Structure of cortical cytoskeleton in fibers of mouse muscle cells after being exposed to a 30-day space flight on board the BION-M1 biosatellite

I. V. Ogneva,1,2 M. V. Maximova,1,3 and I. M. Larina1

1Department of Molecular and Cell Biomedicine, State Scientific Center of Russian Federation, Institute of Biomedical Problems of the Russian Academy of Sciences, Moscow, Russia; 1M. Sechenov First Moscow State Medical University, Moscow, Russia; and 3Moscow Institute of Physics and Technology (State University), Moscow, Russia

Submitted 11 February 2014; accepted in final form 24 March 2014

Ogneva IV, Maximova MV, Larina IM. Structure of cortical cytoskeleton in fibers of mouse muscle cells after being exposed to a 30-day space flight on board the BION-M1 biosatellite. J Appl Physiol 116: 1315–1323, 2014. First published March 27, 2014; doi:10.1152/japplphysiol.00134.2014.—The aim of the work was to analyze changes in the organization of the cortical cytoskeleton in fibers of the mouse soleus muscle, tibialis anterior muscle and left ventricular cardiomyocytes after completion of a 30-day space flight on board the BION-M1 biosatellite (Russia, 2013). The transversal stiffness of the cortical cytoskeleton of the cardiomyocytes and fibers of the skeletal muscles did not differ significantly within the study groups compared with the vivarium control group. The content of beta- and gamma-actin in the membranous fraction of proteins in the left ventricular cardiomyocytes did not differ significantly within all study groups and correlated with the transversal stiffness. A similar situation was revealed in fibers of the soleus muscle and tibialis anterior muscle. At the same time, the content of beta-actin in the cytoplasmic fraction of proteins was found to be decreased in all types of studied tissues compared with the control levels in the postflight group, with lowered beta-actin gene expression rates in the postflight group. After completion of the space flight, the content of alpha-actinin-4 was found to be reduced in the membranous fraction of proteins from the mouse cardiomyocytes, while its content in the cytoplasmic fraction of proteins did not change significantly. Furthermore, gene expression rates of this protein were decreased at the time of dissection (it was started after 13 h after landing). At the same time, the content of alpha-actinin-1 decreased in the membranous fraction and increased in the cytoplasmic fraction of proteins from the soleus muscle fibers.

CURRENTLY, PATHWAYS INVOLVED IN MECHANOTRANSDUCTION IN CELLS HAVE NOT BEEN FULLY STUDIED. ALSO, THERE HAVE BEEN NO ANSWERS TO THE QUESTIONS WHAT IS THE PRIMARY RECEPTOR AND IN WHAT WAY DOES THE CELL DIFFERENTIATE BETWEEN INCREASED OR DECREASED LOADS (PARTICULARLY, GRAVITATIONAL LOAD).

THE LATTER SEEMS TO BE EXTREMELY IMPORTANT FOR IMPLEMENTING LONG-TERM SPACE FLIGHTS, AS SPENDING PROLONGED PERIODS OF TIME IN MICROGRAVITY HAS NEGATIVE EFFECTS ON ALL BODY SYSTEMS, PARTICULARLY ON THE MUSCULOSKELETAL AND CARDIOVASCULAR SYSTEMS. IN ORDER TO DEVELOP EFFICIENT METHODS OF PROTECTION, A DETAILED STUDY OF MECHANISMS OF GRAVITY STIMULUS REALIZATION IN THE CELLS OF THE SKELETAL MUSCLES AND MYOCARDIUM IS REQUIRED. IT IS POSSIBLE TO SIMULATE MICROGRAVITY CONDITIONS ON THE GROUND USING A NUMBER OF MODELS, PARTICULARLY THE DRY IMMERSION MODEL IN HUMANS AND THE MODEL OF RODENT ANTIORTHOSTATIC SUSPENSION (12, 17). UNDER TERRITORIAL CONDITIONS, THE DIRECT IMPACT OF GRAVITATION CANNOT BE AVOIDED. THIS IS THE ONLY EFFECTIVE TOOL FOR STUDYING THE EFFECT OF WEIGHTLESSNESS ON LIVING BEINGS AS AN ACTUAL SPACE FLIGHT.


