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Functional State of the Neuromotor Apparatus of the Gastrocnemius Muscle in Rat Under Microgravity: Effect of Spinal Cord Stimulation

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Abstract
The aim of this study was the evaluation of the functional state of the neuromotor apparatus of the gastrocnemius muscle in rat under conditions of gravitational unloading, as well as in conditions of gravitational unloading combined with magnetic stimulation of the spinal cord. The electrical potentials of gastrocnemius muscle of the rat evoked by the stimulation of the sciatic nerve were recorded after a week of exposure the animal in the experimental conditions. Parameters of motor response and H-reflex were evaluated. It was found that gravitational unloading caused an increase of the reflex excitability of the motor centers of gastrocnemius muscle of the rat and magnetic stimulation of the spinal cord combined with unloading increased the intensity of transformations. In addition, it was registered the changes of the functional state of the muscle under conditions of gravitational unloading combined with the stimulation of the spinal cord. The detected transformations were probably associated with the activation of adaptation processes in the new motor environment (simulation of the microgravity, restriction of peripheral afferentation including the support afferentation).

Keywords Microgravity · Hindlimb unloading · Motor center · Spinal cord stimulation · Electromyography

1 Introduction

In a number of studies performed in conditions of real weightlessness and in model ground-based experiments, it was shown that gravitational unloading was accompanied by alterations in the morphofunctional state of all links of the neuromotor apparatus [1–3]. Moreover, the changes of the structures of the motor apparatus observed in a case of various locomotor disorders in the gravity demonstrate similar alterations that observed in hypogravity. Thus, the exploration of outer space and the widespread prevalence of pathologies, accompanying changes in motor qualities, make obtain necessary to new knowledge about the mechanisms of motor reorganization and to search for new effective methods of rehabilitation. Currently, the effectiveness of various methods of stimulation of spinal neural networks in the post-traumatic reorganization of motor function is studied [4, 5]. It was shown that the magnetic [6] and electrical [7, 8] stimulation of the spinal cord activated the neurons responsible for locomotor activity and can be used in neurorehabilitation.

The aim of the work was to evaluate the functional state of the neuromotor apparatus of the gastrocnemius muscle of the rat under conditions of microgravity, as well as in conditions of microgravity, combined with magnetic stimulation of the spinal cord.

2 Material and Methods

2.1 Animals

In this study, we used male Wistar rats at the age of 8 weeks, weighing 200–220 g ($n = 18$). All procedures strictly corresponded to the bioethical norms of the 1975 Helsinki Declaration and were approved by the Commission on Bioethics of KFU.

A week before the start and during the experiments, each rat was housed in a separate specialized cage at room temperature of 23 °C, with a 12 h/12 h light/dark cycle, with access to water and food ad libitum. Randomly, the animals were divided into three groups: intact animals (INTACT, $n = 5$); animals with gravitational unloading of the hind limbs, 7 days (UN,

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$n = 7$); and animals with gravitational unloading of the hind limbs, 7 days, combined with daily magnetic stimulation of the spinal cord (UN + MS, $n = 6$). All experimental effects were carried out at the same time of day. The identity of each rat in each experimental group remained encoded until the end of the experiment and analysis of the data.

2.2 Experimental Procedures

Gravitational unloading was modeled according to a standard procedure [9, 10]. Rats were elevated by the tail in an anti-orthostatic position (≈ 30°), so that the animal's hindlimbs could not touch the floor and cage walls while the rats could move freely on their forelimbs.

Magnetic stimulation of the spinal cord was performed with a magnetic stimulator "Neuro-MVP-4" (Neurosoft, Russia), through a coil (outer diameter 100 mm). The center of the coil was placed 3–5 mm from the dorsal surface of the animal's body along the middle line in accordance with the level of vertebrae L1–S1. The position of the coil was constantly monitored by the researcher.

