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COMPLEX SYSTEMS BIOPHYSICS

Effect of Vibrostimulation of Foot and Supporting Afferentation on Functional State of Shin Muscles in Rats during Hindlimb Unloading

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Abstract—The goal of this work was to study the influence of daily 3-hr vibrostimulation of the supporting zones of the foot and 3-hr of usual locomotion on the functional state of the soleus, gastrocnemius, and tibial anterior muscles in rats after 7 and 14 days of hindlimb unloading. After 7 days of unloading the soleus weight decreased, while the weight of gastrocnemius and tibial anterior remained unchanged. After 14 days of hindlimb unloading a weight reduction was observed in all studied muscles. Vibrostimulation of the foot and supporting afferentation prevented the loss of weight in gastrocnemius and tibial anterior during the first week of unloading and reduced the negative effect of hindlimb unloading in 14 days. Our results demonstrate that the amplitude of the *M*-response after 7 days of hindlimb unloading was decreased predominantly in soleus as compared to gastrocnemius and tibial anterior. By the 14 day we observed restoration of the amplitude of the *M*-response in gastrocnemius and tibial anterior, and the increased amplitude of the *M*-response in soleus. Different techniques for foot stimulation in rats during hindlimb unloading demonstrated the positive effect in regard to *M*-response restoration – the amplitude of the *M*-response was increased in all studied muscles. Thus, our results demonstrate that foot vibrostimulation or supporting afferentation can completely prevent atrophy caused by hindlimb unloading in gastrocnemius and tibial anterior and decrease atrophy in soleus in rats.

Keywords: hindlimb unloading, *M*-response, muscle atrophy, vibrostimulation, supporting afferentation

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INTRODUCTION

Mechanical unloading of skeletal muscles during spaceflights or their ground analogs, such as confinement to bed and hindlimb unloading in rodents, causes atrophy of skeletal muscles, which especially affects the antigravity musculature of lower limbs [1, 2]. Atrophy is characterized by reduction of muscle volume, muscle mass and force, change in histochemical characteristics and expression of contractile proteins, and also reduction of neuromuscular function [3–9]. Effective counteraction to these changes in the motor system has decisive significance for success in space development. Three decades ago a laboratory model of muscle unloading was elaborated – model of antiorthostatic suspension, which is used in various laboratories in order to determine the role of microgravity in muscle atrophy [10]. The model of hindlimb unloading in rat is characterized by a state of hypodynamia–hypokinesia ("suspension" of hind limbs and reduction of motor activity), which imitates some of the effects of weightlessness that are observed in conditions of spaceflight [1, 11].

The magnitudes of these changes (muscle atrophy, muscle force reduction, changes in type of fiber and electrophoretic profiles) come to be specific for muscles and fiber types: greatest in extensors, such as soleus muscle, which usually executes antigravity postural functions [1, 12]. Several different training programs and means of stimulation of foot support receptors have been proposed in order to reduce or prevent muscle atrophy during unloading [13–19]. Nonetheless the used approaches have failed to completely prevent muscle atrophy caused by conditions of unloading. The aim of the present investigation was determination of functional and morphological characteristics of rat hindlimb muscles in conditions of modeled gravitational unloading, and also unloading combined with various means of stimulating the foot support receptors.

EXPERIMENTAL

The investigation was conducted on 41 non-line laboratory rats of 230–260 g mass. All manipulations with animals were conducted with observance of the rules of humane treatment of laboratory animals and with observance of bioethical norms. Sustenance, feeding, looking after animals and taking them out of

