ISSN 0006-3509, Biophysics, 2014, Vol. 59, No. 2, pp. 316–320. © Pleiades Publishing, Inc., 2014.
Original Russian Text © T.V. Baltina, M.V. Kuznetsov, A.A. Yeremeev, M.E. Baltin, 2014, published in Biofizika, 2014, Vol. 59, No. 2, pp. 387–391.

Effect of Vibratory Stimulation of Foot Support Areas in Rats on the Functional State of Leg Muscles and the Content of N2A Titin Isoforms in Gravity Relief

T. V. Baltina, M. V. Kuznetsov, A. A. Yeremeev, and M. E. Baltin
Kazan Federal University, Kazan, 420008 Russia
e-mail: tvbaltina@gmail.com

Received November 1, 2013

Abstract—In this work, we studied the effect of vibratory stimulation of the foot support zones on the functional state of the leg muscles and the content of N2A titin isoforms in rats under simulated microgravity (suspension model). The results of this study showed that vibratory stimulation of the support zones of the rat foot in a gravity discharge may reduce the drop in the amplitude of leg muscle motor response and undesirable reduction of the titin content.

Keywords: gravitational unloading, suspension model, vibratory stimulation, receptors of support zones, motor response, N2A titin isoforms

DOI: 10.1134/S0006350914020031

INTRODUCTION

Investigations of the motor system in weightlessness and conditions modeling it revealed changes of the functional state of all links of the neuromotor apparatus [1–4]. Mechanical unloading of skeletal muscles during space flights or their ground imitation caused atrophy of skeletal muscles, which especially affects the antigravitational musculature of the lower limbs [2, 5]. Atrophy is characterized by reduction of muscle volume, mass, muscle strength, change of histochemical characteristics, and also reduction of neuromuscular function [6–9]. It is shown that atrophic changes in muscles. In particular in m. soleus, are accompanied by significant reduction of the content of titin—a giant sarcomeric protein playing an important role in sustaining a highly ordered sarcomeric structure and contractile function of muscles [10–13].

In the quality of one of the causes of such changes in conditions of microgravity, supposed is restriction of support afferentation [14, 15] and, in all probability, in consequence of this a change of proteolysis and synthesis of a series of proteins. Data obtained upon testing crew members in conditions of spaceflight [16] and in model experiments on ground [17, 18] have shown that an increase of the influx of sensory information to motor centers upon application of mechanical pressure on support zones of the foot leads to activation of the muscles of lower limbs, which is accompanied by reduction or prevention of their atrophy. It is known that usage of a compensator of support unloading led to a decrease of the development of atrophic processes and prevented reduction of titin content in human m. soleus in conditions of “dry” immersion [19, 20]. Characteristics and spatial localization of skin receptors of the foot in man [21, 22] and rat [23] have been described, but information on the potential good of their stimulation for prevention of atrophy in unloading is insufficient.

In this investigation presented are results of change of the parameters of motor response and content of titin N2A isoform in rat shin muscles in conditions of modeled microgravity upon vibratory stimulation of support zones of the foot.

EXPERIMENTAL

The investigation was conducted on non-line laboratory rats of 180–200 g mass in accordance with the rules of treating laboratory animals with observance of all bioethical norms.

In the quality of a model of gravitational unloading use was made of a method of suspension described in work [24].

In the experiment the animals were divided into three groups:

“control” – intact animals ($n = 6$);

“GU” – gravitational unloading from 7 to 35 days ($n = 36$);

“GU+VS” – 7-day gravitational unloading in combination with daily vibratory stimulation of support zones of the foot ($n = 12$).

Vibratory stimulation was actualized in cycles – six times each in the course of three hours every day in conditions of 7-day gravitational unloading (duration of one cycle of vibratory stimulation constituted 20 min, interval between cycles – 10 min, vibration amplitude – 0.5 mm; frequency – 50 Hz). Use was made of a simulator of original design, which was developed at the Chair of human and animal physiology, KFU.

In 7, 14, 21 and 35 days of impact of gravitational unloading we registered the electrophysiological indices of the triceps muscle of calf. Determined were the maximal amplitude and the threshold of emergence of responses [25].

