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THE INFLUENCE OF INCREASING AND DECREASING FREQUENCY OF STIMULATION ON THE CONTRACTION OF MOTOR UNITS IN RAT MEDIAL GASTROCNEMIUS MUSCLE

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Motoneurons during their activity can generate firings at changing rate. Therefore, the present paper aimed at studying the evoked contractions of different types of motor units to stimulation at progressively decreasing or increasing interpulse intervals. The influence of both patterns of stimulation on the tension, the duration and the tension-time area of motor units in rat medial gastrocnemius muscle was studied. The decreasing stimulation frequency resulted in generation of a bigger tension-time area although a slightly lower tension and a shorter duration of a contraction were observed in majority of studied units. For fast units a bigger tension-time area observed during the stimulation at decreasing frequency is probably due to the potentiation resulting from the initial high-frequency stimulation. On the other hand, at increasing frequency of stimulation the influence of the initial low-frequency stimulation can depress the tension-time area. The decreasing rate of stimulation during relaxation appeared to support the tension more effectively in slow-twitch motor units than in fast ones. Therefore, slow motor units are well suited to support the tension of tonic contractions.

Key words: *motor unit, motor control, stimulation pattern.*

INTRODUCTION

The tension of contracting motor units depends on the firing rate of motoneurons. Therefore, the relationship between the stimulation frequency and the motor unit tension was studied in some experiments (1–3). However, the constant rate of stimulation was applied as a standard method although during normal activity motoneurons generate firings at varying successive time intervals (4–7). The influence of a pattern of stimuli on the motor unit contraction was also described in some papers. In these studies the influence of two pulses at a short interpulse interval (doublet) at the beginning of stimulation on the tension generated by muscle fibers was described (8–10).

These studies revealed that slow motor units are more sensitive to this pattern of stimulation than fast ones. However, in other series of our experiments the influence of decreasing or increasing only one interpulse interval during the tetanus was studied and the tension of fast units showed significantly higher sensibility to each change in the interpulse interval than the tension of slow units (11). Therefore, these observations proved that the regulation of motor unit tension is a complex process. Until now, the influence of linearly varying frequency of stimulation on motor unit tension was investigated in only one study (12) although this kind of experiments can explain some physiological mechanisms responsible for generation of tension during activity of motor units. In numerous studies of motor unit behaviour it was observed that firing rate of motoneurons rise or decline in parallel to the tension increase or decrease, respectively (13—15). Therefore, the aim of present paper was to investigate and compare the contractile responses of various types of motor units to stimulation at progressively decreasing or increasing interpulse intervals with the goal of providing further insight into the process of the control of the motor unit tension. These effects were tested on the three main types of motor units.

MATERIALS AND METHODS

The study was performed on 9 adult Wistar female rats (average weight 291 ± 33 g) under pentobarbital anaesthesia (Verbutal, initial dose of 30 mg/kg I.P., supplemented as required by controlling pinna reflexes and the diameter of pupils). The Principles of laboratory animal care, as approved by the Council of The American Physiological Society and Polish law on animal protection were followed. After experiments, the animals were killed with an overdose of pentobarbital.

Details of surgery were described in the previous paper (16). Briefly, the medial head of gastrocnemius muscle was partly isolated from surrounding tissues and the Achilles tendon was prepared to connect to the force transducer. Blood vessels and the branch of sciatic nerve to medial gastrocnemius were left intact. All other branches of the sciatic nerve were cut. Laminectomy was performed at L2-S1 vertebrae and ventral as well dorsal roots were cut proximally to the spinal cord. The animal was immobilized with several steel clamps: on the tibia, on the processus of the sacral bone and on the L1 vertebra. The spinal cord was covered with paraffin oil in a pool formed of the skin whereas the hindlimb was immersed in a special pool filled with this oil. The rectal temperature of the animal and the temperature of paraffin oil were kept automatically at $37 \pm 1^\circ\text{C}$.