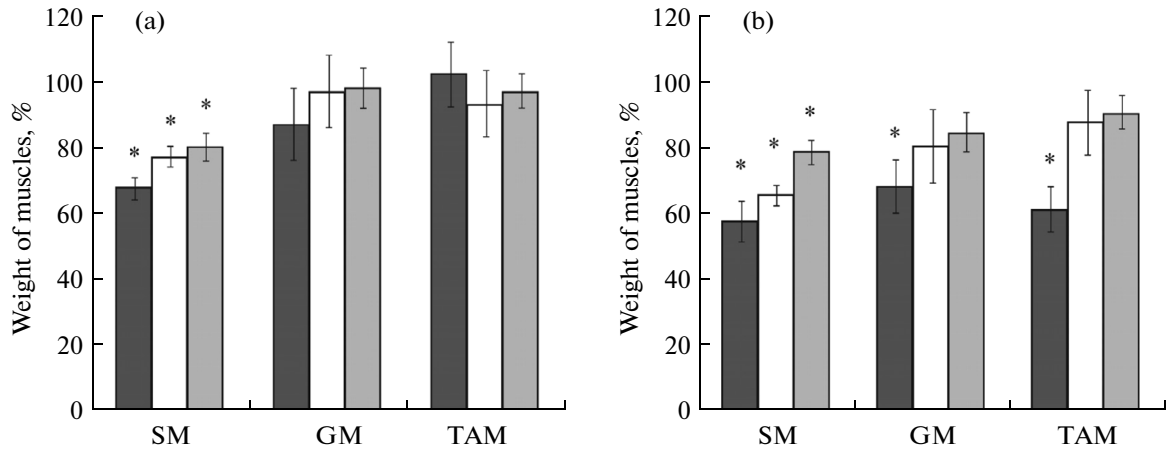

Fig. 1. Change of muscle weight upon combined influence of 7-day (a) and 14-day (b) gravitational unloading with various means of foot stimulation: dark bars – gravitational unloading, white – in combination with vibrostimulation, gray – with support afferentation. Along the ordinate axis – weight of muscles expressed in %, taking as 100% the values of intact animals. * – $p < 0.05$.

experiment were actualized in correspondence with the acting requirements of the Decree of the Ministry and medium special education of the USSR no. 742 of 13.11.1984 approving "Rules of conducting works with the use of experimental animals" and Directive 2010/63/UE on the protection of animals used of scientific purposes of September 22, 2010.

In the quality of a model of gravitational unloading use was made of a modified suspension model of Morey-Holton E.R [20].

In the experiment the animals were divided into seven groups:

1. "Control" – intact animals ($n = 5$);

2. "GU7" – 7-day gravitational unloading (suspension) ($n = 7$);

3. "GU14" – 14-day gravitational unloading ($n = 5$);

4. "GU7 + VS" – 7-day gravitational unloading combined with daily bilateral 3-h vibrostimulation of foot support zones ($n = 7$);

5. "GU14 + VS" – 14-day gravitational unloading combined with daily bilateral 3-h vibrostimulation of foot support zones ($n = 5$);

6. "GU7 + SA" – 7-day gravitational unloading combined with daily support afferentation (daily 3-h support presentation – "planting", usual movement) ($n = 7$);

7. "GU14 + SA" – 14-day gravitational unloading combined with daily support afferentation ($n = 6$);

For delivering a stimulus, amplifying and registering the responses of muscles use was made of an experimental setup on the basis of electromyograph MG-42 ("Medikor") and a Pentium processor. With the aid of bipolar needle electrodes we registered the electric responses of shin muscles evoked by stimulating the sciatic nerve with single rectangular pulses of 0.5 ms duration at a rate of 0.5 pulse/min. The intensity of stimuli varied from 0.3 to 50 V. We determined the maximal amplitude of M-responses.

Conditions of vibrostimulation are described in our precious communication [21]. Oscillation amplitude – 0.5 mm; frequency – 50 Hz. Vibrostimulation was actualized with a vibrostimulator of original design, which was developed at the Chair of human and animal physiology, KFU.

Statistical processing was performed with the use of applied software package "Origin." The reliability of results was determined by the Student's t-test.

RESULTS

Change in muscle weight upon gravitational unloading and combined with various means of foot stimulation. A 7-day gravitational unloading of rat hind limbs led to reduction of the weight of soleus muscle (SM) and in a lesser degree – gastrocnemius (GM) and tibial anterior (TAM) muscles (Fig. 1a, dark bars). However in 14 days of gravitational loading we observed reduction of the weight of all investigated muscles (Fig. 1b, dark bars). Thus the weight of SM made 57 ± 5% of control, GM – 68 ± 8%, TAM – 61 ± 5% ($p < 0.05$).

