peak tension of the last component. In both types of fast units (fast fatigable and fast resistant) the changes in the fusion index were similar to those in tetanic tension: first they increased and then decreased. However, the increase of the fusion index was longer than that of tension and the subsequent decrease in the fusion index was smaller than that of tetanic tension. Furthermore, the initial increase in the fusion index of fast fatigable motor units was greater than in the tension. The dependence of the fusion index on twitch time enables the analysis of the influence of changes in the twitch time on changes in tension of unfused tetani observed during activity of fast motor units.

Key words: motor unit, tetanic fusion, contraction, fatigue

INTRODUCTION

Changes in tension of motor unit unfused tetani are associated with changes in their fusion. In our previous paper (Celichowski and Grottel 1995) the relationship between stimulation frequency and tetanic fusion was described. In the referred paper we introduced a measure of tetanic fusion, the "fusion index", which we defined as the *a/b* ratio, where *a* is the tension to which motor unit relaxed before the last component of tetanus (the distance from the baseline to the maximal relaxation before the last component of tetanus) whereas *b* is the peak tension of the last component (Fig. 1). In an earlier paper (Celichowski 1992b) from our laboratory and in papers of other authors (Gardiner and Olha 1987, Gordon et al. 1990b), changes in tension and time course of tetani during the fatigue test of motor units were described. For fast motor units, marked changes in tension as well as in the shape of tetani were found. During these experiments, changes in fusion of tetani associated with changes in tension were observed, especially in FF (fast fatigable) type motor units. However, measurements of changes in tetanic fusion were not made. For a better understanding of the activity of motor units, especially of the mechanisms of changes in tension during fatigue, a detailed description of changes in tetanic fusion and comparison of these changes to changes in tetanic tension could be very useful. Such an analysis became the aim of the present paper. The measurements of tetani fusion and of their tension were made on records of tetani evoked during the fatigue test. The experiments were performed on motor units of the rat medial gastrocnemius muscle. The tetani of fast motor units of this muscle at a standard frequency of stimulation during the fatigue test (40 Hz) are unfused. The tetani of slow units at this frequency throughout the fatigue test are fused or nearly fused. Therefore, only fast motor units were included in the present analysis.

METHODS

Experiments were performed on female Wistar rats (average weight 310±45 g), anaesthetized with pentobarbital (30 mg/kg i.p., supplemented as required by controling the shape of pupils and pinna reflexes). The duration of one experiment amounted up to 12 h. The investigated medial gastrocnemius muscle was partly isolated while the innervation and blood vessels were left intact. The Achilles tendon was connected to the force transducer. All muscles of the hindlimb except the medial gastrocnemius were denervated. The hindlimb was immobilized with a steel clamp and an additional clamp on the sacral bone immobilized the animal. Laminectomy was performed at the level of L2-S1 segments and the ventral as well as dorsal roots were cut close to the spinal cord. The muscles and spinal cord were covered with warm paraffin oil (36-38^{o}C) and animal rectal temperature was kept in the same range. The functional isolation of single motor units was performed by teasing the L5 ventral root into very thin filaments. The muscle activity evoked during stimulation of these filaments had to be of the "all or none" type with respect both to the tension and the muscle fibre action potential (Celichowski 1992a, Kanda and Hashizume 1992). The stimulation with pulses of 0.1 ms duration and amplitude up to 0.5 V was used. The tension was recorded with an inductive force transducer under isometric conditions, while the muscle was stretched up to 100 mN passive tension. Such a stretch enables to record the highest twitch tension for a majority of motor units in the investigated muscle (C lichowski and Grottel 1992). Isolated motor units were successively stimulated: (1) with single pulses, evoking single twitches, (2) with 500 ms train of stimuli at 40 Hz, evoking unfused tetanus, (3) with 200 ms train of stimuli at 150 Hz, evoking fused tetanus, (4) during the fatigue test, with 330 ms trains of stimuli at 40 Hz, repeated every second for 4 min (Burke et al. 1973). Contractile records and electromyographic activity were photographed from the oscilloscope screen and stored on an recorder. The investigated motor units were classified as "fast" when showing a "sag" in unfused tetanus at 40 Hz stimulation, whereas others were classified as "slow" (Grottel and Celichowski 1990).

