

The time course of the last contractions during incompletely fused tetani of motor units in rat skeletal muscle

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Abstract. The time course of the contraction and the relaxation of individual contractions during incompletely fused tetani of motor units were analyzed. Investigations were performed on fast fatigable (FF), fast resistant (FR) and slow (S) motor units of the rat medial gastrocnemius muscle. Stimulation of a motor unit with a series of nine trains of stimuli at a frequency from 10 to 150 Hz was used and tetani fused to a variable degree were recorded. For fast motor units the procedure was repeated twice and observations were made on potentiated tetani in the second series of stimulation. For each tetanus, the amplitude of the tension increase, the peak amplitude of the contraction, the contraction time and the half-relaxation time were measured in the last contraction of the tension recording. It was observed in all three types of motor units that the last contraction was prolonged in parallel with the increase of fusion of a tetanus. In this contraction, the contraction time slightly decreased whereas the half-relaxation time strongly prolonged. The prolongation of the half-relaxation time was the strongest in tetani of slow units. Moreover, for the last contraction in a tetanus, the rate of changes in tension were studied. The rate of increase in tension during the contraction decreased in parallel with the increase of fusion of a tetanus, whereas the maximal rate of the tension decrease during the relaxation was found in tetani with fusion indices of 0.79, 0.98 and 0.95 for FF, FR and S motor units, respectively. Changes in the time course of contractions in tetani fused to a variable degree can shed light on processes of summation of contractions in unfused tetani at the level of individual motor units.

Key words: motor unit, unfused tetanus, rat, medial gastrocnemius, contraction

INTRODUCTION

During voluntary activity of a skeletal muscle motor units contract in unfused tetani (Kernell 1979, 1984, Hennig and Lømo 1985, 1987). A summation of individual contractions in an unfused tetanus has an influence on the force output. Changes in motoneuronal firing rates enable the regulation of force generated by motor units in a muscle, although it is known that oscillations in the tension of unfused contractions are the source of physiological tremor which accompanies each voluntary activity (Allum et al. 1978, Stein and Lee 1991). Therefore studies of the time course of unfused tetani are very important for our knowledge of motor control processes. In our laboratory the effect of changes in the stimulation pattern on the tension produced by motor units has been investigated (Grottel and Celichowski 1999). Results of decreasing as well as of increasing one interpulse interval have been observed in tetani fused to a variable degree. However, the time course of individual contractions within a tetanus has not been widely investigated. In their pioneer paper, Burke et al. (1976) have described changes in the time to peak in successive contractions during a tetanus with a sag phenomenon in fast motor units in a cat muscle. In a recent study, the course of a sag during unfused tetanic contractions in motor units of the rat triceps surae has been reinvestigated by Carp et al. (1999). The authors have focused their analysis especially on changes of the time to force peak during the sag in a tetanus. However, the time course of relaxation has not been described although this is an important property, which influences the summation of contractions and determines the possibility of regulating tension by varying motoneuronal firing rate (Grottel and Celichowski 1999). Moreover, the time course of relaxation influences the efficiency of a contraction. The tension-time area is the parameter that enables the estimation of work performed by contracting motor units. It has been shown that the area is related to the energy utilized by an active skeletal muscle during its contraction (Jöbsis and Duffield 1967). Therefore, in the present paper we have focused our analysis on the time course of the last contraction during a tetanus because for this contraction a full course of both tension increase and relaxation can be observed. The study was performed on the three types of motor units and their tetanic contractions evoked at different frequencies of stimulation (from sub-fused twitches to fully fused tetani) were analyzed. In particular, we concentrated on

incompletely fused tetani with the fusion indices of 0.5, 0.7 and 0.9. In our previous papers we showed that the tetanus with a fusion index of 0.9 is the contraction with the maximum tension-time area per pulse, i.e., achieved at the lowest metabolic cost (Celichowski et al. 2000). On the other hand, this tetanus has relatively low susceptibility to regulation of tension by changes in the pattern of motoneuronal firing. The tetanus most sensitive to changes in the firing rate of a motoneuron is the tetanus with an fusion index of 0.7, approximately (Celichowski and Grottel 2001).

METHODS

Materials

Experiments were performed on twelve adult female Wistar rats having a mean weight 286 ± 28 g. Principles of laboratory animal care, as approved by the Council of the American Physiological Society, were followed while abiding by Polish laws on animal protection. Animals were anesthetized initially with a 30 mg/kg i.p. dose of pentobarbital (Vetbutal), supplemented after three hours with additional doses of 5 mg/kg per one hour i.p. The depth of anesthesia was monitored by control of the pinna reflex and the shape of pupils. After experiments, animals were killed with an overdose of pentobarbital.