The use of two regimes of rat foot stimulation in conditions of gravitational unloading (Figs. 1a, 1b; vibrostimulation– white bars, support afferentation – gray bars) led to reduction of its negative effect. The weight of all muscles upon combined stimulation and 14-day unloading was larger than without stimulation (Figs. 1a and 1b).

Change in the amplitude of *M*-response of rat shin muscles upon gravitational unloading combined with various means of stimulation of foot receptors. The amplitude of *M*-response of rat SM in seven days after gravitational unloading made 43 ± 15% of control ($p < 0.05$). The amplitude of *M*-response of rat GM 89 ±

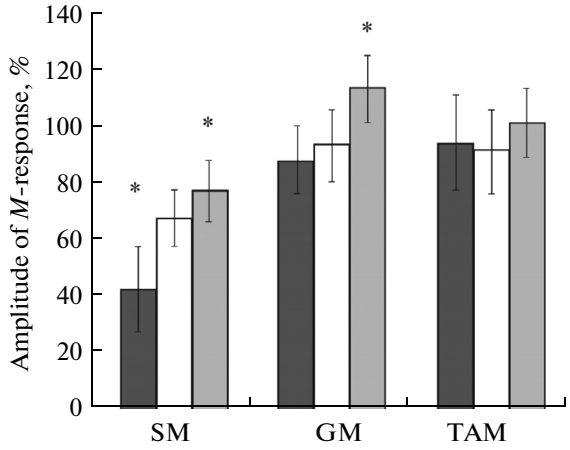

Fig. 2. Change in amplitude of *M*-response of rat shin muscles upon 7-day gravitational unloading (dark bars) and combined influence of foot vibrostimulation (white bars) and support afferentation (gray bars). Along the ordinate axis is amplitude of M-response in % of control taken as 100%; * – $p < 0.05$.

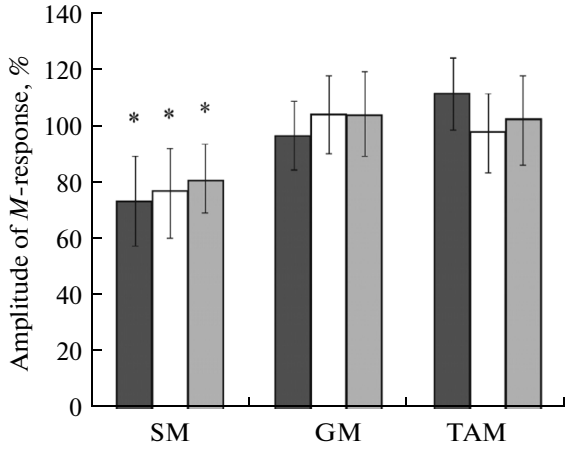

Fig. 3. Change in amplitude of *M*-response of rat shin muscles upon 14-day gravitational unloading (dark bars) and combined influence of foot vibrostimulation (white bars) and support afferentation (gray bars). Along the ordinate axis is amplitude of M-response in % of control taken as 100%; * – $p < 0.05$.

10% relative to control values ($p > 0.05$). The amplitude of *M*-response of rat TAM in seven days after gravitational unloading made 95 ± 11% as compared with control ($p > 0.05$) (Fig. 2). As evident from Fig. 2, use of vibrostimulation and support against the background of unloading led to restitution of *M*-response of GM and TAM to control values, the amplitude of *M*-response of SM increased by 20% and made 68 ± 12%.

In 14 d of gravitational unloading the amplitude of *M*-response of GM and TAM recovered to control values (Fig. 3). Combined stimulation of foot did not exert significant influences on the amplitude of *M*-response of investigated muscles. The amplitude of *M*-response of SM in 14 d of gravitational unloading increased by 30%, additional foot vibrostimulation and support afferentation did not exert influence on the amplitude of M-response of SM (Fig. 3).