In this study only fast motor units were taken into analysis. These fast motor units were classified as

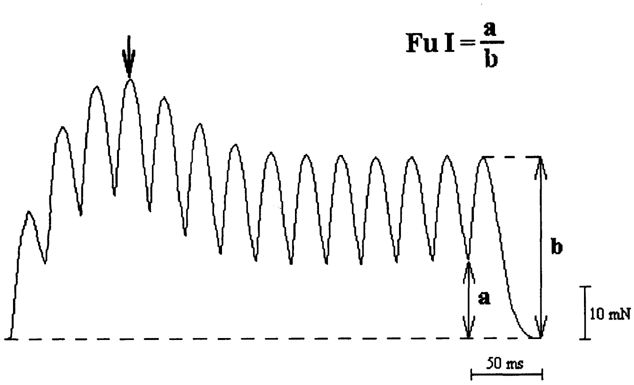

Fig. 1. Calculation of the fusion index (FuI) as *a/b* ratio for unfused tetanus of FF motor unit. Moreover, the arrow shows the position of tetanic peak (the fourth one response) within the tetanus before "sag".

FF (fast fatigable) or FR (fast resistant) according to fatigue index value, which was lower than 0.5 for FF units and higher than 0.5 for FR units (Kernell et al. 1983, Kanda et al. 1985, Grottel and Celichowski 1990). The fatigue index was expressed as the ratio of tetanic tension developed 2 min after maximal potentiation of the tetani was reached to the tension of maximally potentiated tetanus (Kernell et al. 1975, Kernell and Monster 1982). The contraction time (time between the force onset and its peak), half-relaxation time (interval between the peak force and decline to one-half of peak force) and twitch tension were all measured from single twitch records (Grottel and Celichowski 1990). In records of 40 Hz unfused tetanus before the fatigue test, the position of tetanic peak before the sag was determined (Fig. 1) (Celichowski 1992a). For tetani recorded during the fatigue test the tension (from the baseline to the peak of tetanus) and the fusion index were measured. The fusion index was expressed as the *a/b* ratio, where *a* was the tension to which motor unit relaxed before the last component of tetanus whereas *b* was the peak tension of the last component of the tetanus (Fig. 1) (Celichowski and Grottel 1995). These measures of tension and fusion index were made in the following tetani : 1st, 2nd, 5th, 10th, 20th, 30th, 40th, 50th, 60th, 90th, 120th, 150th, 180th, 210th and 240th. The fusion index was also measured in 40 Hz unfused tetanus before the fatigue test. The presented material comprise 50 fast motor units, 26 of the FF type and 24 of the FR type. The Student's *t*-test was used for statistical analysis.

RESULTS

Table I gives the mean values and the variability range of investigated properties of FF and FR motor units. The results of comparison of the measured properties for FF and FR units are also given in the

TABLE I

Mean values, their standard deviations and the ranges (in parentheses) of the contraction time (CT), the half-relaxation time (HRT), the twitch tension (TwT), the maximum tetanic tension (TetT), the fatigue index (FI), the fusion index (FuI) for 40 Hz unfused tetanus recorded before fatigue test and of the timing of peak (the number of the highest response) before sag in this tetanus (Peak), for FF and FR motor units. Below, results of Student's *t*-test for differences between values for FF and FR units; **, difference significant at $P<0.01$; *, difference significant at $P<0.05$; NS, difference non-significant

	CT	HRT (ms)	TwT (mN)	TetT (mN)	FI	FuI	Peak
FF (*n*=26)	15.2±1.4 (13.0-19.0)	11.0±1.3 (8.5-14.0)	26.51±3.1 (7.6-54.5)	137.0±69.0 (37.0-272.7)	0.14±0.10 (0.0-0.41)	0.48±0.26 (0.10-0.93)	2.6±1.2 (1-6)
FR (*n*=24)	16.5±1.8 (14.5-20.0)	12.9±2.3 (9.5-19.5)	13.6±9.9 (2.0-50.0)	78.8±39.2 (11.2-168.0)	0.74±0.12 (0.57-1.00)	0.63±0.27 (0.16-0.95)	3.8±1.5 (2-8)
Studen's *t*-test	*	**	**	**	**	NS	**

Table. Positive correlation was found between the fusion index for the 40 Hz tetanus recorded before the fatigue test and the contraction time (r=0.588, P<0.01). Moreover, there was a correlation between the fusion index and the timing of tetanic peak before sag in the tetanus (r=0.392, P<0.01).