Surgery

A laminectomy was performed at $L_2 - S_1$ vertebrae and ventral as well as dorsal roots of spinal nerves were isolated and cut as proximal as possible to the spinal cord. The vertebral column was immobilized with clamps attached to spinal processes of L_1 and S_2 vertebrae. The spinal cord was covered with paraffin oil that filled a pool formed by skin flaps. All muscles of the hind limb innervated by the sciatic nerve were denervated, except the medial gastrocnemius to be studied. The distal part of this muscle was dissected free while the innervation and blood vessels were left intact. The hind limb was immobilized with a steel clamp attached to the tibia and was immersed in warm paraffin oil in a special pool. The temperature of both the animal and the paraffin oil were automatically maintained at $37 \pm 1^\circ\text{C}$ using a thermoregulator (with a thermometric resistor Pt-100, model CP-211). The L_5 ventral root was subdivided into very thin filaments to reach the func-

tional isolation of single motor units. The "all-or-none" twitch contraction and muscle-action potential were used as criteria of a single motor-unit isolation (Grottel and Celichowski 1990, Kanda and Hashizume 1992).

STIMULATOR

Thin filaments of ventral roots were electrically stimulated with a bipolar silver electrode. The electrode was connected to a stimulator (Grass Instrument Company, a dual channel square pulse stimulator model S88) which generated 0.1 ms rectangular pulses of variable amplitudes (up to 0.5 V), in a frequency range of 1-150 Hz.

TENSION

The medial gastrocnemius muscle was connected to an inductive force transducer (measurement range from 0 to 1,250 mN, deformation of the active element of the sensor of 100 μm per 100 mN force, the transducer model FT-510) through the Achilles tendon. The muscle was stretched up to a passive tension of 100 mN, optimal for recording of the maximal tension of single motor unit twitches (Celichowski and Grottel 1992).

EMG

Action potentials from the muscle studied were recorded with a pair of silver thin-wires inserted into the muscle and amplified using a low-noise multi-channel preamplifier (WPI, model ISO-DAM8-A, with a high-pass filter at 0.1 Hz and a low-pass filter at 10 Hz). The tension and EMG were monitored on an oscilloscope screen and stored on a computer disc using an analogue-to-digital 12-bit converter (RTI-800 Utilities). The sampling frequency was 20 kHz.

Stimulation mode

A special computer program was used to create the applied pattern of stimuli. Each motor unit was first stimulated with five pulses at 1 Hz and the twitch force was averaged. Then, each unit was stimulated with a series of 500 ms trains of stimuli at: 10, 20, 30, 40, 50, 60, 75, 100, 150 Hz. The series ended with the stimulation with five pulses at 1 Hz (the averaged twitch was recorded). Intervals of 10 s were used between successive trains. For fast motor units the procedure was repeated, because it was observed that during the first series of

stimulation a potentiation developed (Jami et al. 1982, Powers and Binder 1991, Macintosh and Willis 2000). This phenomenon influences the analyzed course of a tetanus. Therefore, in the present paper the first and the second series of trains of stimuli will be referred as "*the first series*" and "*the second series*", respectively. To eliminate the influence of potentiation the main analysis will concern the second series. At the end of the recordings, each of the studied motor units was subjected to the fatigue test (stimulation with trains of 14 stimuli at 40 Hz, repeated every second during 4 min) (Burke et al. 1973).

Classification of motor units

Slow/fast division of motor units was based on a "sag" in the profile of the 40 Hz unfused tetanus. Motor units with a sag were classified as fast, whereas those with no sag were classified as slow (S) (Burke et al. 1973, Grottel and Celichowski 1990). Measurements of the peak tetanic tension recorded during the fatigue test enabled us to calculate the fatigue index (the ratio of the tension reached 2 minutes after the initial maximum to the maximal tension at the beginning of the test) (Kernell et al. 1983, Grottel and Celichowski 1990). Fast motor units with a fatigue index below 0.5 were classified as fast fatigable (FF), whereas those with an index over 0.5 were classified as fast resistant (FR).

Parameters

The twitch tension (T_{Tw} , measured from the baseline to the peak), the contraction time (CT_{Tw} , the time interval from the beginning of a twitch to the peak tension) and the half-relaxation time (HRT_{Tw} , the time from the peak to half-maximum tension) were measured for averaged twitch recordings. The rates of tension increase ($V_{Tw/CT}$) and tension decrease ($V_{Tw/HRT}$) were calculated as a ratio of twitch tension to contraction time and as a ratio of a half of the twitch tension to the half-relaxation time, respectively. Additionally, in fast motor units the influence of twitch potentiation was evaluated as ratios of values of parameters calculated for the twitch recorded before and after each series (Table II). The maximum tetanic tension (T_{max}) was measured on the fully fused tetanus recording (at 150 Hz stimulation).