The stimulation of very thin filaments of ventral roots (mainly L5) enabled the functional isolation of single motor units. The evoked activity was accepted as the contraction of a single motor unit when both the contraction and the action potential were of "all or none" type. The tension was recorded under isometric conditions, in a muscle stretched up to the passive tension of 100 mN (17). Muscle fiber action potentials were recorded using silver bipolar electrode inserted into the muscle. Filaments of the ventral root were stimulated with rectangular, electrical pulses (duration 0.1 ms, amplitude up to 0.5 V).

Isolated units were stimulated with: 1/single pulses repeated 5 times at 1 Hz frequency (the averaged twitch was recorded), 2/500 ms series of pulses at 40 Hz (an unfused tetanus was recorded), 3/200 ms series of pulses at 150 Hz (a fused tetanus was recorded), 4/ten 20-pulses trains at 40 Hz (inducing a potentiation of units studied) (11), 5/ramp-frequency stimulations (stimulation with a linearly increasing frequency from 20 to 150 Hz during 383 ms, and then the reverse pattern with a decreasing frequency) (Fig. 1), 6/series of 14 pulses at 40 Hz repeated every second, during 4 minutes (fatigue test) (18). All stimulations were separated by 10-seconds intervals. Units studied were classified as fast-twitch according to the presence of a "sag" in the 40 Hz unfused tetanus whereas "non-sagging" units were classified as slow-twitch (19). The fatigue index was calculated to the ratio of tetanic tension reached by a motor unit two minutes after the tension potentiated to a maximum at the beginning of the fatigue test to this maximal initial tension (20). Fast units were divided into fast resistant (FR) and fast fatigable (FF) according to the fatigue index which exceeded 0.5 for FR units and was lower than 0.5 for FF units (19). In the present experiments 178 motor units were studied, 27 S, 86 FR and 65 of FF type.

The contraction time (from the beginning of a twitch to the peak of a tension record), the half-relaxation time (from the peak to the moment when the tension decreased to a half of the peak value) and the twitch tension (the peak amplitude) were calculated from the averaged twitch record. Moreover, the maximum tension was calculated for the fused tetanus. For ramp-frequency tetani, the peak tension and the moment when this peak occurred were analyzed (Fig. 1). The tension-time area (area between the baseline and the tension record) was also calculated. Finally, for statistical evaluation of obtained results ANOVA Kruskal-Wallis, Mann Whitney U Test and Wilcoxon Matched Pairs Test were used.

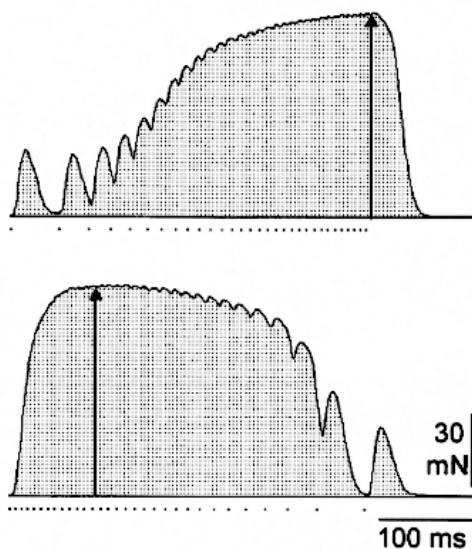


Fig. 1. Examples of studied tetani of FF motor unit evoked at linearly increasing (upper recording) and decreasing (lower recording) stimulation frequencies. Arrows show the peak of tension recording and the moment when the peak occurred. The dotted area is the tension-time area. Points under the records denote the stimulation patterns.

RESULTS

The main contractile properties of studied motor units are summarized in *Table 1*.

Table 1. The main properties (mean values and standard deviations) of motor units studied. CT, the contraction time; HRT, the half-relaxation time; TwT, the twitch tension; TetT, the tetanic tension; FI, the fatigue index.

