

Tetanic depression: a phenomenon influencing the production of tension in fast-twitch motor units in rat medial gastrocnemius

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Abstract. The influence of an increase or a decrease in the stimulation frequency on tension development during a tetanus was studied in motor units of the rat medial gastrocnemius muscle. These effects were tested in one tetanus evoked at two frequencies of stimulation, a lower immediately followed by a higher one or the reverse. For all fast motor units it was observed that after the first part of a tetanus at a lower frequency of stimulation the tension of the following part, of the better fused contraction, was depressed. This effect was called a tetanic depression. When the lower stimulation frequency was followed by the higher one, the depression was visible in some motor units only whereas in the remaining units a potentiation of the second part of the tetanus was visible. The tetanic depression was larger in fast resistant than in fast fatigable motor units. In slow motor units tetanic depression was not observed. The tetanic depression is a phenomenon which can influence the production of contractile tension by fast motor units.

Key words: motor unit, contraction, tetanic depression, medial gastrocnemius, rat

Fast-twitch motor units are more effective in regulating tension than slow ones because they are very sensitive to each change, even in one interpulse interval within the pattern of motoneuronal firing (Grottel and Celichowski 1999). On the other hand, the tension of fast motor units is not as stable as that of slow units and it changes during activity. The twitch of fast units shows post-tetanic potentiation (Bagust et al. 1974, Stephens and Stuart 1975, Parry and DiCori 1989) and the tension of their unfused tetanus can also potentiate (Kernell et al. 1975, Gordon et al. 1990). This tetanus shows a "sag" profile (Burke et al. 1973, Grottel and Celichowski 1990). Fast motor units are also less fatigue resistant than slow ones (Burke et al. 1973). All of these effects are responsible for the production of tension during voluntary contractions and they are the mechanisms participating in motor control processes underlying the regulation of muscle contraction.

In our laboratory the influence of stimulation patterns on the contraction of all types of motor units is studied (Celichowski and Grottel 1998, Grottel and Celichowski 1999). In the last series of experiments we have found that in all fast motor units studied, a slightly fused tetanus depressed the tension in the next part of a better fused tetanus. This phenomenon was called the tetanic depression. The main intention of this paper is to describe this phenomenon.

Experiments were performed on 4 adult Wistar rats (females, weight 250-280 g) under pentobarbital anesthesia (Vetbutal, 30 mg kg⁻¹ I.P., supplemented when required based on pupil size and corneal and limb withdrawal reflexes). The surgery and animal care conformed to "The Principles of Laboratory Animal Care". The Polish Law on Animal Protection was followed and the experiments were approved by the University Ethical Committee. After the experiments, the animals were killed with an overdose of pentobarbital.

Motor units in the medial gastrocnemius muscle were studied. The muscle was partly isolated from surrounding tissues whereas muscle innervation and blood vessels were left intact. The remaining muscles innervated by the sciatic nerve were denervated. A laminectomy at the L2-S1 segments was performed and ventral as well as dorsal roots were cut proximal to the spinal cord. The animal was immobilized with steel clamps on the tibia, the sacral bone and the L1 vertebra. The operated hindlimb was immersed in paraffin oil, which also covered the isolated spinal cord. The temperature of this oil was automatically kept at $37 \pm 1^\circ\text{C}$. The functional isolation

of single motor units was achieved by splitting the ventral root (L5) into very thin filaments which were stimulated with electrical rectangular pulses (amplitude up to 0.5 V, duration 0.1 ms). "All-or-none" twitch contraction and muscle fiber action potential were used as criteria for single motor unit isolation.

The studied muscle was connected to an inductive force transducer through the Achilles tendon. The tension was measured under isometric conditions with the muscle stretched up to a passive tension of 100 mN, optimal for the motor unit twitch contraction in this muscle (Celichowski and Grottel 1992). Muscle fiber action potentials were recorded with a bipolar silver electrode inserted into the muscle. Both the contractile tension and action potentials were stored on a computer disc using an AD converter (sampling rate 1 kHz for tension and 10 kHz for action potentials).

All units were stimulated with the following protocol: 1) 10 stimuli at 1 Hz (averaged twitch was recorded); 2) 500 ms train of stimuli at 40 Hz (the unfused tetanus was recorded); 3) 200 ms train of stimuli at 150 Hz (the fused tetanus was recorded). Units with a "sag" in the profile of an unfused tetanus were accepted as fast-twitch, whereas non-sagging units were accepted as a slow-twitch (S) (Grottel and Celichowski 1990). The tension of fast units was potentiated by 5 tetani (stimulations with a 500 ms trains at 40 Hz) before the main protocol; this potentiation assured that the shape of the potentiated tetani in the next part of experiment was more constant (Celichowski 1992, Grottel and Celichowski 1999). The twitch of a fast motor unit was increased after this protocol by $35.6 \pm 15.3\%$ and $30.9 \pm 11.3\%$ (mean \pm S.D.) as compared to the initial value for fast fatigable (FF) and fast resistant (FR) units, respectively (difference non significant, $P > 0.05$, Student *t*-test).