The fusion index measured for successive tetani during the fatigue test showed changes. Figure 2 presents records of FF and FR motor unit tetani and corresponding values of the fusion index.

Average time courses of changes in tetanic tension and fusion index during the fatigue test for both types of fast motor units are shown in Fig. 3. In general, changes in both investigated tetanic parameters are similar. For FF motor units, showing stronger changes in tension (Burke et al. 1973, Gardiner and Olha 1987), greater changes in the fusion index were also observed. For these units, an increase of the fusion index by 182.6±202.8% (mean±SD) of its initial value was observed during the first 34.2±12.1 s. Then the fusion index decreased and during 120 s of the fatigue test the decrease amounted to 0.54±0.28 (64.4±35.6% of the highest value). This kind of fusion index changes was observed in 14 FF units. The remaining 12 units of this type showed additionally a decrease of the fusion index in the second tetanus as compared to the first one, and the decrease could be observed until the 5th tetanus. Then, an increase in the fusion index was observed in these units.

For the majority of FR motor units, a decrease of the fusion index in the second tetanus was initially observed (Figs. 2 and 3). The fusion index then increased during 51.5±27.0 s by 83.4±82.7% as compared to the value in the 2nd tetanus. In the following part of the fatigue test the fusion index decreased by a mean of 0.13±0.12 (16.6±16.5% of the highest value) during 120 s. The initial decrease in the fusion index (in the 2nd tetanus as compared to the 1st one) was observed in 19 out of the 24 FR motor units. The fusion index of the remaining 5 units increased from the first tetanus and then decreased after several tens of seconds.

Similarly as in some FF units, the initial decrease of the fusion index of some FR units could also be observed during up to 5 s.

The comparison of mean values of the increase in the fusion index and of its duration as well as of the decrease in the index observed during 120 s of fatigue test for both FF and FR units showed significant differences. The difference in the increase of the fusion index was significant at P<0.05 whereas

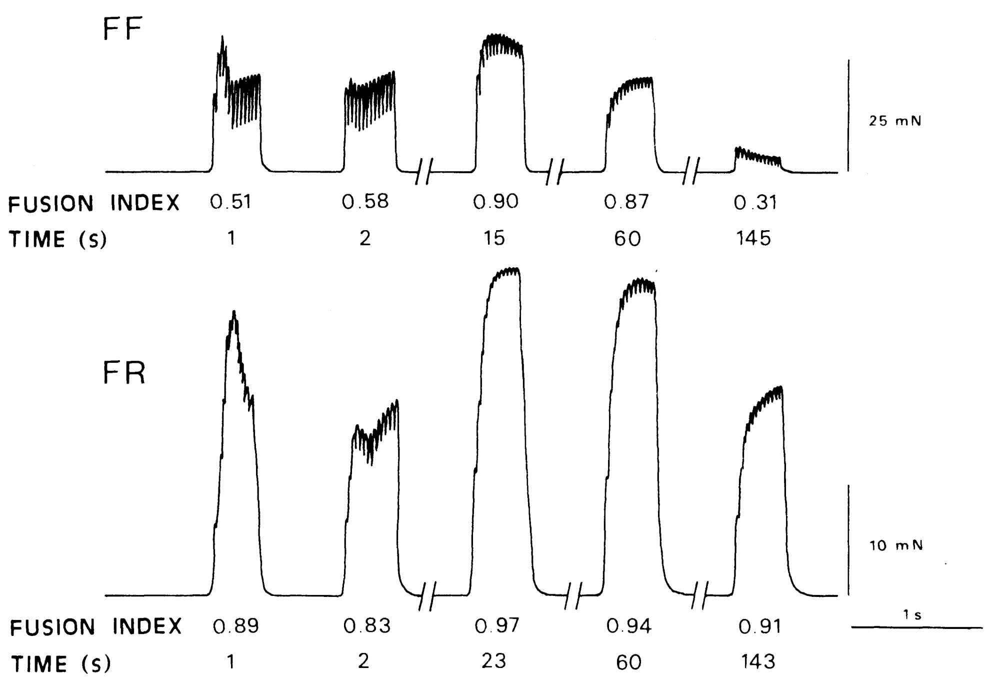

Fig. 2. Representative tetani (fragments of fatigue test) of one FF motor unit (upper records) and one FR unit (lower records). The fusion indices corresponding to these tetani as well as the time of recording (in seconds) measured from the onset of fatigue test, are given under each tetanus. The tetani recorded in 15 s (FF unit) and in 23 s (FR unit) are maximally potentiated i.e. of the highest peak amplitude.