Motor unit type		CT (ms)	HRT (ms)	TwT (mN)	TetT (mN)	FI
FR	(n = 65)	13.7 ± 2.4	14.2 ± 4.2	51.1 ± 28.6	151.0 ± 69.2	0.21 ± 0.13
FR	(n = 86)	14.3 ± 2.6	16.2 ± 5.0	14.7 ± 13.1	70.2 ± 47.0	0.86 ± 0.10
S	(n = 27)	24.8 ± 5.9	37.1 ± 10.5	4.03 ± 2.08	27.7 ± 12.6	0.95 ± 0.04

The analysis of the tension-time area in tetani evoked at a variable frequency of stimulation revealed that for all motor units of the three types it was bigger when the decreasing frequency was applied (for FF, FR and S units difference significant at $P < 0.001$, Wilcoxon test) (*Fig. 2*, *Table 2*).

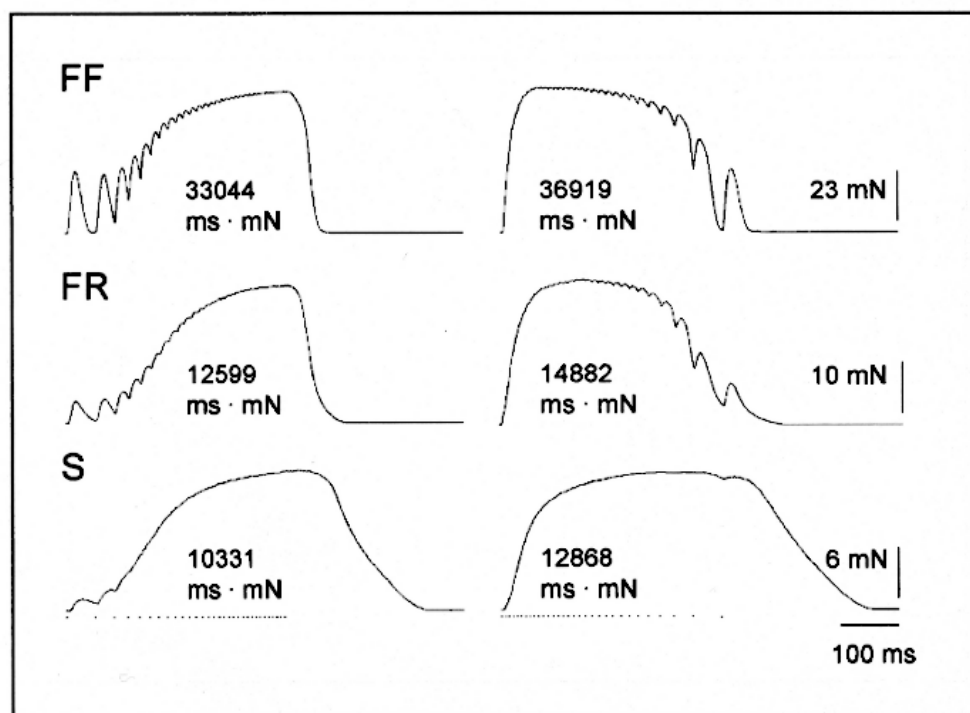


Fig. 2. Examples of tetani of the three motor units of FF, FR and S types recorded at the increasing (left) and the decreasing (right) frequencies of stimulation. Values of the tension-time area are given under the recordings. Points under the recordings denote the stimulation patterns.

Table 2. Properties of tetani recorded at the increasing (higher part) and the decreasing (lower part) frequencies of stimulation. Below both parts of the Table results of ANOVA and Mann Whitney test are given separately for recordings at increasing and decreasing frequency of stimulation.

Significance of differences: *** $P < 0.001$, NS — not significant, $P > 0.05$.