The following stimulus protocol formed the main part of the experiment: 1) 300 ms train of stimuli at 40 Hz; 2) 300 ms train of stimuli at 60 Hz; 3) 600 ms train of stimuli at two frequencies, 300 ms at 40 Hz and 300 ms at 60 Hz; 4) 600 ms train of stimuli at two frequencies, 300 ms at 60 Hz and 300 ms at 40 Hz; 5) 300 ms train of stimuli at 40 Hz; 6) 300 ms train of stimuli at 60 Hz. The time intervals between successive tetani were 10 s. Motor unit averaged twitch was recorded after the above protocol. For slow units lower-frequency and longer trains of stimuli were applied (700 ms trains at 15 or 30 Hz instead of 300 ms trains at 40 or 60 Hz used in the protocol for fast motor units; the duration of the two-frequencies stimulation at 15-30 Hz or 30-15 Hz was 1400 ms).

For each motor unit the experiment ended with a fatigue test (stimulation with trains of 14 stimuli at 40 Hz, repeated every second within 4 minutes) (Burke et al. 1973). Analysis of tetanic tension performed during this test enabled us to calculate the fatigue index, the ratio of tension reached 2 min after the initial maximum to this maximal tension at the beginning of the test (Kernell et al. 1983). Fast units with the fatigue index under 0.5 were classified as FF, whereas those with an index over 0.5 as FR (Grottel and Celichowski 1990). The present material comprises 54 motor units (15 FF, 25 FR and 14 S type units).

Figure 1 presents an example of the records of three motor unit tetani evoked by applied patterns of stimuli. For fast units the effect of tetanic depression is visible in the two-frequencies tetani, when the higher-frequency contraction followed the contraction evoked at a lower-frequency of stimulation (40-60 Hz tetanus). This phenomenon was visible as the depression in the tension of the 60 Hz part of the contraction. When the reverse or-

der of frequencies was applied (60-40 Hz tetanus) and in a constant-rate tetanus at 60 Hz stimulation, the peak tension was higher. The diminution of the peak values of the 40-60 Hz tetani amounted to $9.2 \pm 4.1\%$ and $5.2 \pm 3.3\%$ (mean values \pm S.D.) for FR and FF motor units, respectively (significant difference, $P < 0.01$, Student *t*-test). For all motor units studied action potentials recorded during tetani with a depression did not display a reduction in amplitude (Fig. 1).

In a majority of 60-40 Hz tetani a depressing influence of the first part of contraction on the second part of this tetanus was also observed (Fig. 1, FR unit). The depression measured at the end of contraction amounted to $12.6 \pm 7.4\%$ and $3.7 \pm 2.0\%$ for 21 FR and 9 FF units (difference significant, $P < 0.01$). For the remaining 4 FR and 6 FF type motor units (Fig. 1, FF unit) the tension of the second part of the 60-40 Hz tetanus was potentiated as compared to the control 40 Hz tetani by a mean of 6.0% and 11.1%, respectively (difference not significant, $P > 0.05$). The shape of described tetani was also ana-