For incompletely fused tetani, the time course of the last contraction within the tetanus was analyzed. The amplitude of the increase of a tension (F_{Tet}), the peak

amplitude of a contraction (T_{Tet}), the contraction time (CT_{Tet}) and the half-relaxation time (HRT_{Tet}) were measured on tension recordings, for tetani evoked at stimulation frequencies in a range from 10 to 100 Hz (Fig. 1). The rate of tension increase ($V_{\text{Tet}/CT}$) and of tension decrease ($V_{\text{Tet}/HRT}$) were calculated as the ratio of F_{Tet} to CT_{Tet} and the ratio of a half of T_{Tet} to HRT_{Tet} , respectively (Fig. 1). Additionally, the fusion index (FuI) was calculated as indicated in Fig. 1 (Bakels and Kernell 1995, Celichowski and Grottel 1995). Moreover, in order to compare changes in analyzed parameters of the last contraction in tetani fused to a variable degree, for different motor units, a normalization procedure was used for the tension increase, the peak amplitude of a contraction, the contraction time and the half-relaxation time. For slow units the relative values of analyzed parameters were presented as a ratio of these values in unfused contractions to those in a single twitch. For fast units, instead of this single twitch recorded before the analyzed series, non-fused twitches at 20 Hz frequency of stimulation were used. At 30 Hz a slightly fused tetanus was observed in a majority of fast units. We decided to compare analyzed values for unfused tetani to these

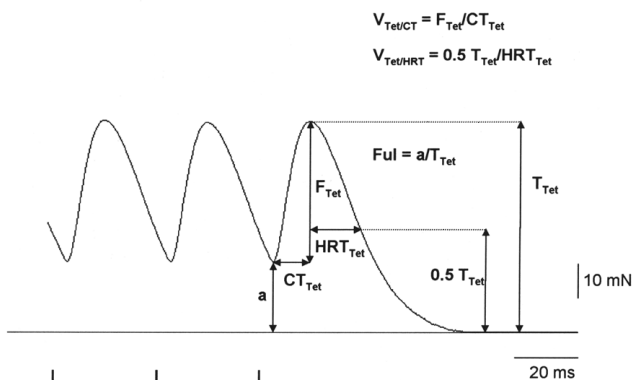


Fig. 1. Parameters calculated for the last contraction within an unfused tetanus. The last part of an unfused tetanus of FF motor unit evoked at 30 Hz frequency stimulation. CT_{Tet} , the contraction time, HRT_{Tet} , the half-relaxation time, F_{Tet} , the amplitude of the increase of tension during the last contraction, T_{Tet} , the peak amplitude of the last contraction, $V_{\text{Tet}/CT}$, the rate of the tension increase, $V_{\text{Tet}/HRT}$, the rate of the tension decrease during the relaxation, FuI, the fusion index calculated as the ratio of the distance from the baseline to the lowest tension before the last contraction within the tetanus (a) to the peak amplitude of the contraction (T_{Tet}). Under the recording, bars show the three last pulses of stimulation. Over the unfused tetanus, equations present methods of calculation of velocities of contraction and relaxation and of the fusion index.

for the last twitch at 20 Hz stimulation because of the staircase effect (Bryant and Leslie 1987, Rankin et al. 1988, Macintosh et al. 1994) visible in non-fused twitches of fast units. The staircase effect mainly decreases the amplitude of the twitch before summation of contractions into an incompletely tetanus begins.

Seventy seven motor units of the rat medial gastrocnemius muscle (26 FF, 34 FR, 17 S type units) were observed in the present study.

RESULTS

Main contractile properties of studied motor units are summarized in Table I. In fast motor units a potentiation developed during the first series of stimulation. During the second series a further development of potentiation was not visible. The data characterizing the potentiation of fast units after the first and the second series of stimuli are presented in Table II. In slow units there was no twitch potentiation. In fast units the potentiation resulted in an increase of the twitch tension, an increase of rate of changes in tension during both contraction and relaxation and a slight prolongation of the time courses. Potentiation was more pronounced in FF than in FR motor units. Therefore, for fast motor units the analysis of data focused on the second series to eliminate the influence of development of this phenomenon on the time course of the last contraction (Fig. 3).

The study was performed using a series of trains of stimuli at increasing frequencies which evoked contractions fused to a variable degree (from twitches to fully fused tetani). Time parameters and the tension of the last contractions during tetani of increasing fusion index were analyzed. Examples of fragments of recordings of tetanic tension performed after the last stimulus are illustrated for the three types of motor units in Fig. 2. It is shown that the contraction time shortened whereas the half-relaxation time prolonged in parallel with the increase of fusion of tetani.

