Effect of Hindlimb Unloading Induced Atrophy on Muscle Afferents in Rat Soleus

Hidemi FUJINO\textsuperscript{1}, Hisaharu KOHZUKI\textsuperscript{1}, Isao TAKEDA\textsuperscript{1}, Hiromitsu TASAKI\textsuperscript{1}, Hiroyo KONDO\textsuperscript{2}, Torao ISHIDA\textsuperscript{2}, Fumihiko KAJIYA\textsuperscript{3}

\textsuperscript{1}Department of Physical Therapy, Faculty of Health Science, Suzuka University of Medical Science
\textsuperscript{2}Institute of Traditional Chinese Medicine, Suzuka University of Medical Science
\textsuperscript{3}Department of Cardiovascular Physiology, Okayama University Graduate School of Medicine and Dentistry

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Abstract

The changes of muscle afferents during stimulation of ramp and hold stretching were studied in the rat soleus muscle with hindlimb unloading induced atrophy. Ramp and hold stretch of the muscle induced by passive motion of ankle joint. Muscular atrophy was induced by the hindlimb unloading for two weeks (HU). The HU resulted in a significant reduction of muscle weight (p < 0.05). Muscle afferents discharge in the resting state in the 0° ankle position was 48±16 imp/ sec in the HU and 27±2 imp/ sec in the control (CONT), being increased in the HU. Dynamic responses immediately after dorsiflexion of the ankle to 90° were 229±22 imp/ sec in the HU and 146±8 imp/ sec in the CONT group, being significantly increased in the HU group (p < 0.05). These results indicate that following muscular atrophy achieved through two weeks of HU, the muscle afferents were changed. We suggest that awareness of changes of proprioceptive sensibility could provide a useful clue for advancing various therapeutic programs for physical therapy of patients with atrophic muscles.
INTRODUCTION

Rat hindlimb unloading is a popular model to investigate the atrophy of slow twitch muscle. This model introduced by Morey and induced that decreases in the muscle fiber area and the quantity of myogenic proteins, transformation to fast type of the myosin heavy chain type and changes in contraction functions such as twitch and tetanus. Also, many of these reports concerned the contraction function, morphology, and biochemistry of skeletal muscle fibers, and little is known concerning changes due to disuse atrophy including those of the nervous system. There are only a few studies on muscle afferents (Group Ia nerve activities). Reports to date on muscle spindle functions in atrophied skeletal muscles include increases in muscle afferents at rest in muscles atrophied by cast fixation and marked changes in denervated and tenotomized muscles. All these results have been obtained in separated muscles or by pulling one end of the muscle, and details of in vivo muscle afferents associated with joint motions remain unknown.

In this study, therefore, we measured muscle afferents associated with joint motions, and analyzed what changes occur in muscle afferents in muscles atrophied by disuse caused by hindlimb unloading.

MATERIALS AND METHODS

1) Animals

Male ten-week-old Wistar rats with an average initial body weight of 293±3 g were used. The experiments were conducted in accordance with a guiding principles for the care and use of animals in the field of physiological sciences of the physiological society of Japan. Fourteen male rats were randomly divided into a control group (n=5, CONT) and an atrophy group (n=5, HU).

2) Muscle atrophy procedure

In the HU group, the buttocks were lifted by a jacket made of a soft material worn from the trunk to the pelvic zone according to the method of Musacchia et al.. The animals of both groups were cared in individual cages throughout the study, being given food and water ad libitum. After a 2-week hindlimb unloading period, the animals were anesthetized with pentobarbital (50 mg/kg, i.p.), and muscle afferents of the soleus muscle were measured. After the end of the experiment, the wet weight of soleus muscle was measured.

3) Measurement of muscle afferents

After tracheotomy, heparin (1,000 U/kg, i.p.) was administered under artificial ventilation, and a cannula was inserted into the carotid artery for monitoring of the systemic blood pressure. Next, the nerve supplying the soleus muscle was identified where it entered the soleus muscle under the stereoscopic microscope, exposed up to the division of the tibial nerve, and cut. The perineurium at its distal end was detached, the exposed nerve was placed on a platinum bipolar electrode, and afferent nerve activities were drawn in situ. The nerve and electrode were kept warm by filling fluid paraffin and petrolatum in a pool prepared by incising the skin and lifting it. The activities detected were amplified with a bioelectricity amplifier (AVB-21, Nihon Kohden), impulses that responded stretch stimulation were selected with a wind slicer (EN-601J, Nihon Kohden), and converted to pulse waves. The spike frequency per unit time was determined from these pulse waves. Also, to discriminate them
from impulses from the tendon organs, cessation of the activities during twitch was confirmed⁹. The analogue signals of afferent nerve activities, pulse waves, and frequency of discharge were converted to digital signals at 20 kHz (Powerlab 16/sp, ADInstrument) and analyzed with a personal computer (PowerMac G4, Apple computer).

The ankle was moved passively using a rat exerciser, which was a computer-controlled apparatus prepared in cooperation with Okayama University of Science. After the foot and crus of the rat were fixed to the exerciser, programmed ramp exercise was conducted. In this study, the maximum plantar flexion of the ankle was regarded as the 0° ankle position, and 90° dorsiflexion was performed starting at this position. The program of ramp exercise was maintaining the 0° position for 5 seconds, dorsiflexion from 0° to 90° at an angle velocity of 90°/sec, maintaining the 90° dorsiflexed position for 5 seconds, and returning to 0° at the same angle velocity. The signals of joint angle were also converted to digital signals at 20 kHz similarly to afferent nerve activities and input into the personal computer. In this study, afferent nerve activities from the soleus nerve during passive movements of the ankle were expressed as the frequency of discharge per second (imp/sec).