For investigation of the isoform composition of titin we took samples of soleus muscle from each group of rats ($n = 18$). SDS-gel electrophoresis was conducted a method described in work [26]. Densitometric processing of gels was conducted with the aid of computer program Total Lab 1.11. The amount of titin was evaluated in relation to the content of myosin heavy chains [13]. Immunoblotting of titin was conducted by method [27]. Use was made of monoclonal antibodies T11 (Sigma, USA) to a region of titin molecule in the I-disk of the sarcomere. In the quality of secondary antibodies conjugated with horseradish peroxidase, use was made of rabbit antibodies against IgG of mice. Protein bands were revealed with the aid of 3,3'-diaminobenzidine.

In statistical analysis of results use was made of the Student's *t*-test. The criterion of significance was taken to be a level – 0.05.

RESULTS

Influence of hindlimb gravitational unloading on amplitude of motor responses of triceps muscle of rat calf. The amplitude of motor response of rat triceps muscle in seven days after unloading constituted 111 ± 7% as compared with control ($p > 0.05$). Subsequently the amplitude increased and by 14th day constituted 112 ± 8% ($p > 0.05$); in 21 days – 114 ± 9% ($p > 0.05$); in 35 days – 131 ± 11% as compared with control ($p < 0.05$). Results are presented in Fig. 1.

Inasmuch as we did not obtain reliable changes in the amplitude of responses of the triceps muscle of calf in the first weeks of experiment, although from literature data it is known that in this period muscle atrophy already develops, further we conducted analysis of the amplitude of motor responses of separate heads of this muscle.

We restricted ourselves to 7th and 14th day of gravitational unloading, inasmuch as it has been shown that maximal atrophy in the muscles of rat hind limb in these conditions is observed in this temporal interval [17].

The amplitude of motor response of the medial head of the rat gastrocnemius muscle in seven days after unloading constituted 74 ± 15% ($p < 0.05$), as compared with control. Subsequently the amplitude of response increased and by 14th day constituted 85 ± 16% ($p < 0.05$). The amplitude of motor response of the lateral head of rat gastrocnemius muscle in seven days after unloading constituted 104 ± 10% ($p > 0.05$), on the 14th day – 105 ± 12% ($p > 0.05$) in relation to control values.

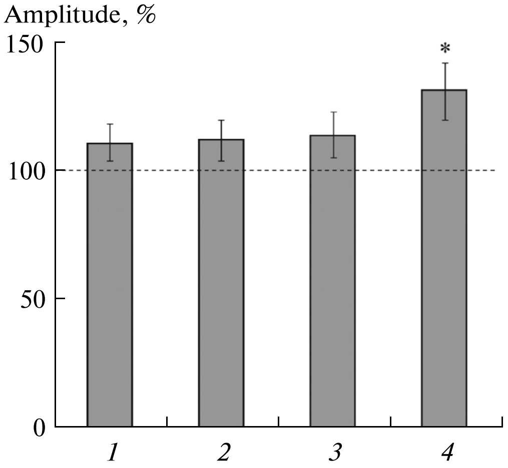

Fig. 1. Values of amplitude of motor response of the triceps muscle of rat calf at different terms of gravitational unloading. Along the abscissa axis, denoted are days of impact of support unloading: *1* – 7th day, *2* – 14th day, *3* – 21st day, *4* – 35th day; along the ordinate axis – values of amplitude of motor response, expressed in percent relative to control. Interrupted line denotes control values taken as 100%. * – Reliability, $p < 0.05$.

On the seventh day of gravitational unloading the amplitude of motor response of soleus muscle constituted 43 ± 11% ($p < 0.05$) as compared with control. Subsequently the amplitude increased and by 14th day constituted 74 ± 16% ($p < 0.05$) as compared with control. Results are presented in Fig. 2.

Influence of 3-hour vibratory stimulation of support zones of the foot of rat on amplitude of motor responses and on titin content in triceps muscle of calf at a background of gravitational unloading. Upon combined influence of 3-hour vibratory stimulation of support zones of the foot and 7-day gravitational unloading the maximal amplitude of motor response of the lateral head of gastrocnemius muscle did not change as compared with control, while the amplitude of response of the medial head of gastrocnemius muscle increased and constituted 118 ± 12% of control values ($p < 0.05$). The amplitude of motor response of soleus muscle constituted 68 ± 15% as compared with data obtained upon investigation of intact animals ($p < 0.05$). Results are presented in Fig. 3.