Stimulation was performed daily for 90 min in 10-min series at an interval of 10 min; the amplitude of the stimuli was a threshold for contraction of the gastrocnemius muscle; and the frequency was − 3 Hz. When the rat lumbar spinal cord is stimulated with low-frequency electromagnetic pulses (but not high-frequency), interneuron networks are activated, providing locomotion [11]. Also, the frequency of stimulation we used did not cause any defensive behavior in animals.

2.3 EMG Recordings

After a week of exposure to the experimental conditions, the electrical responses of the rat gastrocnemius muscle caused by stimulation of the sciatic nerve were recorded. Preliminarily, the animals were anesthetized with a mixture of Zoletil 100 0.5 mg/kg and Xylazalum 0.05 ml/kg intraperitoneal injection. The hanged animals were anesthetized when the hind limbs remained unloaded and were not subjected to support.

For electromyographic testing, a research facility based on an 8-channel stimulator, a 16-channel amplifier «A-M systems» (Calsborg, WA, USA), and DataWave software packages (Loveland, CO, USA) are used. Digitized data was stored on the computer. Stimulation was performed with a bipolar electrode, which was placed on a pre-prepared sciatic nerve in the middle between the knee and the hip joint. Single stimulus of 0.5 ms duration, 0.25 to 20 V intensity, was used. The interval between stimuli was at least 30 s in order to exclude the effects of the preceding stimulus. The registration of the induced responses was carried out with needle electrodes made of stainless steel, which were inserted into the middle part of the muscle. The depth of the placement of electrodes, the position of the posterior limbs of the rat, and the angles in the joints that determine the extent of gastrocnemius extension, strictly controlled by the experimenter, exclude the influence of each of these parameters for the recorded electrophysiological characteristics.

The motor (M) response, which was the electrical potential of the muscle, formed upon stimulation of the efferents and the H-reflex, which was the reaction of spinal motoneurons to afferent stimulation, were recorded. The threshold of occurrence, maximum amplitude, latency, and duration of evoked potentials were determined. The ratio of the maximum amplitudes of the H-reflex and motor responses [(Hmax/Mmax) × 100%] was calculated.

2.4 Statistical Analysis

All results were expressed as mean ± sem. Differences between the groups were statistically analyzed using the ANOVA variance analysis. Level of significance was $p < 0.05$.

3 Results and Discussion

Electromyographic research allows us to study with great accuracy the dynamics of the reorganization of motor units and makes it possible to judge the functional state of any link in the neuromotor apparatus. In the conducted experiments, it was found that the time characteristics of the recorded potentials of the gastrocnemius muscle (latent period, duration) in the experimental groups (INTACT, UN, UN + MS) did not differ significantly (data not shown). However, significant changes in the thresholds and amplitudes of the evoked potentials of gastrocnemius muscle were found.

To carry out microgravity studies, the data in the UN and UN + MS groups were compared with the data obtained in the group of intact animals. It was found that in the UN and UN + MS groups, the threshold of M-response and H-reflex of rat gastrocnemius muscle was significantly decreased ($p < 0.05$); the amplitude of the M-response in the UN group did not differ from the value in the INTACT group ($p > 0.05$); in the UN + MS group, the amplitude of the recorded potentials increased ($p < 0.05$). At values of the Hmax/Mmax ratio, a significant increase ($p < 0.05$) in the experimental groups with hindlimbs unloading was also observed. The results are shown in Fig. 1.