In addition, the state of other proteins from the contractile apparatus was studied. Vikhlyantsev et al. (44) measured titin levels in muscle and myocardium samples of Mongolian gerbils exposed to a 12-day flight onboard the FOTON-M3 (Russia, 2007) satellite. An approximately 2-fold increase in the long-to-short titin isoforms ratio (N2BA/N2B) was observed in the postflight group of gerbils. Also, there were changes in the secondary structure of titin obtained from the left ventricle myocardium of the postflight animals compared with the control. Moreover, an increase of myocardium titin phosphorylation rates was detected in the gerbils exposed to the effects of microgravity. Furthermore, a decrease of titin-induced activation of actomyosin ATPase was found in the postflight gerbils (44).

However, all of the previously obtained experimental data are mainly related to the state of the contractile apparatus of skeletal muscles and myocardium cells. To date, the causes of negative changes in muscle cells, resulting in a further decrease of their functional properties, have not been made clear. We have obtained evidence from a series of earlier studies suggesting the role of the submembranous cytoskeleton in primary mechanoreception in skeletal muscle and myocardium cells (19–24). It should be noted, however, that these results were not obtained under zero gravity conditions but under conditions of changes in cell orientation with respect to the Earth’s gravity field. Thus the implementation of studies under the influence of natural microgravity (during a space flight) will allow us to identify the precise mechanisms in the development of skeletal muscle and myocardium cell responses when exposed to space flights, as well as the role of nonmuscle isoforms of actinin in the provision of mechanotransduction in these cells.

**MATERIALS AND METHODS**

Experiments were performed on the left ventricular myocardium (LV), soleus muscle (Sol), and tibialis anterior muscle (TA) samples obtained from C57 black mice, which were killed within 13–16.5 h of the BION-M1 biosatellite landing (the space flight lasted for 30 days from 19 April up to 19 May 2013, Russia). There were six animals (n = 6) in this main study group, which was designated as group F. During the space flight, the animals were provided with paste-like feed with an energy value of 361.4 kcal/100 g of dried feed.

The following control groups were formed.

- There were eight animals in control group V1 (n = 8), which were housed in the animal breeding facility (vivarium) during the space flight of the BION-M1 biosatellite.
- Control group S consisted of seven animals (n = 7), which served as asynchronous control animals and were left under 3-mo surveillance (since the date of the biosatellite launch). These animals were housed in a model of a space flight vehicle and underwent the entire cycle of prelaunch preparation tests (including the 72-h prelaunch housing of the animals in the spacecraft with all conditions of a space flight artificially reproduced, including the dietary pattern and gas composition of the air inflated). The age of the animals at the start of the synchronous experiment was similar to the age of the animals of the main study group. They were provided with the same feed as the group F animals.
- Group V2 consisted of seven animals (n = 7) and served as the vivarium control group (in relation to the animals in control group S). The formation of this group was justified by the necessity to avoid the effects of seasonal fluctuations on the studied parameters.

All of the animal experimental procedures were approved by the Commission on Biomedical Ethics of the State Scientific Center of the Russian Federation, Institute for Biomedical Problems, the Russian Academy of Sciences.

**Transversal stiffness measurements using atomic force microscopy.** Cardiomyocytes were isolated from a sample of the left ventricular tissue of the mouse using the standard technique (14, 47) but without the use of Triton X-100. The soleus and tibialis anterior muscles were extirpated from one tendon to another according to the technique which had been previously described by Stevens et al. (37). Before the experiments took place, the samples were stored at −20°C in a buffer containing relaxing solution in equal volumes of R (20 mM MOPS, 170 mM of potassium propionate, 2.5 mM of magnesium acetate, 5 mM of K2EGTA, and 2.5 mM of ATP) and glycerol.

On the day of the experiment, the samples were transferred into solution R, where glycerinized cardiomyocytes and muscle fibers were isolated.

To measure the transversal stiffness, the isolated cells were attached to the bottom of the liquid cell of the atomic force microscope, fixing their ends with the special Fluka shellac wax-free glue (Sigma, Munich, Germany). The measurements were performed in the contact mode with the indentation depth of 150 nm, in accordance with the technique which was previously described in detail (20).

The results obtained were processed in a specially designed program in the MatLab 6.5 environment.

**Determination of relative protein contents using gel electrophoresis-immunoblot analysis.** To determine the protein content, samples of the left ventricular myocardium, soleus muscle and tibialis anterior muscle were frozen at the temperature of liquid nitrogen. The method described by Vitorino et al. (45) was used to prepare tissue extracts and to obtain the membrane and cytoplasmic fractions of proteins.

Denaturing polyacrylamide gel electrophoresis was performed using the Laemmli technique and the Bio-Rad system (Hercules, CA). Based on the measurements of concentration using spectroscopic methods, the same amount of protein was applied to each well to estimate the relative protein content. Transfer to nitrocellulose membrane was carried out as described in the method of Towbin et al. (41).