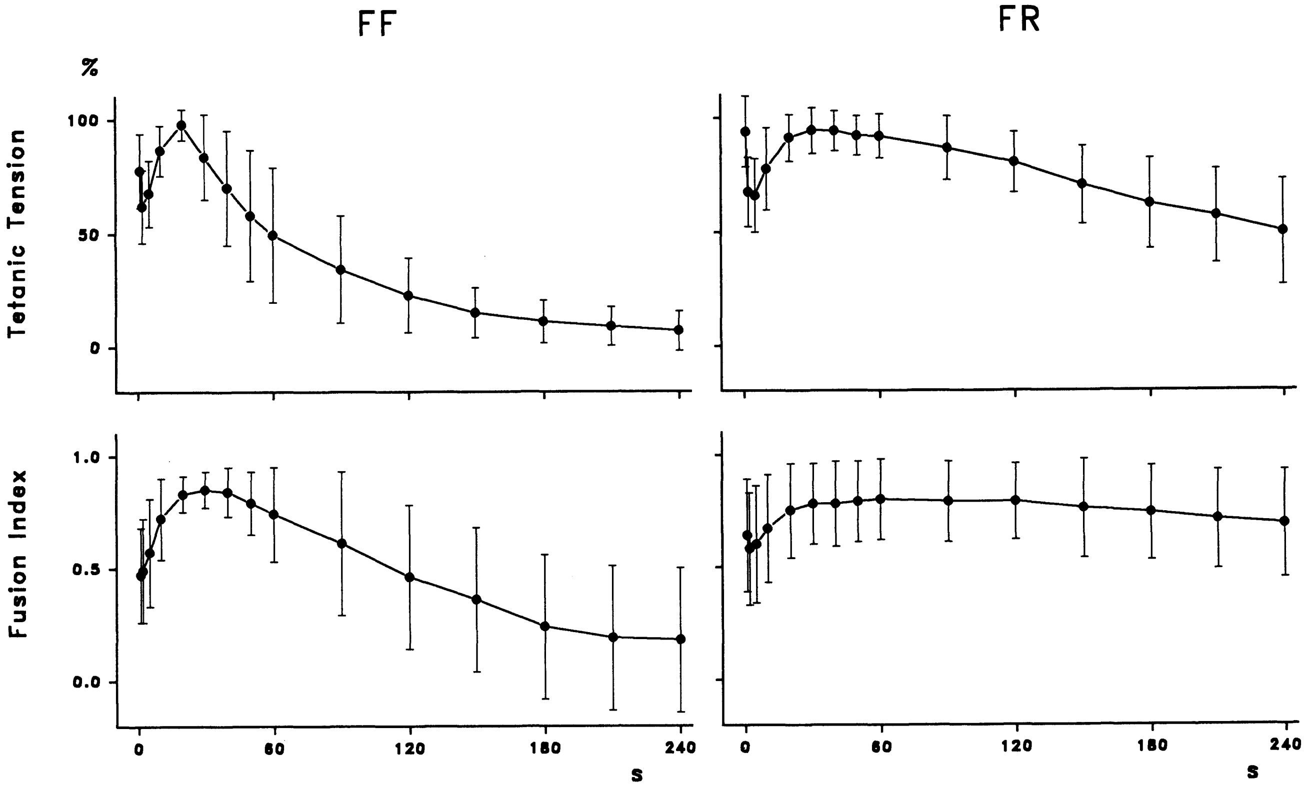

Fig. 3. Averaged changes in tension (upper plots) and in fusion index (lower plots) as a function of time during the fatigue test, for FF (left column) and FR (right column) motor units. Each point give the mean values and their standard deviations for all investigated motor units (n=26 for FF units and n=24 for FR units). For each motor unit their maximally potentiated tetanus was considered as 100% for calculations of tetanic tension (upper plots).

its duration and further decrease were different at $P<0.01$.