4) Statistical analyses

The data were expressed as the mean ± standard error of each group. The relationship between the joint angle and the frequency of discharges in each group was examined by regression analysis, and a regression equation was obtained. Comparisons among the groups were made by the Mann-Whitney U-test. A probability of less than 5% was regarded as significant.

RESULTS

1) Wet weight of soleus muscle

The wet weight of the soleus muscle was 97±10 mg in the HU group and was significantly smaller than 198±9 mg in the CONT group.

2) Changes in muscle afferents

Muscle afferents discharge in the resting state in the 0° ankle position was 48±16 imp/sec in the HU group and 27±2 imp/sec in the CONT group, being increased in the HU group (Figs. 2A, C and 3A). Dynamic responses immediately after dorsiflexion of the ankle to 90° were 229±22 imp/sec in the HU

![Fig. 1 Representative example of the muscle afferents during motion of ankle joint](image-url)

The top row represents muscle afferents and the bottom row joint motion ankle. The upper column (A) represent muscle afferents during dorsiflexion of ankle joint (0° → 90°) and the lower column (B) during plantar flexion (90° → 0°).
group and 146±8 imp/sec in the CONT group, being significantly increased in the HU group (Fig. 3B). Static responses when 90° flexion of the ankle was maintained were 209±24 imp/sec in the HU group and 90±15 imp/sec in the CONT group, being significantly increased in the HU group similarly to dynamic responses (Fig. 3B). However, when dynamic and static responses were compared, static responses were significantly smaller than dynamic responses in the CONT group, but static responses were similar to dynamic responses in the HU group (Fig. 3B).

**DISCUSSION**

In this study, changes in muscle afferents associated with disuse atrophy of the muscle by hindlimb unloading were evaluated by measuring the responses at rest and responses during joint

![Fig. 2 Representative example of the effect of hindlimb unloading.](image)

The left and right columns represent muscle afferents at ankle joint 0° (A, C) and 90° (B, D), respectively. The top row represents muscle afferents of CONT and the bottom row HU muscle.

![Fig. 3 Influence of hindlimb unloading on muscle afferents](image)

Values are means ± SEM. Values obtained from soleus muscles of CONT (open bar) and HU (closed bar).

A: The frequency of muscle afferent activity per minute, measured at ankle joint 0 degree.

B: The frequency of muscle afferent activity per minute, measured during ramp (Dynamic) and hold (Static) stretching soleus muscle at ankle joint 90 degrees.

* Significantly different from control mean (P<0.05). ** Significantly different from dynamic mean (P<0.05).
motions. When the ankle was rested at 0°, muscle afferents were increased in the HU group compared with the CONT group (Figs. 2 and 3). The 0° ankle position was plantar flexion, in which the soleus muscle was relaxed. Therefore, muscle spindles are considered to have discharged spontaneously and not to have been responding to mechanical stimulation. In this study, the frequency of discharge of soleus muscle afferents was increased in the relaxed resting state in the HU group. Matthews reported that muscle afferents in the resting state are controlled by the γ system, but the effect of the γ system is excluded in this study, because the muscle was denervated. Other possibilities are a decrease in the threshold of discharge or an increase in the sensitivity of muscle afferents. Nordstrom et al. observed a similar increase in muscle afferents in cats. These results suggest that muscle afferents themselves develop functional changes when the muscle is atrophied.

Next, analysis of changes in muscle afferents associated with joint motions showed significant increases in muscle afferents associated with progressive dorsiflexion of the ankle in the CONT group (Fig. 3). In the HU group, however, muscle afferents increased more markedly than in the CONT group. Thus, in the HU group, muscle afferents may have responded excessively to joint movements, with impairment of the position and motor sensibilities.

We considered that these phenomena are caused by the following two factors. The first is the possibility of functional changes in muscle spindles themselves associated with muscle atrophy, and the second is the possibility that muscle afferents are dependent on mechanical properties of the skeletal muscle, because muscle spindles are organs that are embedded in muscle tissues and are part of them.

Boyd et al. showed that viscoelasticity of the muscle is a major factor in the occurrence of dynamic index in muscle afferents and explained responses of muscle afferents in a skeletal muscle stretched in its entirety using a mechanical model. Extrafusal fibers, which have a much greater quantity of contractile components than intrafusal muscle fibers, show marked viscosity when stretched. Therefore, if viscosity is assumed to be in parallel with elasticity, only elasticity can be considered in intrafusal fibers, particularly, at sensory nerve terminals. When this model is stretched, it shows responses resembling those of isolated muscle spindles, but the values of dynamic and static components are unequal because of the difference in viscoelasticity. In summary, adaptation of the frequency of muscle afferents is considered to be dependent primarily on viscoelastic characteristics of muscle fibers including both extrafusal and intrafusal fibers.

From these observations, we hypothesized that these phenomena are related to viscoelasticity of the muscle. According to Boyd, also, characteristics of muscle afferents are considered to depend primarily on viscoelastic properties of intrafusal and extrafusal muscle fibers. Therefore, the increase in the muscle viscoelasticity in the HU group may have affected afferent nerve activities.

In conclusion, muscle afferents associated with joint movements were markedly changed in muscles atrophied by hindlimb unloading. Muscle afferents are important organs in the motor control mechanism, and these changes may impair the control of body movements.

REFERENCES

1) Thompsem, P., and Luco, JV. Changes of weight and neuromuscular transmission in mus-
4) Horsley, V. Sort note on sense organs and on the preservation of muscle spindles in conditions of extreme muscular atrophy, following the section of the motor nerve. Brain 20 : 375–376, 1897.