To compare the influence of the fusion degree on parameters analyzed for different types of motor units we approximated the studied values for tetani with fusion indices of 0.5, 0.7 and 0.9. For approximation of each fusion index (i.e., 0.5, 0.7 or 0.9) the data for two "neighboring" tetani (slightly more and slightly less fused) were used. We assumed a linear relationship between fusion index and the approximated parameter. The results of approximation are presented in Table III. The prolongation of the last contraction occurring in parallel

Table I

Main contractile properties of studied motor units							
Motor unit type	T _{Tw} (mN)	CT _{Tw} (ms)	HRT _{Tw} (ms)	V _{Tw/CT} (N/s)	V _{Tw/HRT} (N/s)	T _{max} (mN)	FI
FF (n = 26)	28.6 ± 13.7	12.8 ± 2.2	11.6 ± 4.2	2.24 ± 0.99	1.29 ± 0.61	117.2 ± 47.2	0.24 ± 0.12
FR (n = 34)	11.2 ± 6.5	15.5 ± 2.7	15.7 ± 4.4	0.85 ± 0.63	0.44 ± 0.33	57.9 ± 22.9	0.84 ± 0.15
S (n = 17)	5.2 ± 2.4	27.6 ± 6.6	38.5 ± 11.2	0.2 ± 0.14	0.08 ± 0.07	33.9 ± 14.6	0.94 ± 0.06

Mean values ± SD for properties of the three types of motor units (FF, fast fatigable; FR, fast resistant and S, slow). Twitch properties concern the twitch recorded at the beginning of experiment. T_{Tw}, the twitch tension; CT_{Tw}, the contraction time; HRT_{Tw}, the half-relaxation time; V_{Tw/CT}, the rate of tension increase during the contraction time; V_{Tw/HRT}, the rate of tension decrease during the half-relaxation time; T_{max}, the peak tension of maximal tetanus; FI, the fatigue index.

Table II

The influence of potentiation on properties of the twitch of fast motor units recorded after the first and the second series of trains

Motor unit type		T _{Tw}	CT _{Tw}	HRT _{Tw}	V _{Tw/CT}	V _{Tw/HRT}
FF	the first series	1.63	1.10	1.08	1.49	1.55
		± 0.16	± 0.10	± 0.17	± 0.16	± 0.28
	the second series	1.02	0.97	1.07	1.06	0.96
		± 0.02	± 0.09	± 0.08	± 0.11	± 0.07
Mann-Whitney test:		***	***	*	***	***
FR	the first series	1.38	1.02	1.08	1.17	1.05
		± 0.18	± 0.09	± 0.09	± 0.60	± 0.55
	the second series	1.03	1.04	1.05	0.97	0.99
		± 0.03	± 0.08	± 0.08	± 0.09	± 0.07
Mann-Whitney test:		***	NS	NS	***	***

The mean values ± SD of studied parameters of the twitch recorded after the first and the second series are presented as relative values (the ratio of properties calculated for the twitch recorded after the first or the second series to values obtained before the first or the second series, respectively). Relative values above 1.0 indicate the increase of the analyzed parameter resulting from the potentiation during a given series of stimulations. T_{Tw}, the twitch tension; CT_{Tw}, the contraction time; HRT_{Tw}, the half-relaxation time; V_{Tw/CT}, the rate of tension increase; V_{Tw/HRT}, the rate of tension decrease; ***, difference significant, $P < 0.001$; *, difference significant, $P < 0.05$; NS, difference non-significant, $P > 0.05$.

