

Changes in the Compound Muscle Action Potentials (CMAPs) of Rats Following High Frequency Repetitive Stimulations : Differences in Incremental Responses in Amplitude between Two Different Muscles

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ABSTRACT. Although it has been known that high frequency repetitive stimulations of the peripheral nerves of normal humans at more than 10 Hz lead to gradual increases in the amplitude of their compound muscle action potentials (CMAPs), the mechanism of this phenomenon remains unknown. Our previous studies confirmed the occurrence of this phenomenon in normal rats, and it was suggested that changes in the shape of muscle fibers were related to its appearance. In the present study, we exposed two kinds of muscles (the soleus and gastrocnemius muscles) from 70 normal rats, and stimulated the nerves electrically at two different frequencies; i.e., at 2 and 20 Hz. We compared the changes in the amplitude evoked by 8 stimuli at two different frequencies between the muscles. These two muscles were also examined histochemically. The soleus is composed primarily of type 1 muscle fibers (slow twitch fibers), whereas the gastrocnemius consists primarily of type 2 muscle fibers (fast twitch fibers). The relationships between the percentages of each muscle fiber type and increasing rates in the amplitude of CMAPs were analyzed. Following high frequency (20 Hz) stimulation, the rates of increase in amplitude were greater in the soleus than that in the gastrocnemius. In both muscles, the increasing rates in amplitude correlated well with the percentage of type 1 fibers. It is supposed that muscles rich in type 1 fibers experience greater changes in shape perpendicular to the muscle fiber arrangement following high frequency stimulations, because the speed of contraction and relaxation is slower in these muscles. Based on our findings that the rates of increase in the amplitude of CMAPs were greater in muscles with more type 1 fibers, it is suggested that the normal incremental responses in the amplitude of CMAPs following high frequency stimulations may be attributed to changes in the shape of the muscle fibers.

Key words : CMAPs — muscle fiber types — incremental responses —
high frequency stimulations

Changes in compound muscle action potentials (CMAPs) following repetitive electrical stimulations of peripheral nerves were first reported in 1895 by Jolly as means for diagnosing myasthenia gravis.¹⁾ According to his report, repeated application of faradic currents at short intervals resulted in a sharp decrease in the amplitude of CMAPs in patients with myasthenia

gravis. Harvey and Masland *et al* gave the term “waning” to this decrease in CMAPs.²⁾ In 1957, Lambert and Eaton *et al* applied such repetitive stimulations to patients with Lambert-Eaton myasthenic syndrome (LEMS), which is characterized by muscular weakness in the extremities resembling the symptoms of myasthenia gravis. However, the changes in CMAPs amplitude were found to be in the opposite direction; i.e., there was a gradual increase in CMAPs (a waxing phenomenon).³⁾

Although the repetitive stimulation test is an important clinical evaluation method used for the diagnosis of the diseases described above, the waxing phenomenon of CMAPs can be observed even in healthy individuals following repetitive stimulations at more than 10 Hz.⁴⁻¹³⁾ The mechanism of this phenomenon remains unclarified, although the following precautions have been advised when performing repeated stimulation tests: (1) the stimulus intensity must be supramaximal; (2) the recording electrode must be placed in the center of the muscle belly and the reference electrode must be placed over the distal tendon (belly-tendon recording); (3) the recording electrode must be in close contact with the surface of the muscle; (4) the stimulation electrode must be tightly fixed; (5) muscle contractions must be minimized during the test.¹⁰⁾

Studies conducted in our Department since 1997 have demonstrated that high frequency repetitive stimulations induce the waxing phenomenon of CMAPs in normal rats, as seen in humans. It was suggested that changes in the shape of muscle fibers were related to the appearance of the phenomenon.¹⁴⁾ The ulnar nerve was stimulated repetitively at frequencies of 2 or 20 Hz, and eight CMAPs were recorded from a surface electrode placed on the flexor carpi ulnaris muscle in anesthetized normal rats, and increasing rates in the amplitude of CMAPs were calculated by 8th wave/1st wave. Incremental responses in CMAPs amplitudes were observed notably in the anesthetized rats at the frequency of 20 Hz, and they disappeared following the administration of magnesium sulfate or dantrolene sodium. Magnesium inhibits the inflow of calcium ions into nerve endings and thus suppresses the release of acetylcholine and muscular contractions. Dantrolene inhibits the release of calcium ions from the sarcoplasmic reticulum and thus also suppresses muscular contractions. Considering that the administration of magnesium sulfate or dantrolene sodium, which decreases contractions and the relaxation of muscle fibers, inhibits the incremental responses of CMAPs, it seems that they may be attributed to changes in the shape of the muscles, which serve as generators.