<i>Tetanus recorded at increasing frequency of stimulation</i>				
Motor unit type	The tension-time area (ms · mN)	The peak tension (mN)	The duration of tetanus (ms)	The time to peak (ms)
FF	46 479 ± 26 556	178.7 ± 71.1	464.6 ± 22.3	349.0 ± 42.0
FR	20 314 ± 13 389	69.3 ± 44.7	463.8 ± 23.0	379.6 ± 13.8
S	10 085 ± 4 518	27.3 ± 12.0	622.8 ± 75.2	394.7 ± 14.3
ANOVA	H = 51.2 ***	H = 93.0 ***	H = 66.6 ***	H = 55.5 ***
Mann-Whitney				
FF-FR	***	***	NS	***
FF-S	***	***	***	***
FR-S	***	***	***	***
<i>Tetanus recorded at decreasing frequency of stimulation</i>				
FF	51 310 ± 28 040	182.0 ± 73.1	453.1 ± 21.4	102.9 ± 45.3
FR	21 394 ± 14 381	67.1 ± 46.6	449.1 ± 21.1	159.2 ± 34.9
S	11 473 ± 5 351	26.6 ± 12.6	621.2 ± 69.9	277.7 ± 41.3
ANOVA	H = 39.8 ***	H = 97.3 ***	H = 68.0 ***	H = 97.3 ***
Mann-Whitney				
FF-FR	***	***	NS	***
FF-S	***	***	***	***
FR-S	***	***	***	***

Moreover, the peak tension in FR and S units was slightly higher in tetani recorded at the increasing frequency of stimulation, whereas in FF units — at the decreasing frequency ($P < 0.001$ for FR and S units) (Table 2). For fast motor units the tetanus recorded at the increasing frequency of stimulation was characterized by longer duration than at the decreasing frequency ($P < 0.001$ for FF and FR units). The same observation concerned the time to peak in tension records for the three types of units ($P < 0.001$).

The influence of the stimulation pattern on the contraction of motor units studied was additionally analyzed as the ratio of the tension-time area in the tetanus recorded at the decreasing frequency to the tetanus at the increasing frequency of stimulation. Values of the ratio amounted to 112.2 ± 9.2 , 106.0 ± 8.6 and 113.2 ± 8.5 for FF, FR and S units, respectively (differences significant, $H = 26.9$, $P < 0.001$ ANOVA; differences FF-FR and FR-S significant at $P < 0.001$, Mann Whitney test).

Moreover, the influence of the decreasing frequency of stimulation on the relaxation was analyzed and the time course of relaxation after cessation of

stimulation in the tetanus at the increasing frequency of stimulation was compared to the course of relaxation prolonged by the stimulation at decreasing frequency (Fig. 3). It was observed that this stimulation effectively increased the tension-time area measured after the peak by $357 \pm 221\%$, $383 \pm 198\%$ and $101 \pm 70\%$ for FF, FR and S units respectively. However, because of evidently different time occurrence of the peak in tension before the relaxation (Table 2) these effects were due to 17.0 ± 4.8 pulses in FF units, 11.0 ± 3.1 pulses in FR units and only 3.5 ± 1.8 pulses in S motor units. The analysis of these effects per one pulse revealed that one pulse during the relaxation increased the tension-time area by 1714 ± 1136 ms·mN, 892 ± 595 ms·mN and 821 ± 563 ms·mN for FF, FR and S motor units, respectively (differences significant, $H = 36.2$, $P < 0.001$ ANOVA; differences FF-FR and FF-S significant at $P < 0.001$, Wilcoxon test).