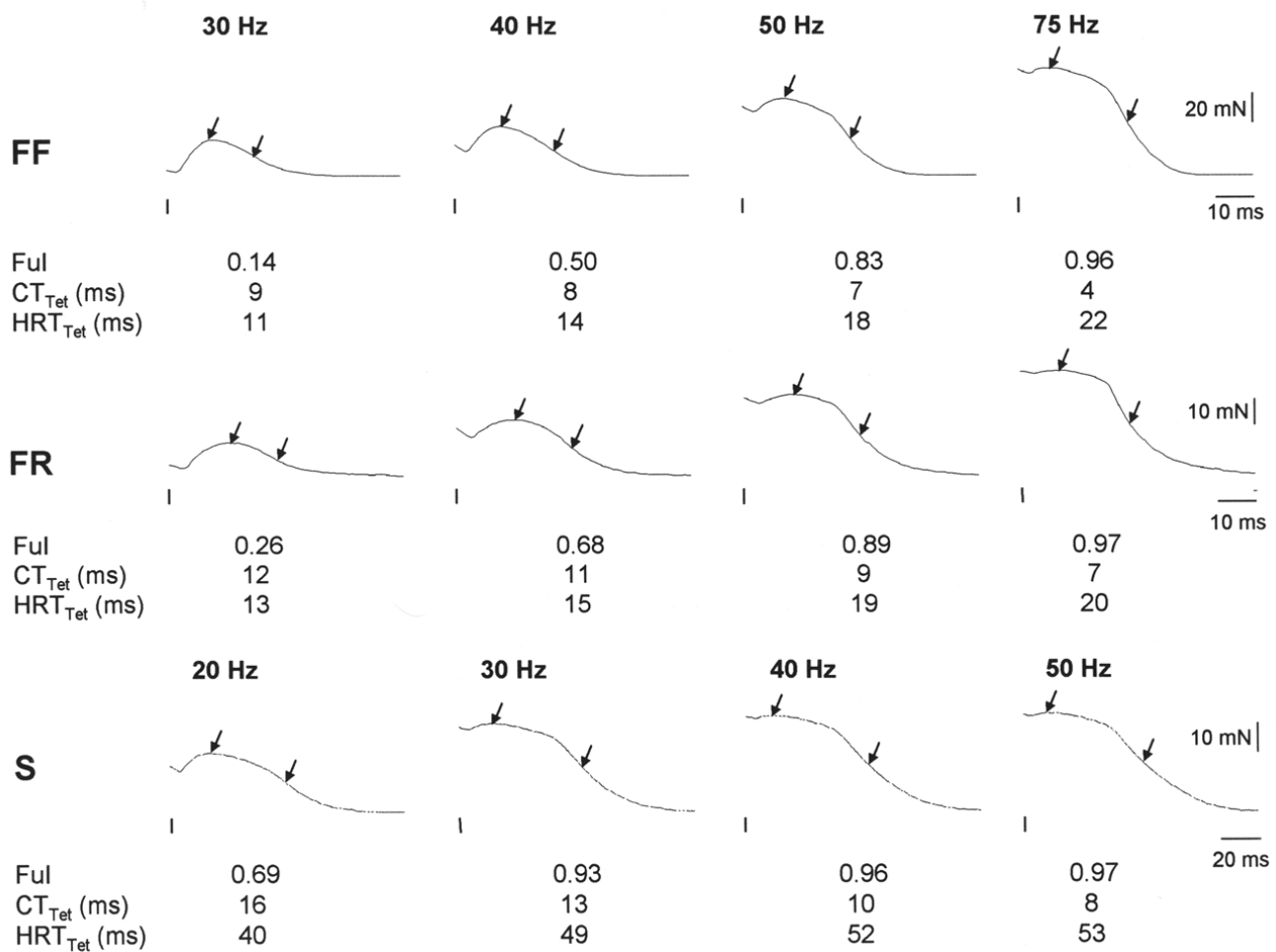


Fig. 2. Fragments of tetani evoked by last stimuli for fast fatigable (FF), fast resistant (FR) and slow (S) motor units. For fast units the second series of stimulation and for slow unit the first series of stimulation were used. Trains of stimuli at 30, 40, 50, 60, 75 Hz and at 20, 30, 40, 50 Hz were used for fast and slow motor units, respectively. The arrows point to the time period when the half-relaxation time was calculated. Under recordings, bars show the time position of the last pulse in a train of stimuli. The values of the fusion index (Ful), the contraction time (CT_{Tet}) and the half-relaxation time (HRT_{Tet}) are presented under recordings.

to the increase of the fusion index was the greatest in slow units. These units showed the weakest shortening of contraction time and the greatest prolongation of half-relaxation time for each of the analyzed fusion indices.

The relationship between the fusion index vs. the tension increase, the peak amplitude of a contraction, the contraction time and the half-relaxation time was analyzed. The normalized values of these properties as a function of the fusion index are illustrated in Fig. 3. With increasing fusion index normalized tension increase diminished, whereas the peak amplitude of a contraction increased. Moreover, the contraction time shortened whereas the half-relaxation time prolonged with the increase in the fusion index.

The rate of changes of tension in the last contraction during incompletely fused tetani was also analyzed. Figure 4 gives an example of the normalized rate of tension increase and tension decrease as a function of the fusion index for FF motor unit. The rate of the tension increase diminished with the increase of the fusion index. However, the rate of the tension decrease first rose and peaked for the fusion index of approximately 0.9 and then decreased. This situation was observed in 44 of the studied units, whereas in the 33 remaining motor units (9 FF, 23 FR and 1 S type) the latter decrease of maximal rate was not observed. The maximal rate of the tension decrease was observed in tetani with the fusion index of 0.79 ± 0.26 , 0.98 ± 0.01 and 0.95 ± 0.04 for FF, FR and S units, respectively (differences significant, ANOVA

Table III

The normalized values of the analyzed parameters of unfused tetani						
Motor unit type	CT _{Tet}			HRT _{Tet}		
	FuI = 0.5	FuI = 0.7	FuI = 0.9	FuI = 0.5	FuI = 0.7	FuI = 0.9
FF	0.85 ± 0.06	0.76 ± 0.09	0.55 ± 0.09	1.23 ± 0.10	1.38 ± 0.11	1.63 ± 0.17
FR	0.84 ± 0.09	0.79 ± 0.11	0.64 ± 0.10	1.03 ± 0.06	1.12 ± 0.06	1.30 ± 0.12
S	1.10 ± 0.14	0.95 ± 0.17	0.85 ± 0.18	1.49 ± 0.22	1.72 ± 0.30	2.00 ± 0.38
ANOVA Kruskal-Wallis rank test:						
	<i>H</i> =56.3 ^{***}	<i>H</i> =23.2 ^{***}	<i>H</i> =48.6 ^{***}	<i>H</i> =68.4 ^{***}	<i>H</i> =64.8 ^{***}	<i>H</i> =64.4 ^{***}
Mann-Whitney test						
FF-FR	NS	NS	**	***	***	***
FR-S	***	***	***	***	***	***
FF-S	***	***	***	***	***	***