Increasing rates in the amplitude of CMAPs in human muscles evoked by high frequency repetitive stimulations have been reported to be greater in the upper extremities than in the lower extremities.¹⁵⁾ In other words, the incremental responses differed among different skeletal muscles in the same individuals. Incremental responses in the same kind of muscles may also differ among different individuals. In consideration of the fact that the soleus and gastrocnemius muscles differ greatly from each other in terms of the percentages of muscle fiber types (fast twitch fibers and slow twitch fibers), in the present study, we compared the increasing rates in CMAPs amplitudes, evoked by repetitive stimulations between these two muscles. We also examined the muscles histochemically and analyzed the relationships

between the percentages of muscle fiber types and the increasing rates of CMAPs following high frequency repetitive stimulations.

MATERIALS AND METHODS

Seventy male Wistar rats were used for this study. Twenty rats, weighing 226 g on average (range: 185-250 g) and 7.5 weeks old on average (range: 7-10 weeks), were used for the first experiment. Fifty rats, weighing 237 g on average (range: 190-260 g) and 7.8 weeks old on average (range: 7-11 weeks), were used for the second experiment. Prior to the study, approval of the Experimentation Committee of Kawasaki Medical School was obtained (approval No. 99-122 and 02-082) regarding treatment of the experimental animals and the protocol of the experiments, pursuant to the ethical rules of the school. All animals were reared in animals quarters where the temperature was kept at 25°C and the light of the room was switched on for 14 hours each day. The animals were allowed free access to a solid diet (MF, Oriental Yeast Co.) and water. The experimental methods we employed were as follows.

The animals were anesthetized intraperitoneally with 30 mg/kg of pentobarbital sodium (Nembutal®). The experiment was started after confirming with painful stimuli that adequate depth of anesthesia had been induced. Adequate depth of anesthesia was maintained to avoid the influence of voluntary contraction during the experiment.

To expose the nerves and muscles, the skin on the posterior area of bilateral lower extremities was incised extensively, and the fascia of hamstrings were torn in the middle. Connective tissue was exfoliated gently between the biceps femoris and the semitendinosus muscles with the fingers to avoid injury to the nerves and vessels, and the entire gastrocnemius muscle, the sciatic nerves, tibial nerves, and common peroneal nerves were exposed. The common peroneal nerve of each animal was cut at its bifurcation of the sciatic nerve to remove the influence of volume conduction from other muscles when stimulating the tibial nerve.

To minimize the motion of the joints caused by the contraction of the gastrocnemius, an indwelling venous needle (Elaster F®; 18 G, 51 mm, Hakko-Denki Co.) was inserted from the anterior plane of the tibia into the femur, and the knee joint was fixed at an angle of about 90 degrees. The ankle joint was manually fixed at an angle of about 0 degrees.

Experiment 1 : Relationship between the frequency of stimulations, muscle types, and increasing rates of CMAPs amplitude

Before CMAPs of the gastrocnemius were measured, the soleus below the gastrocnemius was resected. The stimulation electrode was placed on the tibial nerve, the recording electrode was fixed on the middle of the medial head of the gastrocnemius, and the reference electrode was fixed on the common calcaneal tendon (belly-tendon recording) (Fig 1a). A pair of hook-shaped electrodes (OH99-046, Unique Medical) were used as the stimulation electrode (Fig 1b). The recording and reference electrodes used in this study were order-made surface electrodes under which a fishhook was attached for fixation (OS95-093A, Unique Medical). Each surface electrode