Therewith in the soleus muscle in rats upon combined influence of 3-hour vibratory stimulation of support zones of the foot and 7-day gravitational unloading (group "GU+VS" in figure) the content of titin N2A isoform declined only by 11% as compared with the titin content in soleus muscle of rats of the

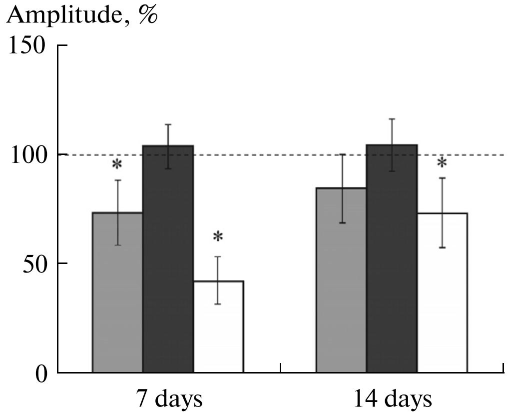

Fig. 2. Values of amplitude of motor response of rat calf muscles at different terms of gravitational unloading. Along the abscissa axis, denoted are days of impact of support unloading; along the ordinate axis – values of amplitude of motor response, expressed in percent relative to control. Interrupted line denotes control values taken as 100%; by light-gray color, denoted are values of amplitude of motor response of the medial head of gastrocnemius muscle; by dark-gray color – of the lateral head of gastrocnemius muscle, white – soleus muscle.

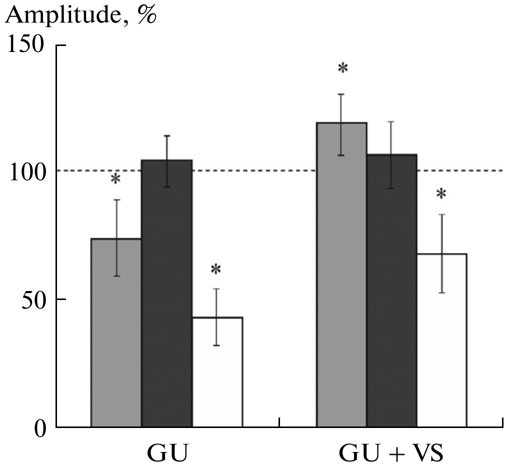

Fig. 3. Values of amplitude of motor responses of rat calf muscles upon combined influence of vibratory stimulation of support zones of the foot and 7-day gravitational unloading. GU – group with gravitational unloading, GU+VS – group with combination of gravitational unloading with vibratory stimulation of support zones of the foot. The rest of designations as in Fig. 2.

control group. The increase of the relative amount of titin N2A isoform was accompanied by reduction of the content of T2-fragment in soleus muscle in rat of group with application of vibratory stimulation of support zones of the foot ("GU + VS") by 1.5 times as compared with the group without stimulation ("GU"). Results are presented in Fig. 4.

DISCUSSION

The results of conducted investigation showed that the amplitude of motor response of triceps muscle of rat calf essentially did not differ in the control group of rats and animals staying in conditions of gravitational unloading. However muscle atrophy must have manifested itself upon suspension as reduction of the amplitude of motor responses on the 7th–14th day, which was observed in non-used muscles [28]. In our experiments we have not obtained a similar effect upon registration of responses from whole muscle. Taking into account that gravitational unloading differently influence the muscles of lower limbs of the rat [29, 30], we decided to conduct picking up a motor response for each head of the muscle.

Separate testing of heads of the triceps muscle of calf has shown that the amplitude of motor response of the medial head of gastrocnemius and soleus muscles of rat in conditions of modeled gravitational unloading declines in relation to control values, at that in the soleus muscle such a decline is more expressed. For explaining some the effects of unloading in the literature they discuss a decrease of support afferentation, playing a role in regulation of posture and locomotions [14, 30, 31]. The authors of work [1] supposed that reduction of contractile properties of skeletal muscles upon transition to weightlessness is conditioned by reflectory reduction of muscular tone, conditioned in its turn by elimination of support. Subsequently this supposition was confirmed in a series of investigations, having shown that reduction of velocity-force characteristics in conditions of microgravity essentially differs in various muscles, being more expressed in tonic muscles ("antigravitational") [5, 8]. In experiments on rats it was shown that electromyographic activity of soleus muscle declines as soon as after 15 min of suspension [30, 32]. Analogous changes of electromyographic activity of soleus and gastrocnemius muscles were observed in monkeys after spaceflight [33]. Our results have also shown a reduction of the amplitude of motor response in soleus muscle of rats, which, in all probability, comes as a consequence of trophic changes in the muscle [19]. However a contribution into this reduction might have been made also by a decrease in the content of titin, playing an important role in sustaining the sarcomeric structure and contractile function of muscles [34].