Data concern the second series of trains of stimuli for fast units and the first series for slow units. Values were approximated for tetani fused to a fusion index (FuI) of 0.5, 0.7 and 0.9. CT_{Tet}, the contraction time; HRT_{Tet}, the half-relaxation time. ***, difference significant, $P < 0.001$; **, difference significant, $P < 0.01$; NS, difference non-significant, $P > 0.05$.

Kruskal-Wallis rank test, $H=39.5$, $P < 0.001$; the difference between FF and two other types of motor units significant, $P < 0.01$, Mann-Whitney test).

DISCUSSION

The present experiments were aimed at studying the time course of individual contractions during incompletely fused tetani. The last contraction during a tetanus with a full relaxation was used for this analysis. Two series of trains of stimuli with increasing frequency were used. In the first series potentiation developed in fast but not in slow units (Jami et al. 1982). For fast units in the second series of stimulation the tension was potentiated, but a comparison of twitches recorded before and after this series revealed that the tension was relatively stable and potentiation or fatigue failed to develop. Similar results were obtained in our previous paper (Celichowski and Grottel 1997). Therefore, the present analysis of the time course of the last contractions of fast units focused on the second series to eliminate the influence of potentiation. The procedure allowed a comparison of the time course of last contractions during tetani of slow and fast motor units. The potentiation of fast motor units tension before the main part of experiment with the tension recording was applied in several studies (e.g., Burke et al. 1973, Grottel and Celichowski 1999).

During natural activity of muscles their motor units contract in unfused tetani (Grimby et al. 1979, Nordström et al. 1989). However, the time course of unfused tetani has not been widely studied, although it is changing during activity as an effect of potentiation, fatigue and other phenomena (Gardiner and Olha 1987, Celichowski 1992, Celichowski et al. 1996, Celichowski 2001). In a previous paper (Celichowski and Grottel 1997) the relationship between twitch properties and the tension-frequency curve was studied for the three types of motor units. Results have revealed that changes of contraction and half-relaxation times of the twitch influence the summation of contractions in an unfused tetanus and significantly modify the course of the tension-frequency relationship. In FF motor units the greatest changes in tetanic tension have been observed. At any given stimulation frequency the relative tension of an evoked tetanus depends mainly on motor unit contraction and relaxation times and therefore tetanic tension is different for the three types of motor units (Kernell et al. 1983). However, the time course of individual contractions during tetani evoked at different frequencies of stimulation has not been studied previously. The unfused tetanus results from a summation of individual contractions, the successive contraction beginning during the relaxation of the preceding one. Therefore, the tension and the fusion of a tetanus depend mainly on the relaxation time course. The analysis of this relaxation elucidates the mechanisms