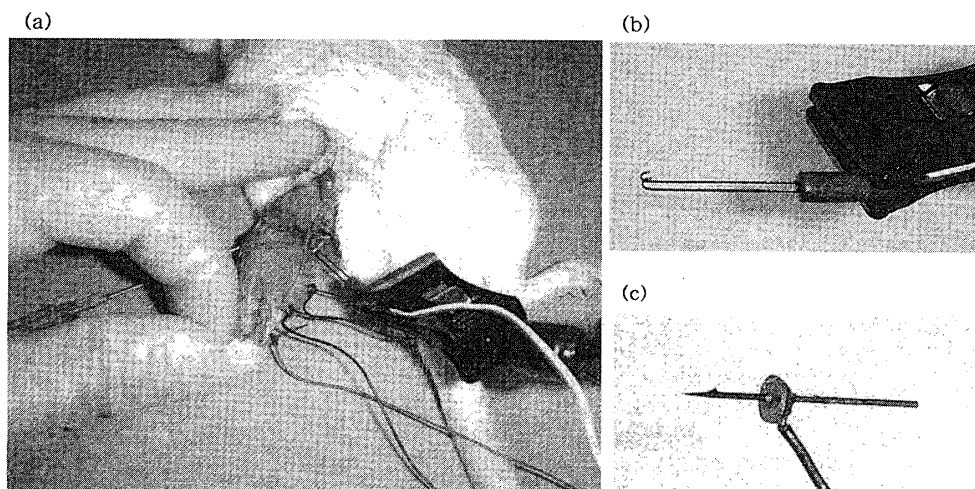


Fig 1. Placement of electrodes (a), including the stimulation electrode (b), the pick-up electrode or reference electrode (c). CMAPs were recorded from the gastrocnemius in this case. The stimulation electrode was placed on the tibial nerve, the recording electrode was fixed on the middle of the medial head of the gastrocnemius, and the reference electrode was fixed on the common calcaneal tendon.

was 3 mm in diameter (Fig 1c).

The CMAPs from the soleus were measured on the side opposite to that used for measurement from the gastrocnemius. Before measuring the CMAPs of the soleus, the same side of the gastrocnemius was resected, and the soleus and the nerves branching into the muscle were exposed. The stimulation electrode was placed on the nerve branch in the soleus, the recording electrode was fixed on the middle of the soleus, and the reference electrode was fixed on the common calcaneal tendon (belly-tendon recording).

Each nerve was stimulated repetitively supramaximally eight times, and eight CMAPs were evoked. The duration of the electrical stimulation was 0.2 msec, and the stimulation frequencies were 2 Hz and 20 Hz. Low and high frequency filters used to record the CMAPs were 20 Hz-3 KHz. The Neuropack-4 (Nihon Kohden Co.) was used for recording action potentials in this study, and the program of analysis was in the repetitive stimulation mode.

After recording CMAPs, peak-to-peak amplitudes were measured. Increasing rates (%) in the amplitude of the CMAPs evoked by repetitive stimulations were calculated by the 8th wave/1st wave \times 100 for the two muscles. The paired-t test was used to compare the eighth to the first waves following stimulations at each frequency for each muscle. The increasing rates of the soleus were compared with those of the gastrocnemius at a frequency of 20 Hz using the paired t-test. Values were expressed as means \pm the standard deviation (SD). Statistical significance was defined as a p-value of less than 0.05. StatView-J 4.11 for Macintosh was used for statistical analysis.

Experiment 2: Relationship between the percentages of muscle fiber types and the increasing rates in the amplitude of CMAPs

The method used to record CMAPs in the first experiment was also

used to record them from the soleus and gastrocnemius muscles in the second experiment. The stimulation frequency used in the second experiment was 20 Hz, the higher of the two frequencies used in the first experiment.

Immediately after the measurement of CMAPs, the medial heads of the gastrocnemius and the soleus were extracted. The muscle samples were stood upright on a piece of cork using tragacanth gum mixed with water, and were frozen in isopentane cooled adequately in advance in liquid nitrogen. The frozen samples were cut with a cryostat (-20°C) into 10 mm sections. Reduced nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-TR) staining was used to distinguish different muscle fiber types. The numbers of muscle fibers of each type were counted and the percentage of type 1 fibers was calculated in each muscle sample. Simple regression analysis was performed to test the correlation between the percentage of type 1 fibers and the increasing rates in the amplitude of CMAPs following repetitive stimulations at a frequency of 20 Hz in each muscle. StatWiew-J 4.11 for Macintosh was used for statistical analysis.