The decrease in the threshold of the M-response of rat gastrocnemius muscle observed in our experiments could not be due to synaptic or muscle mechanisms. In some experiments with microgravity, it was shown a significant reduction of the amount of synaptic vesicles in the terminals of neuromuscular synapses [12] and a decrease of the excitability of the muscles [13]. The recorded alterations in the M-response threshold was probably a consequence of an increase in the excitability of the efferents of the corresponding spinal

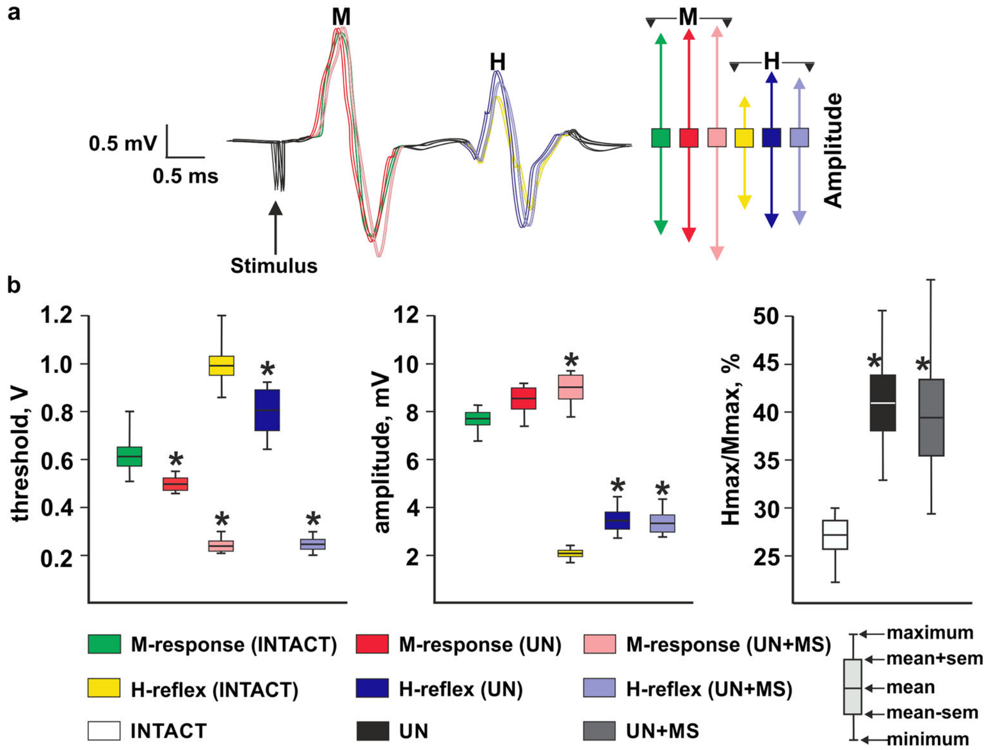

Fig. 1 Parameters of evoked potentials of the rat gastrocnemius muscle. **a** Potentials recorded in the gastrocnemius of the rat with stimulation of the sciatic nerve. **b** Comparative analysis of the parameters of the recorded potentials. *Differences with the INTACT group are significant, $p < 0.05$

motoneurons as a result of a change in their functional state. Data on morphological and functional transformations in motoneurons innervating skeletal muscles [14] testify to the neurogenic origin of changes in skeletal musculature during microgravity. The high rate of development of hypogravitational motor transformations also indicated a reflex nature. It was shown that after only 20 s of exposure to microgravity, the electrical activity of the soleus rat muscle drastically changed [15]. Our evaluation of parameters of the H-reflex of the gastrocnemius muscle, which was an analog of the monosynaptic reflex to stretching, is used to assess the reflex excitability of spinal motor neurons [16, 17], demonstrating the change in the state of the corresponding motor center. The decrease in the threshold and the increase in the amplitude of the H-reflex detected during our experiments can be caused by an increase in the excitability of α-motoneurons of the gastrocnemius muscle. The change in the state of motoneurons was confirmed by an estimate of the Hmax/Mmax ratio, because this ratio accurately reflected the proportion of the excited α-motoneurons in the pool of motoneurons [18]. The reason for the increase in the reflex excitability of the motor centers may be a structural remodeling of spinal circuitries [19], because of a restriction of peripheral afferentation, including the support afferentation. The importance of the support afferentation in modulating the functional state of neuromotor systems has been confirmed by a number of studies [20, 21]. It was assumed that the processes developing in the muscle are closely related with central reactions. The increase in the amplitude of motor potentials in the UN + MS group appeared to be due to an increase in the synchronicity of the reaction of motor units to the stimulus as a result of their reorganization after changes in motor conditions. It was shown that under the