Measurements of tetanic tension revealed that tension of the first tetani was usually higher than that of the second ones. This phenomenon was accompanied by a clear "sag" in the first tetanus (Fig. 2) (Celichowski 1992b). For all FF and FR units the tension of further tetani became potentiated (for FF units by a mean of 75.6±47.3% in relation to tension of the 2nd tetanus, whereas for FR units - by 83.4±71.3%). The increase could be observed for FF units during 19.2±5.2 s and for FR units - during 34.5±18.7 s. Then the tension decreased and during 120 s of the fatigue test of FF units 84.7±10.5% decrease of the tension was observed whereas for FR units the decrease amounted to 25.4±12.3%.

The comparison of changes in the fusion index to changes in tetanic tension, observed during the fatigue test of both types of motor units, revealed that the initial increase of the fusion index and of tension for FR units did not differ, whereas the increase of the fusion index for FF units was higher ($P<0.05$). The increase in the fusion index was longer than in tension for both types of motor units ($P<0.01$ for FF and $P<0.05$ for FR units). However, the decrease in the fusion index during 120 s of fatigue test was smaller than in the tension ($P<0.01$ for FF and $P<0.05$ for FR units).

DISCUSSION

The present study aimed to describe the changes in the fusion index of tetani during a fatigue test. These changes were also compared to changes in tetanic tension, described in more detail in a previous paper (Celichowski 1992b). Comparison of changes in tension to changes in the fusion index enables to estimate the influence of changes in the twitch time (contraction and relaxation) on the change in tetanic tension. The tension of unfused tetani is influenced not only by changes in tension of twitches, which are components of the tetanus, but also by changes in contraction and/or relaxation time. The correlation between the fusion index and the contraction time found for 40 Hz tetanus as well as the correlation between the course of fusion index - stimulation frequency curve and contraction time described previously (Celichowski and Grottel 1995) suggest that changes in the fusion index are influenced mainly by changes in contraction and relaxation times of single twitches. Thus, the initial increase in the fusion index is probably accompanied by a prolongation of contraction and/or relaxation, associated with potentiation of the twitch (Bagust et al. 1974, Hultman and Sjöholm 1983, Jami et al. 1983, Bergström and Hultman 1986, Kukulka et al. 1986, Dubose et al. 1987, Rankin et al. 1988). Moreover, it is well known that the tension decrease usually follows the initial potentiation during activity of fast motor units. This decrease can be accompanied by shortening of the twitch (Burke et al. 1974, Gordon et al. 1990a, Celichowski and Grottel 1996) when the fatigue develops. This shortening could be responsible for the decrease in fusion index, observed concomitantly with the decrease in tetanic tension. However, regular measurements of changes in twitch course during fatigue test were not made up today.

The observed changes in the fusion index were greater for FF than for FR motor units. This observation suggests that changes in twitch time of FF units are greater than in FR units.

The present results showed that during the fatigue test the increase of the fusion index lasted longer than that of tetanic tension. This result shows that the twitch time increases longer than the tension of tetani and/or the tension of twitches being components of the tetanus. In a previous paper from our laboratory (Celichowski 1992b), the shorter increase of tension of the first tetanic component (corresponding to a single twitch tension) than increase of peak tetanic tension was observed.

A parallel decrease in the fusion index accompanied the decrease in tetanic tension. This observation indicates that this tension decrease results partly from shortening of twitch time.

The initial increase in the fusion index observed in the FF group was higher than the rise in tetanic tension. Probably during this period of activity of FF units the prolongation of twitch time is an important factor of the potentiation of unfused tetani. In FR units, where the increase both in the fusion index and in tension were similar, this prolongation of the twitch should be less significant.

Our previous suggestion that the time course of "sag" in unfused tetanus depends on the fusion of this tetanus (Celichowski 1992a) was fully confirmed by the correlation of the fusion index with the timing of a peak before the "sag" within a tetanus. On the other hand, this correlation confirms that the sag is a relative criterion (depending on the conditions of the experiment) of fast - slow classification of motor units (Burke et al. 1973, Burke 1981).

ABBREVIATIONS

FF	fast fatigable
FR	fast resistant

ACKNOWLEDGEMENT

The study was supported by grant of the State Committee of Scientific Research.

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Received 7 December 1995, accepted 22 July 1996