RESULTS

Experiment 1 : Relationship between the frequency of stimulations, muscle types, and increasing rates of CMAPs amplitude

The mean increasing rate in amplitude of CMAPs was $99.8 \pm 0.8\%$ for the soleus and $100.4 \pm 0.6\%$ for the gastrocnemius during repetitive stimulations at 2 Hz. The amplitude of eight CMAPs did not change significantly. Following high frequency stimulations at 20 Hz, both muscles showed significant increases in the amplitude of 8th wave of CMAPs, with the increasing rate being $119.2 \pm 20\%$ for the soleus and $110.0 \pm 0.8\%$ for the gastrocnemius. Figure 2 shows examples of incremental responses in the amplitude observed in both muscles. Gradual increases in the amplitude of the CMAPs are evident in the figure.

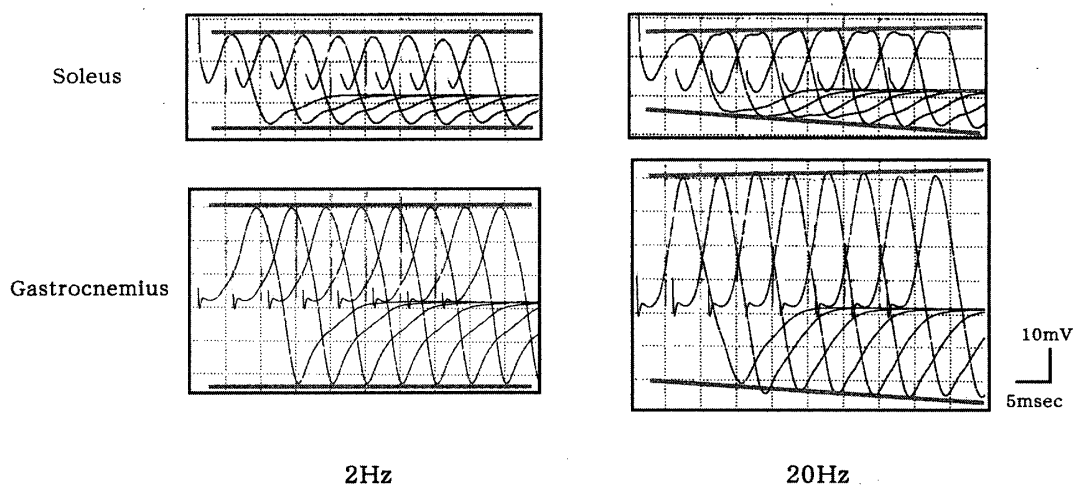


Fig 2. Examples of CMAPs following repetitive stimulation. The amplitude of CMAPs did not change significantly at 2 Hz. Following high frequency stimulations at 20 Hz, both muscles showed significant gradual increases in the amplitude of the CMAPs.

TABLE 1. Increasing rates in the amplitude of CMAPs (%)

Stimulation frequency Muscles	2 Hz	20 Hz
Soleus	99.8±0.8	119.2±2.0
Gastrocnemius	100.4±0.6	110.0±0.8

Increasing rates in the amplitude of CMAPs (%) = [amplitude of 8th wave of CMAPs (mV)/amplitude of 1st wave of CMAPs (mV)] × 100.
Double asterisk (**) shows $p < 0.01$ ($n = 20$).

The increasing rates in the amplitude of the CMAPs following stimulations at 20 Hz were significantly greater for the soleus than that for the gastrocnemius (Table 1).

Experiment 2 : Relationship between the percentages of muscle fiber types and the increasing rates in the amplitude of CMAPs

Most of the muscle fibers in the soleus were type 1 fibers that were stained dark by NADH-TR staining. The gastrocnemius had few type 1 fibers, and was mainly composed of light stained muscle fibers (type 2B) and intermediately stained fibers (type 2A) (Fig 3).

The mean percentage of type 1 fibers among all the fibers of the soleus ($n = 50$) was $82.1 \pm 5.6\%$ (range : 70.3-94.2%), while that for the gastrocnemius ($n = 50$) was $25.4 \pm 3.2\%$ (range : 20.0-31.9%). The increasing rates in the amplitude of the CMAPs following stimulations at a frequency of 20 Hz were significantly greater for the soleus ($121.4 \pm 3.9\%$; range 116.0-132.1%) than for the gastrocnemius ($109.6 \pm 1.8\%$; range 106.0-113.9%), similar to the results observed in the first experiment.