In order to determine each protein, the specific monoclonal primary antibodies based on mouse immunoglobulins were used (Santa Cruz Biotechnology, Santa Cruz, CA) in dilutions recommended by the manufacturer: 1:300 for beta-actin (ACTB); 1:100 for gamma-actin (ACTG); 1:100 for alpha-actinin-1(ACTN-1); and 1:100 for alpha-actinin-2 (ACTN-2). As secondary antibodies, biotinylated goat antibodies were used against mice IgG (Santa Cruz Biotechnology) using a dilution of 1:5,000.

Next, the membranes were treated with a solution of streptavidin conjugated with horseradish peroxidase (Sigma, Germany) in a dilution of 1:5,000. Protein bands were detected using 3,3’-diaminobenzidine (Merck, White House Station, NJ).

**Real-time PCR (RT-PCR) for mRNA quantification.** In order to determine the expression rates of the genes encoding the studied proteins, total RNA was isolated from the frozen samples of the mouse left ventricular myocardium and tibialis anterior muscle, using the RNeasy Micro Kit (Qiagen, Hilden, Germany), in accordance with the manufacturer’s instructions. Reverse transcription was conducted using d(T)15 as the primers and 500 ng of RNA. To evaluate the expression rates of the studied genes, real-time PCR (RT-PCR) was performed using the specific primers selected using the Primer3Plus software (Table 1).

Expression rates of the corresponding genes were not determined in the fibers of the soleus muscle, because of the absence of sufficient materials for such studies.

**Statistical analysis.** The results obtained during the experiments were statistically processed using ANOVA, using the post hoc t-test with the confidence level P < 0.05 to evaluate the significance of differences between the groups. The data were represented as M ± SE, where M is the mean value and SE is the mean error.
RESULTS

Comparative characteristics of the studied groups of animals (Table 2). The results for weight indicated no differences between the studied groups of animals.

The mean weight of the tibialis anterior muscle reduced by 18.2% in group F ($P < 0.05$) compared with group VI and increased by 18.5% in group $S$ ($P < 0.05$). Moreover, the mean weight of the soleus muscle also decreased by 21.9% in group $F$ ($P < 0.05$) compared with group VI.

It should be noted that heart and adrenal gland weights did not statistically differ between the postflight and control animals, but these parameters tended to be increased in group $F$.

Left ventricular cardiomyocytes. The results of this study are shown in Table 3. The data obtained in all studied groups indicated that the transversal stiffness of the cortical cytoskeleton did not differ from the control level (group $VI$).

The content of beta-actin in the membranous fraction of proteins was found to be similar across the study groups (Fig. 1A). However, in group $F$, the relative content of beta-actin within the cytoplasmic fraction of proteins reduced by 38% ($P < 0.05$) compared with group $VI$. Similarly, expression rates of the gene encoding beta-actin were found to be decreased by 26% compared with the corresponding control value ($P < 0.05$) (Fig. 4A).

The content of gamma-actin in both protein fractions (as well as expression rates of the corresponding gene) did not differ between groups $F$, $V2$, and $S$ and the reference value registered for group $VI$ (Fig. 4A).

Alpha-actinin-1 concentrations within both protein fractions of the left ventricular cardiomyocytes did not differ between any of the study groups and the control level (Fig. 1C). At the same time, expression of the corresponding gene appeared to be elevated by 34% ($P < 0.05$) in group $F$ when compared with group $VI$ (Fig. 4A).

Within the membranous fraction of proteins of the left ventricular cardiomyocytes, the content of alpha-actinin-4 was reduced by 28% in mice of group $F$ ($P < 0.05$) compared with group $VI$ (Fig. 1D). No differences were detected for the content of alpha-actinin-4 within the cytoplasmic fraction of proteins. Alpha-actinin-4 gene expression rates changed exclusively in group $F$, where it was shown to be reduced by 18% ($P < 0.05$) compared with group $VI$ (Fig. 4A).

Soleus muscle fibers. The results of the study are shown in Table 4. The values of the transversal stiffness registered across all study groups did not differ from the control value obtained in group $VI$.

Beta-actin content within the membranous fraction of proteins of the soleus muscle did not differ from its content in group $VI$ either (Fig. 2A). However, within the cytoplasmic fraction of proteins, the content of beta-actin was decreased by 16% in group $F$ compared with group $VI$ ($P < 0.05$).