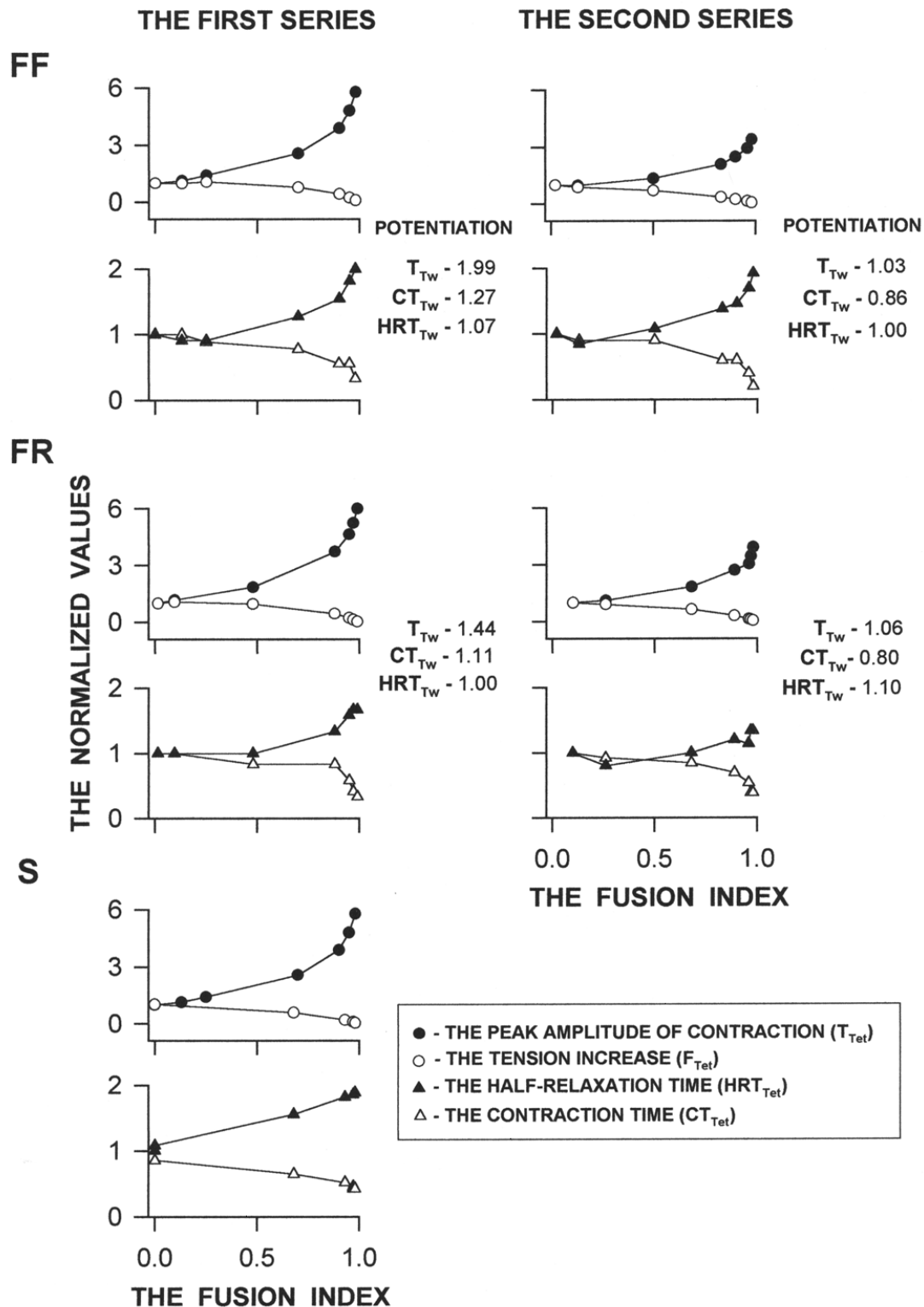


Fig. 3. The normalized values of the peak amplitude of the contraction, the tension increase, the half-relaxation time and the contraction time as a function of the fusion index. Plots for the three types of motor units, for the first series of stimulation (left) and for fast units for the second series (right). The same units as those presented in Figure 2 are shown. Relative values of parameters of potentiated twitches for fast motor units are given to right of plots. These values were calculated as ratios of these parameters, calculated for the twitch recordings after and before each series.

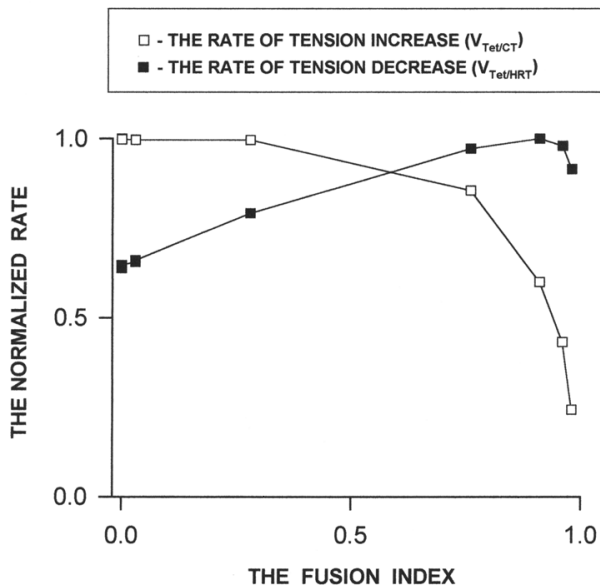


Fig. 4. The normalized rate of the tension increase and decrease in the last contraction during the tetanus as a function of the fusion index. The rate was normalized to 1.0 for maximal values.

underlying the summation of successive contraction during the unfused tetanus.

Results described in this paper revealed that the contraction time slightly shortened whereas the half-relaxation time significantly prolonged as tetanic fusion index increased. In slow units there was only a slight shortening of the contraction time, but the greatest prolongation of the half-relaxation time for each of the analyzed fusion indices (0.5, 0.7, 0.9). This indicates that in unfused tetani of slow motor units the tension of successive contractions can summate very effectively. Therefore, slow units are characterized by a lower twitch-to-tetanus tension ratio (twitch/tetanus ratio) (Burke et al. 1973, Stephens and Stuart 1975, Gardiner and Olha 1987, Celichowski and Grottel 1993). Moreover, results concerning prolongation of contractions explain also another phenomenon. It is known that motor units are characterized by more than linear summation of individual contractions in an unfused tetanus (called "tetanic potentiation", Piotrkiewicz et al. 1986) and this phenomenon is more pronounced in slow than in fast units. Finally, observations that contractions in tetani of slow motor units undergo the greatest prolongation are in agreement with our earlier observation that slow units are less susceptible to changes in a pattern of moto-

neuronal firing than fast units (Grottel and Celichowski 1999). This low sensitivity to firing pattern is a result of significant prolongation of the relaxation, which, on the other hand, enables these units to maintain force effectively even at low firing rate of their motoneurons.