DISCUSSION

Effects of 7- and 14-day hindlimb unloading of rats in our investigations were comparable with results of other researchers [1, 22, 23]. In the period of 7-day suspension there was a decrease in the wet weight of SM, while the weight of muscles of a faster type (GM and TAM) did not change. Atrophy of muscles in real conditions of spaceflight also takes place quickly, with reduction to 37% muscle mass in the course of one week [2]. In rats the antigravity slow muscles atrophy more quickly, extensor faster than flexors [24–27]. In 14 d after suspension we observed a decrease of wet weight of all investigated muscles. It is shown that spaceflight causes degeneration of slow fibers into fast and practically does not influence the fast fibers [2]. Therefore it may be supposed that loss of SM weight in the first week of gravitational unloading in the first place is caused by reduction of protein synthesis. Further loss of weight of SM and fast muscles is connected with degradation of myofibrils, which reaches a maximum in 9–15 d [28]. Our investigations showed that foot vibrostimulation and support afferentation may hinder loss of weight of fast muscles exactly in the first week of unloading. However in 14 d a combination of limb unloading with stimulation only attenuate the effect of suspension. On the whole, effects of foot stimulation can be evaluated also in regard of SM. Thus application of brief daily vibration of SM tendon in the course of 14 d of gravitational unloading partly prevented its atrophy (weight loss to 75%, in our investigation – 78%) and loss of contraction velocity and maximal muscle force (93 and 59% respectively) [16]. Everyday support for 2 or 4 h in the course of four weeks partly prevented atrophy of rat SM (32 and 35% reduction of muscle weight, respectively, as compared with 75 and 78% in the present investigation) [29]. Our results touching on prophylaxis of reduction of the muscle mass of SM during the use of support afferentation were similar with running a treadmill at a rate of 20 m/min in the course of 1.5 h [30]. But it must be noted that in spite of that support afferentation had a better effect of counteracting unloading, we still did not obtain 100% prevention of reduction of SM weight upon 3-h planting per day either in 7-day or in 14-day unloadings, as it has been shown in works of B. Sun with coauthors [31].

The amplitude of *M*-response in the first seven days of gravitational unloading decreased in a greater degree in SM than in GM and TAM. Subsequently we observed recovery of the amplitude of *M*-response in GM and TAM to control values. The amplitude of *M*-response of SM increased but not substantially.

Gravitational unloading, as it is supposed, causing atrophic changes in muscles, must lead to reduction of the amplitude of *M*-response, classically observed in unused muscles [32]. However the amplitude of *M*-response of GM and TAM increased and reached control values after 14 days of gravitational unloading, despite the decrease in the wet weight of muscles. As it appears, insubstantial atrophy of these muscles in the first two weeks of unloading may explain why the amplitude of *M*-response was not essentially altered. Our conditions of foot stimulation in rats had a positive effect in restitution of the amplitude of *M*-response of all investigated muscles. Support afferentation almost twice prevented the drop in the amplitude of *M*-response in SM and GM in the first week of gravitational unloading. The changes noted by us may be evoked by reduction of support afferentation or with a decrease of activation of motoneurons. For example, integrated electromyography from muscles of rat hind limbs recorded more than 24 h significantly declines in the course of several days after the beginning of unloading, after which it returns to the values of control [33]. As it seems, motoneurons experience only temporary reduction of activity, which lasts only several days, after which they restore the initial level of activation. This allows suggesting that changes in muscles as a result of gravitational unloading do not depend on activation of motoneurons and come to be the source of its change [34]. The stimulation of foot surface used by us may activate corresponding pathways and motor neurons, evoking contraction of muscles imitating the usual conditions of sole stimulation. Possibly, use of such tricks may become an effective rehabilitation tool for clinical groups of the population, such as bed patients or elderly people.

In this way, our investigations have shown that vibrostimulation of foot in rat or support afferentation are capable of counteracting atrophy of gastrocnemius and tibial anterior muscles evoked by gravitational unloading, and reducing its effects on soleus muscle.

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