There was a significant positive correlation between the percentage of type 1 fibers and the increasing rates in the amplitude of the CMAPs for the soleus ($r = 0.79$, $p < 0.01$). The means that the increasing rates became greater as the percentage of type 1 fibers increased (Fig 4). There was a

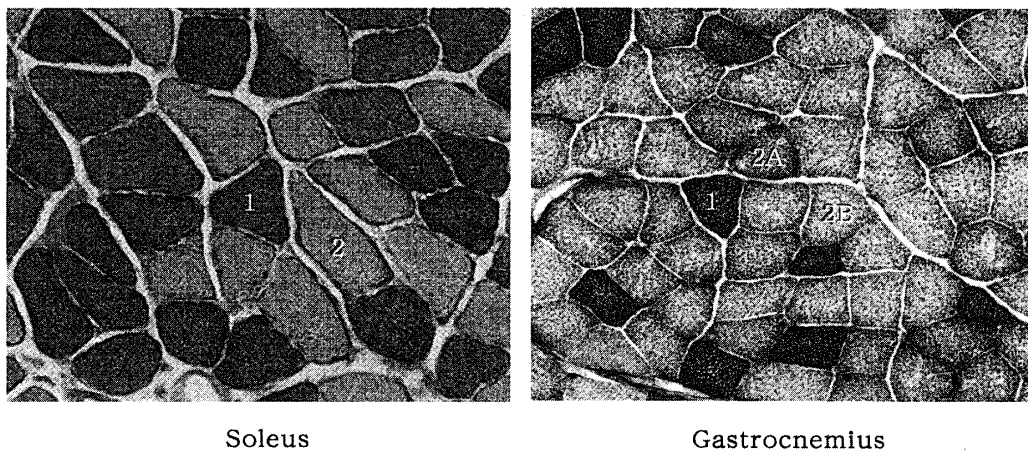


Fig 3. Examples of NADH-TR staining ($\times 200$). The dark stained fibers are type 1, light stained fibers are type 2B, and intermediately stained fibers are type 2A.

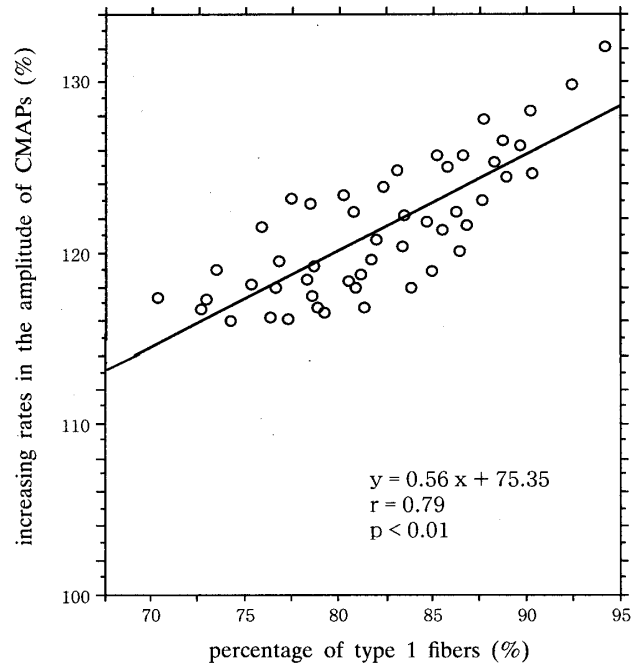


Fig 4. Relationship between the percentage of type 1 fibers and increasing rates in the amplitude of CMAPs in the soleus.