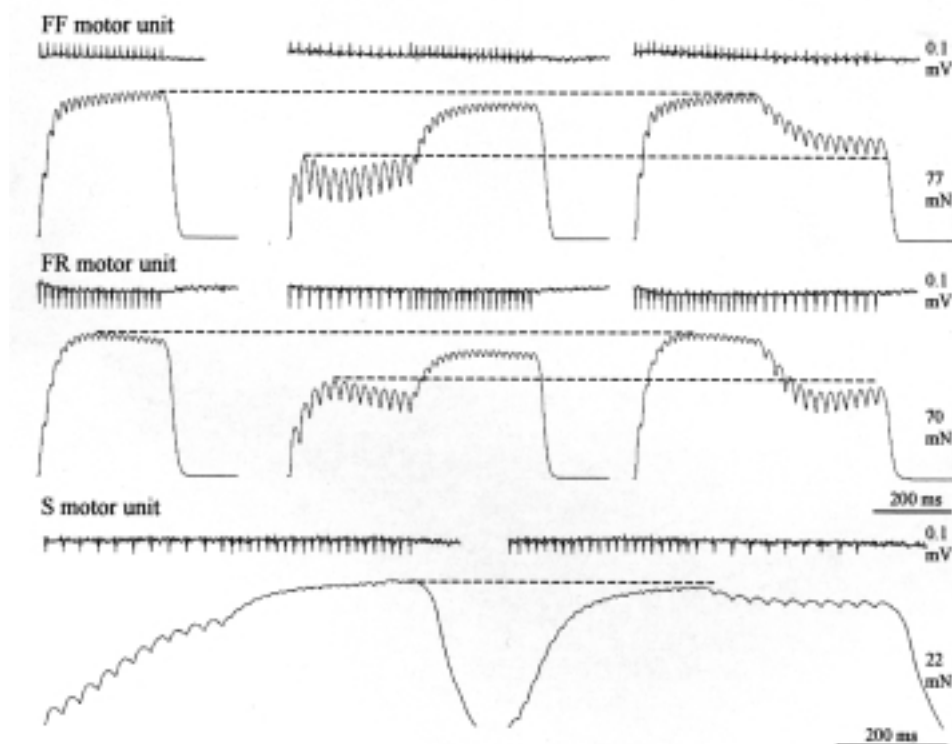


Fig. 1. Examples of FF, FR and S motor unit tetani (lower trace) and muscle fiber action potentials (higher trace) evoked by the applied trains of stimuli. For fast units, the left tetanus was recorded at 60 Hz stimulation, the middle recording is a 40-60 Hz tetanus and the right one is a 60-40 Hz tetanus. The higher dashed horizontal line denotes a peak tension of a 60 Hz tetanus recorded before and after a 40-60 Hz tetanus with a tetanic depression. The lower line denotes a peak tension of a 40 Hz tetanus to make apparent a potentiation (FF unit) or a depression (FR unit) in the 40 Hz part of a contraction of the a 60-40 Hz tetanus. For slow unit the left record is a 15-30 Hz tetanus and the right one is a 30-15 Hz tetanus. The horizontal dashed line denotes a peak tension of the a 15-30 Hz tetanus.

lyzed. The sag profile was visible in 8 FF and 17 FR motor units at 40 Hz stimulation (Fig. 1, FF and FR unit). Moreover, for 5 out of these 17 FR units the sag profile was additionally visible at the second applied frequency i.e. 60 Hz (Fig. 1, FR unit). For slow motor units a tetanic depression was not observed (Fig. 1). The peak tension reached at the two applied patterns of stimulation was the same or slightly (1-3%) lower when the lower frequency followed the higher one (as in the case presented in Fig. 1).

The depression of motor unit contractile tension was observed in several studies, but in all these experiments it was due to fatigue or to the after-effects of fatigue (Jami et al. 1983, Bevan et al. 1993). The described tetanic depression is a phenomenon participating in the production of tension by contracting fast motor units. Therefore, it should be taken into account in discussions concerning motor control processes. It can be expected that when motoneurons increase the firing rate, as at the beginning of activity (De Luca et al. 1996, van Bolhuis et al. 1997, Adam et al. 1998), the tetanic depression can slow the development of the tension. On the other hand, when motoneurons decrease the rate of firing, mechanisms similar to those producing the post-tetanic potentiation (Bagust et al. 1974, Stephens and Stuart 1975) or the catch effect (Burke et al. 1970) can slow down the relaxation and support the developed tension. Therefore, all these phenomena can be responsible for differences in the course of tension-frequency relationships obtained by Binder-Macleod and Clamann (1989) in experiments with a linearly increasing and decreasing frequency of stimulation. A higher tension at the same instantaneous frequency of stimulation was observed when the decreasing frequency was applied. In the present study it was observed that in the 60-40 Hz tetani both phenomena, potentiation or depression, were visible in the 40 Hz part of tetani. Probably the duration of the first part of a two-frequency tetanus can be a factor influencing the final effect visible in the second part: prolonged stimulation at a high frequency could produce more potentiation of the second part of the contraction.

Fast-twitch motor units are characterized by a high sensitivity to a pattern of motoneuronal firing (see Introduction) and therefore they are a group of units well suited to participate in the precise regulation of contractile tension. However, these units reveal a strong dependence on the preceding activity and many factors are responsible for the course of their contraction. The results obtained in the present study reveal that not only the fatigue and the potentiation (Bagust et al. 1974), the

long-lasting after-effects of preceding fatigue (Jami et al. 1983, Bevan et al. 1993) or the catch effect (Burke et al. 1970) must be taken into account as factors influencing the contraction, but the tetanic depression is one of these factors.

The tetanic depression is probably due to events occurring within muscle cells and not at the neuromuscular junction. The motor unit action potentials do not display a reduction in amplitude (Jami et al. 1982, Bevan et al. 1993). The intracellular mechanism responsible for the described tetanic depression is currently unknown. The reduction of intracellular release of calcium, which is a discussed mechanism of a "sag" in unfused tetanus of fast motor units at a constant-rate stimulation (Celichowski et al. 1999), could also be a factor responsible for the analyzed depression. It is even possible that the tetanic depression has a similar cause as the sag. However, it is intriguing that in some units the sag was observed in two-frequency tetani twice, at the lower and then at the higher frequency of stimulation.

Concluding, the phenomenon of tetanic depression which occurs during unfused fast motor unit contractions was found. This depression is a mechanism which can participate in the production of tension of fast motor units.

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