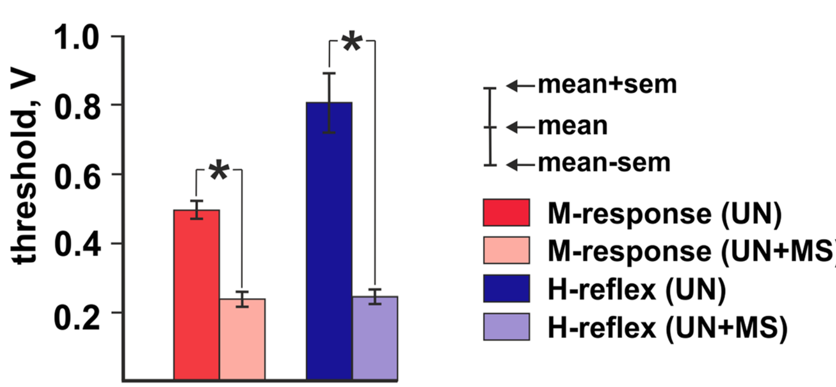

Fig. 2 Comparative analysis of the thresholds of evoked potentials of the rat gastrocnemius muscle. *Differences are significant, $p < 0.05$

conditions of immersion hypokinesia, new motor units can be recruited during the implementation of the movement, and the activated units can increase the frequency of impulses [22]. Also, it is known that the myosin phenotype of muscle changes in microgravity conditions and the content (%) of fast fibers, that have a high speed and force of contraction, increases [23]. An increase in the content (%) of fast fibers in mixed gastrocnemius muscle can increase the synchronism of the response of individual motor units and cause an increase in the amplitude of the M-response.

To identify the effects of magnetic stimulation of the spinal cord under conditions of microgravity, a comparison of the characteristics of the induced responses of gastrocnemius muscle animals, UN and UN + MS groups, was carried out. It was found that when the spinal structures were activated under gravitational unloading conditions, the thresholds of the recorded electrical responses were reduced (see Fig. 2).

The obtained data indicated that under these conditions, an increase in the excitability of the motor neurons of the spinal motor center of GM became more pronounced, and, apparently, this effect was more associated with alterations in the condition of low-threshold motor neurons. It is known that electrical stimulation of the spinal cord modulates segmental activity and activate the reserve capabilities of the neuronal reflex circuits of the spinal cord [24, 25]. The obtained data suggested that the additional activation of the spinal cord with gravitational unloading probably intensified the processes of hypogravitational adaptation of the spinal motor control systems. The increase in reflex activity is a process closely related to the reorganization of the motor function. Lee et al. [19] showed that the increase in reflex responses correlated with the restoration of locomotions after a spinal cord injury in mice. The formation of a new motor skill can determine the activity of various reflex pathways [26].

4 Conclusions

Thus, according to the results of electromyographic studies, we conclude that the increase in the excitability of motoneurons of the gastrocnemius muscle found during gravity discharge may be a consequence of adaptation of the central nervous system to new conditions of motor activity. In the works of Gerasimenko et al. [27]and Angeli et al. [28], it was determined the important role of activation of spinal neural networks in the probability and efficiency of restoring voluntary movements after spinal cord injury. In our experiments, the role of intra-spinal structures was confirmed by data in a series with magnetic stimulation of the spinal cord. The changes in the parameters of the M-response and H-reflex revealed under these conditions indicated a greater increase in the reflex excitability of the corresponding spinal motor centers. Such changes were probably related to the activation of adaptation processes in the new motor environment (simulated microgravity).

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