It is known that mechanical stimulation of support zones of the foot (both in rat and in man), during gravitational unloading can prevent muscular atrophy and reduction of titin content. Apart from that, it was disclosed that upon usage of superficial mechanical pressure on the foot there is prevention of atrophy of muscle fibers of type I and in a lesser degree of muscle fibers of type II [19, 35].

Our results also confirm data of other authors [17, 18] about that mechanical stimulation of support zones of the foot of rat during gravitational unloading

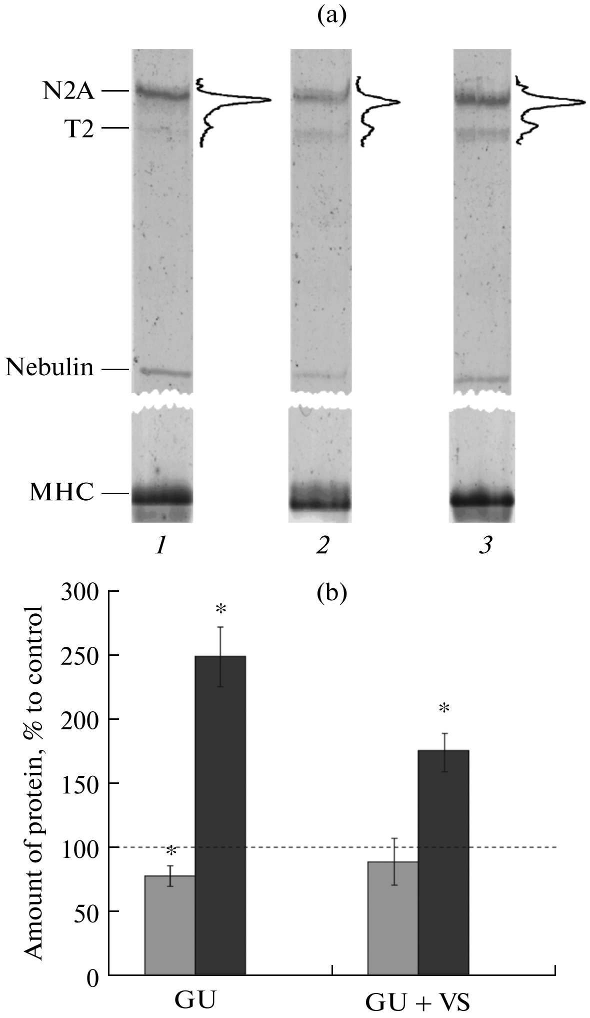

Fig. 4. Changes in isoform composition of titin in rat soleus muscle in conditions of gravitational unloading and in combination of vibratory stimulation of support zones of the foot; (a): *1* – "control"; *2* – "GU"; *3* – "GU + VS". Indicated are protein bands: myosin heavy chains; nebulin – cytoskeletal protein of thin filaments; T2-fragment of titin and N2A isoform of titin and profiles of intensity of optical density of titin bands on the gel. (b) Along the ordinate axis: ratio of amount of titin N2A isoform (light-gray bars) and T2-fragment (dark-gray bars) to myosin heavy chains by results of densitometry of protein bands on electrophoregrams, expressed in % relative to control. The rest of designations as in Fig. 3.

can prevent muscular atrophy and reduce the titin content. In our investigation we have shown that vibratory stimulation of support zones of the foot prevents atrophy of gastrocnemius muscle, the most part of fibers of which is represented by muscle fibers of type II. Most probably this is connected with that the use of vibratory stimulation is directed toward activation of Ruffini endings or Pacinian corpuscles disposed deeper in skin.

In this way, the results of the given investigation have shown that in conditions of gravitational unloading vibratory stimulation of support zones of the foot in rat prevents reduction of the amplitude of motor response and degradation of titin in gastrocnemius and soleus muscle of the rat, which may testify also to reduction of the degree of expression of atrophic changes in these muscles.

ACKNOWLEDGMENTS

The work was supported by the Russian Foundation for Basic Research (13-04-01746a).