Tansey et al. (1996) have observed contractions of single motor units in a cat muscle during centrally-evoked contractions (stimulation of the mesencephalic locomotor region). Results of their experiments suggest that during natural activity tetani of contracting motor units are relatively highly fused, with the tension at the level > 70% of the maximum output, which is probably optimal for the work performed by individual contractions within these tetani. In our previous paper (Celichowski et al. 2000) the tension-time area per pulse has been calculated for tetani evoked at different frequencies of stimulation. We have found that the tetanus with the fusion index of 0.9 is a contraction with the maximum tension-time area per pulse, i.e., achieved at the lowest metabolic cost. The optimal sub-fused tetanus corresponded to about 75% of the maximum tension. In the same study it has also been found that the area per pulse did not correlate with twitch tension. For the weakest slow motor units the area in an optimal tetanus appears to be greater than the area of FR units twice as strong (Celichowski et al. 2000). These observations can be explained by lower frequency of stimulation in an optimal tetanus of S motor units and by a significant prolongation of relaxation in these units, as described in this paper.

In our previous work (Grottel and Celichowski 1999) effects of changes in the pattern of stimulation on the tension produced by motor units have been studied. We have found that motor units of all three types were the most sensitive to the increase in the interpulse interval when the fusion index of a tetanus amounted to 0.75-0.80. In the present paper the velocity of the tension decrease during the relaxation of tetani fused to a variable degree was calculated. The maximal velocity of the tension decrease during the last contraction of fast fatigable motor units was observed in a tetanus with a fusion index of 0.79. In fast fatigable motor units the tetanus fused to 0.8, approximately, has the highest velocity of the tension decrease and it is the most sensitive to the decrease in a motoneuronal firing rate. Therefore, this tetanus can be very effective in a regulation of tension.

It is intriguing to consider the possible mechanisms underlying the observed changes in the time course of

the studied contraction. One of the phenomena observed in the present study is the potentiation of tension. It was shown in numerous studies that in skeletal muscle the mechanism responsible for activity-dependent potentiation is phosphorylation of the regulatory light-chain of myosin, increasing Ca^{2+} sensitivity of contractile mechanism (Grange et al. 1993, Sweeney et al. 1993, Vandenboom and Houston 1996, Macintosh and Willis 2000, Rassier and Macintosh 2000). However, as the main topic of the present article, we analyzed changes in the time course of contraction and relaxation parallel to the increase of tetanic fusion. The rate of the force development, and - to some extent - the time parameters of contraction, are also Ca^{2+} -dependent, at least in fast-twitch muscle fibers (Grange et al. 1995, Vandenboom et al. 1995). The skeletal muscle force output is regulated through Ca^{2+} mediated alterations of the rate at which cross-bridges make the transitions from non-force-generating to force-generating states. The phosphorylation of the regulatory light-chain of myosin contributes to an enhanced rate of isometric twitch force development (Vandenboom et al. 1995, Grange et al. 1995). Additionally, several other factors can influence the duration of contraction and relaxation. Among them, the rate of release and removal of Ca^{2+} from the sarcoplasm, the rate of association and dissociation of Ca^{2+} from troponin C and the rate of cycling of cross-bridges as well as the level of H^+ and inorganic phosphate concentration were discussed (Macintosh et al. 1994, Macintosh and Willis 2000). The observed shortening of the contraction time and prolongation of relaxation parallel to the increase of tetanic fusion suggests that the above intracellular processes are dynamically changing during contractions. The described dependence of the contraction and relaxation times on the fusion of evoked tetani suggests that not only the potentiation but also the motoneuronal firing rate are factors modifying the discussed intracellular processes in muscle fibers.

Summarizing, the last contraction during incompletely fused tetani was prolonged in parallel to an increase of the fusion index. This phenomenon resulted from significant prolongation of the relaxation, although the contraction time slightly shortened. The prolongation was the greatest in slow motor units. Changes in the time course of contractions in incompletely fused tetani might clarify processes participating in motor control at the level of individual motor units.

CONCLUSION

In motor units the prolongation of the last contraction during unfused tetani occurs in parallel to the increase of tetanic fusion. It results from significant prolongation of the relaxation, although the contraction time slightly shortens. These phenomena influence summation of successive contractions into an unfused tetanus.

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