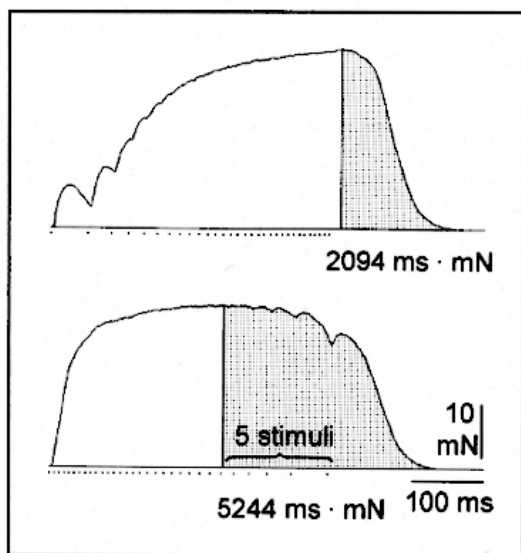


Fig. 3. Estimation of the influence of the decreasing stimulation frequency on the course of a relaxation. An example of S type motor unit. The tension-time area measured after the tension peak in the tetanus at the increasing (upper record) and in the tetanus at the decreasing (lower record) stimulation frequencies were compared. The values of the tension-time areas are given under recordings. In the lower example five stimuli influenced the analyzed parameter: on the average, one pulse increased the tension-time area by 630 ms·mN.

DISCUSSION

The results obtained in the present study confirmed that the contraction of motor units is pattern sensitive (8, 11, 21). The two applied patterns of stimulation appeared to influence mainly on the tension-time area of recorded contractions. The tension-time area was bigger when the decreasing frequency of stimulation was used to evoke the contraction. This result indicates that the decrease in the motoneuronal firing rate has a smaller influence on the contractile tension than the same increase in the motoneuronal

firing. The present study additionally stress that the high frequency at the beginning of stimulation is a very important determinant of the following part of contraction. This observation is consistent with results obtained in experiments testing the influence of a doublet (two stimuli at a very short interpulse at the beginning of stimulation pattern). These experiments revealed that a doublet significantly increased the tension of the following part of contraction (8, 10, 22).

Two phenomena occurring in fast motor units can explain why the tension-time area was bigger when the decreasing frequency stimulation was used. First, well known phenomenon is the potentiation of the tension which occurs after preceding high-frequency tetanic activity (23) and can effectively increase the tension after the initial high-frequency stimulation (24). The second phenomenon could be an influence of a preceding low-frequency stimulation depressing the development of tension of the following higher-frequency tetanus (25). In the present experiments this phenomenon could diminish the tension-time area of fast motor unit tetanus evoked at the increasing frequency of stimulation. For slow units the mechanism similar to this responsible for the "catch effect" (8) could support the tetanic tension at the decreasing frequency of stimulation.

Observations of the influence of the decreasing frequency of stimulation following the fused tetanus revealed that this pattern of stimuli very effectively supports the contractile tension. This effect was analyzed as an increase in the tension-time area per one stimulus during the relaxation, after the peak in tetanic tension. This analysis showed that slow units exerted the strongest effects in relation to their low tension (the analyzed tension-time area per pulse was approximately two-times lower than in FF units whereas the twitch tension of slow units was approximately twelve-times lower than of FF units — *Table 1*). Therefore, this property of slow motor units reveals that they are well suited to participate in tonic activity requiring a stable tension and to effectively support the tension. In the previous study of relationship between the constant-rate stimulation and the tension-time area it was found that the maximum tension-time area per one pulse for different types of motor units occurred in the tetanus fused to the same extend (the fusion index of 0.90) and that the maximum tension-time area per pulse reached similar values as those obtained in the present study (26). However, it should be finally stressed that the studied influence of the decreasing frequency of stimulation on the course of the relaxation was due to different numbers of stimuli in different motor units (lower in slow units and higher in fast ones). Therefore, further studies of the influence of a single pulse generated during various stages of the relaxation on its time course are necessary.

Concluding, it was found that the decreasing frequency of stimulation effectively supports the contractile tension of motor units. This phenomenon was relatively the strongest in slow motor units which are well suited to support the tension of tonic contractions.

Acknowledgements: The study was supported by the State Committee of Scientific Research grant V4P05D 06116.

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Received: July 7, 2000

Accepted: October 18, 2000

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