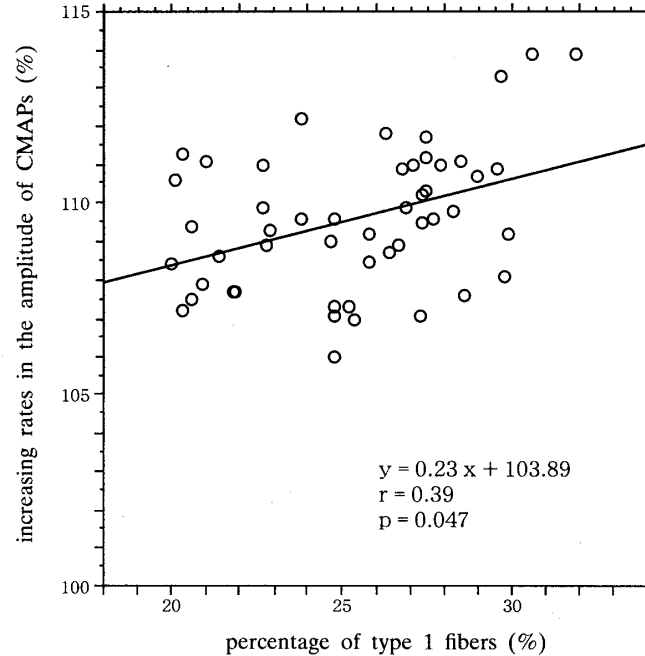


Fig 5. Relationship between the percentage of type 1 fibers and increasing rates in the amplitude of CMAPs in the gastrocnemius.

weak but significant positive correlation between the percentage of type 1 fibers and the increasing rates in the amplitude of the CMAPs for the gastrocnemius ($r=0.39$, $p=0.047$) (Fig 5).

DISCUSSION

Skeletal muscles are divided by their color into red and white muscles. Red muscles are physiologically characterized by an abundance of slow twitch fibers, while white muscles are abundant in fast twitch fibers. Histochemically, the slow twitch fibers are called type 1 muscle fibers and are rich in mitochondria and fat, with high oxidative enzyme activity. The fast twitch fibers are called type 2 muscle fibers and are rich in glycogen, with high anaerobic glycolytic enzyme activity.¹⁶⁾ Regarding the percentages of type 1 and 2 fibers in humans, it is known that most skeletal muscles are composed of a mosaic-like distribution of the two types of muscle fibers, and do not show groups of any particular type of muscle fibers. However, there are some exceptional muscles in which either type 1 or type 2 fibers are predominant.¹⁶⁾ In animals other than humans, however the percentages of muscle fiber types vary considerably from species to species.¹⁷⁻¹⁹⁾

The present study was based on previous findings that repetitive stimulations of the peripheral nerves at frequencies higher than 10 Hz resulted in incremental responses in the amplitude of CMAPs even in healthy individuals, and that the increasing rates differed between the upper and lower extremities. Since the percentages of muscle fiber types are well known for rats, we examined the relationship between the percentages of these types and the incremental responses in the amplitude of the CMAPs for two kinds of muscles in which the percentages were notably different.

When the peripheral nerves were stimulated repetitively at a frequency of 2 Hz, there were no significant changes in the amplitude of the CMAPs for the two muscles. Their amplitude, however, increased gradually following high frequency stimulations at 20 Hz. The incremental responses were significantly greater for the soleus muscle, in which type 1 fibers were predominant, than that for the gastrocnemius, in which type 2 fibers were predominant. The percentages of type 1 fibers in the soleus varied considerably from individual to individual (70.3-94.2%). The increasing rates in the amplitude of the CMAPs became greater as the percentage of type 1 fibers increased. A positive correlation between the percentage of type 1 fibers and the increase in the amplitude of the CMAPs was also shown in the gastrocnemius. These results suggest that the mechanism for the incremental responses in the amplitude of the CMAPs observed in normal muscles is dependent on the percentage of muscle fiber types.

It is supposed that change in the shape of fibers are greater following high frequency stimulations in type 1 fibers, because the speed of contraction and relaxation is slower in these fibers. Since the intervals between two neighboring stimuli are short during high frequency stimulations, the next contraction is induced before the relaxation is completed following the previous stimulus in type 1 fibers. As a result, muscle fibers may remain completely contracted at the end of repetitive stimulations. On the other hand, type 2 fibers contract faster, and become relaxed before the next stimulus reaches them, even if the interstimulus interval is short. Since the soleus is rich in type 1 fibers, it is supposed that the muscle swells more greatly in the direction perpendicular to the