REFERENCES

1. I. Kozlovskaya, I. Dmitrieva, L. Grigorieva, et al., in *Stance and Motion. Facts and Concepts*, Ed. by V. S. Gurfinkel, M. Ye. Ioffe, J. Massion (Plenum, N.Y., 1988), pp. 37–48.
2. V. R. Edgerton and R. R. Roy, Adv. Space Biol. Med. **4**, 33 (1994).
3. M. F. Reschke, W. H. Paloski, J. J. Bloomberg, et al., Brain Res. Rev. **28** (1–2), 102 (1998).
4. E. Nagy, L. Bognar, A. Csengery, et al., Int. Tinnitus J. **6** (2), 120 (2000).
5. R. H. Fitts, D. R. Riley, and J. J. Widrick, J. Appl. Physiol. **89** (2), 823 (2000).
6. K. M. Baldwin, Med. Sci. Sports Exerc. **28** (10), 101 (1996).
7. M. M. Bamman, M. S. Clarke, D. L. Feeback, et al., J. Appl. Physiol. **84** (1), 157 (1998).
8. C. Kourtidou-Papadeli, A. Kyparos, M. Albani, et al., Acta Astronaut. **54** (10), 737 (2004).
9. D. B. Thomason and F. W. Booth, J. Appl. Physiol. **68** (1), 1 (1990).
10. T. Toursel, L. Stevens, H. Granzier, and Y. Mounier, J. Appl. Physiol. **92**, 1465 (2002).
11. B. S. Shenkman, T. L. Nemirovskaya, I. N. Belozerova, et al., J. Gravit. Physiol. **9** (1), 139 (2002).
12. I. M. Vikhlyantsev, S. L. Malyshev, B. S. Shenkman, and Z. A. Podlubnaya, Biophysics **49** (6), 895 (2004).
13. I. M. Vikhlyantsev and Z. A. Podlubnaya, Biophysics **53** (6), 592 (2008).
14. A. I. Grigor'ev, I. B. Kozlovskaya, and B. S. Shenkman, Ros. Fiziol. Zh. Sechenova **90** (5), 508 (2004).
15. R. Roll, J. C. Gilhodes, J. P. Roll, et al., Exp. Brain Res. **122** (4), 393 (1998).
16. C. S. Layne, G. W. Lange, C. J. Pruett, et al., Acta Astronaut. **43** (3–6), 107 (1998).
17. L. De-Doncker, F. Picquet, and M. Falempin, J. Appl. Physiol. **89** (6), 2344 (2000).
18. L. De-Doncker, M. Kasri, and M. Falempin, Exp. Neurol. **201** (2), 368 (2006).
19. B. S. Shenkman, Z. A. Podlubnaya, I. M. Vikhlyantsev, et al., Biophysics **49** (5), 807 (2004).
20. I. M. Vikhlyantsev, Z. A. Podlubnaya, B. S. Shenkman, and I. B. Kozlovskaya, Dokl. RAN **407** (5), 692 (2006).
21. F. A. Sonnenborg, O. K. Andersen, and L. Arendt-Nielsen, Clin. Neurophysiol. **111** (5), 2160 (2000).
22. M. Trulsson, Exp. Brain. Res. **137** (1), 111 (2001).

23. J. W. Leem, W. D. Willis, and J. M. Chung, J. Neurophysiol. **69** (5), 1684 (1993).
24. E. R. Morey-Holton and R. K. Globus, J. Appl. Physiol. **92** (4), 1367 (2002).
25. T. V. Baltina, A. A. Yeremeev, and I. N. Pleshchinskii, Ros. Fiziol. Zh. Sechenova **91** (5), 481(2005).
26. R. Tatsumi and A. Hattori, Anal. Biochem. **224** (1), 28 (1995).
27. H. Towbin, T. Staehlin, and J. Gordon. Proc. Natl. Acad. Sci. USA **76** (9), 4350 (1979).
28. J. Duchateau and K. Hainaut, J. Physiol. (Lond.) **422**, 55 (1990).
29. E. A. Ilyin and V. S. Ogahov, Adv. Space Res. **9** (11), 11 (1989).
30. F. Kawano, A. Ishihara, J. L. Stevens, et al., Am. J. Physiol. Regul. Integr. Comp. Physiol. **287** (1), 76 (2004).
31. V. Dietz, Clin. Neuro-physiol. **114** (8), 1379 (2003).
32. Y. Ohira, T. Nomura, F. Kawano, et al., J. Gravit. Physiol. **9** (2), 49 (2002).
33. R. R. Roy, S. C. Bodine, D. J. Pierotti, et al., J. Gravit. Physiol. **6** (2), 55 (1999).
34. R. Horowits, E. S. Kempner, M. E. Bisher, and R. J. Podolsky, Nature **323** (6084), 160 (1986).
35. A. Kyparos, D. L. Feeback, Ch. S. Layne, et al., J. Appl. Physiol. **99** (2), 739 (2005).