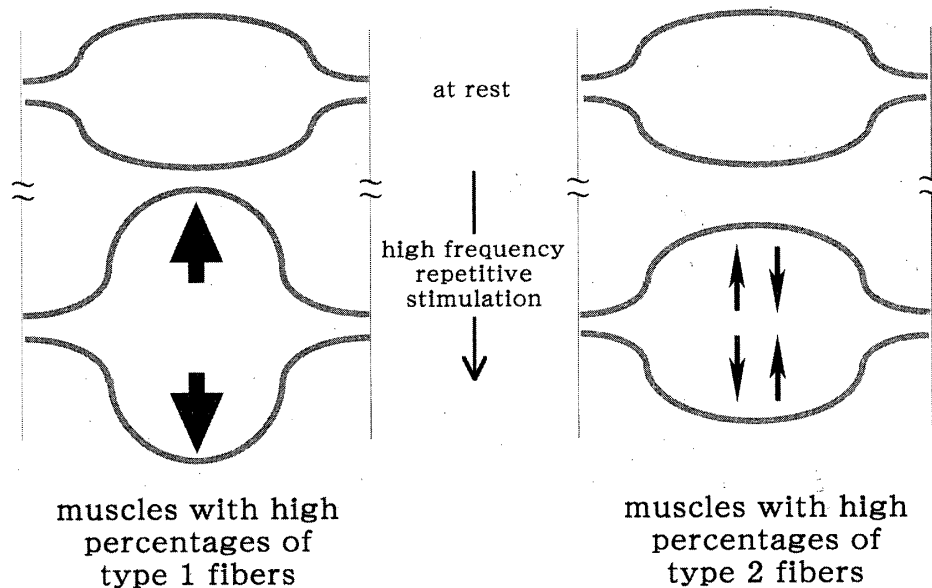


Fig 6. Changes in the shapes of muscle fibers as a result of high frequency stimulation. It is supposed that changes in the shape of fibers are greater following high frequency stimulations in type 1 fibers. Arrows mean the amount of contraction; large arrows indicate greater changes in the shape of muscle.

fiber arrangement (Fig 6). Because there were significant positive correlations between the percentage of type 1 fibers and the increasing rates in the amplitude of the CMAPs following high frequency repetitive stimulations, it is suggested that the incremental responses in the amplitude of the CMAPs in normal muscles are caused by stimulation-induced changes in the shape of the muscles. This is quite consistent with the finding mentioned above that the incremental responses in the amplitude of the CMAPs were suppressed by the administration of magnesium sulfate or dantrolene sodium. These agents decrease the contraction and relaxation of muscle fibers, and inhibit changes in the shape of muscle fibers. In particular, dantrolene sodium suppresses incremental responses in amplitude without reduction in the amplitude of the CMAPs, since the agent does not inhibit the appearance of membranous potentials.²⁰⁾

Integration or frequency analysis of surface electromyography during voluntary contraction of muscles is sometimes used as a means of measuring changes in muscle contractility in the field of rehabilitation medicine. These measurements are based on the assumption that the amplitude and duration of the action potentials of each muscle fiber remain unchanged even when the contraction force changes. In the present study, however, it was revealed that the action potentials of individual muscle fibers changed in amplitude when the muscle contracted at high frequencies. This suggests that conventional measurements using surface electromyography might not be valid, and further studies are needed to prove this hypothesis.

The effects of training or disuse on muscles are important concerns in rehabilitation medicine. There is a consensus that muscle fibers become hypertrophied following training and become atrophied following disuse. There is, however, no widely accepted view concerning how each type of muscle fiber changes. Therefore, our results may have implications for

research on the effects of training and disuse on muscles.

CONCLUSIONS

In the present study using rats, we analyzed the relationship between increasing rates in the amplitude of the CMAPs following high frequency repetitive stimulation of peripheral nerves and the percentages of muscle fiber types. The following novel findings were made: 1) The increasing rates in the amplitude of the CMAPs following high frequency repetitive stimulation were greater for the soleus, which is rich in type 1 fibers, than in the gastrocnemius, which is rich in type 2 fibers; 2) The increasing rates in amplitude became greater as the percentage of type 1 fibers increased; 3) The incremental responses in the amplitude of the CMAPs observed in response to high frequency repetitive stimulation were attributed to changes in the shape of the muscles.

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