The content of gamma-actin did not differ from its reference value across all of the study groups (Fig. 2B).

In group $F$, the concentration of alpha-actinin-1 dropped by 55% within the membranous fraction of proteins ($P < 0.05$) compared with the levels reported for group $VI$. Conversely, it was elevated by 62% within the cytoplasmic fraction of proteins (Fig. 2C).

In the mice of study groups $F$, $V2$, and $S$, the content of alpha-actinin-4 within both protein fractions of the soleus muscle remained similar to the reference value registered for the animals of group $VI$ (Fig. 2D).

Tibialis anterior muscle fibers. The results of the study are represented in Table 5. The transversal stiffness of the tibialis anterior muscle fibers did not change across the study groups, except for group $F$, where it was found to be increased by 12% ($P < 0.05$).

Beta-actin levels, as well as the expression rates of the gene encoding this protein, were similar within all control groups of animals (Fig. 3A). In group $F$, the content of beta-actin was found to be elevated by 55% ($P < 0.05$) within the membranous fraction of proteins and reduced by 24% ($P < 0.05$) within the cytoplasmic fraction of proteins; this was accompanied by a reduction in beta-actin gene expression by 36% ($P < 0.05$) compared with the values for all of the corresponding parameters registered in group $VI$ (Fig. 4B).

Gamma-actin levels did not change within both protein fractions across all study groups (Fig. 3A). However, in group $F$, the expression rate of the gene encoding gamma-actin was found to be reduced by 15% ($P < 0.05$) when compared with group $VI$ (Fig. 4B).

In groups $F$, $V2$ and $S$, the contents of alpha-actinin-1 (Fig. 3C) and alpha-actinin-4 (Fig. 3D) within both (membranous

Table 1. RT-PCR primers and products

<table>
<thead>
<tr>
<th>Gene</th>
<th>Direction</th>
<th>Primer Sequence (5’…3’)</th>
<th>Product Size, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actn4</td>
<td>Forward</td>
<td>gttcagcagcaacctcctc</td>
<td>165</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>ttctttcctcaacctctctca</td>
<td>184</td>
</tr>
<tr>
<td>Actb</td>
<td>Forward</td>
<td>gttgcttttaccccttttc</td>
<td>197</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>gttgctccaaccaacctctct</td>
<td>218</td>
</tr>
<tr>
<td>Actg</td>
<td>Forward</td>
<td>ctgggtgatctcttggaacca</td>
<td>218</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>tcaggagggaganaaccaga</td>
<td>184</td>
</tr>
</tbody>
</table>

Table 2. General parameters

<table>
<thead>
<tr>
<th>Mass</th>
<th>Group VI, n = 8</th>
<th>Group F, n = 6</th>
<th>Group V2, n = 7</th>
<th>Group S, n = 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>M&lt;sub&gt;animals&lt;/sub&gt;, g</td>
<td>28.5 ± 0.4</td>
<td>29.4 ± 1.7</td>
<td>28.9 ± 1.0</td>
<td>29.7 ± 0.8</td>
</tr>
<tr>
<td>M&lt;sub&gt;triceps&lt;/sub&gt; ant. muscle, mg</td>
<td>75.8 ± 2.3</td>
<td>62 ± 3*</td>
<td>80.5 ± 2.9</td>
<td>89.8 ± 2.3*</td>
</tr>
<tr>
<td>M&lt;sub&gt;soleus&lt;/sub&gt; muscle, mg</td>
<td>9.6 ± 0.4</td>
<td>7.5 ± 0.3*</td>
<td>9.8 ± 0.7</td>
<td>9.9 ± 0.5</td>
</tr>
<tr>
<td>M&lt;sub&gt;heart&lt;/sub&gt;, mg</td>
<td>165 ± 14</td>
<td>189 ± 11</td>
<td>162 ± 10</td>
<td>183 ± 19</td>
</tr>
<tr>
<td>M&lt;sub&gt;adrenal glands&lt;/sub&gt;, mg</td>
<td>2.33 ± 0.21</td>
<td>2.56 ± 0.12</td>
<td>1.7 ± 0.4</td>
<td>1.92 ± 0.12</td>
</tr>
</tbody>
</table>

*P < 0.05 as compared to the group $VI$. 

Table 3. Transversal stiffness (pN/nm) of the cortical cytoskeleton of the left ventricle cardiomyocytes

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Group</th>
<th>n</th>
<th>Group</th>
<th>n</th>
<th>Group</th>
<th>n</th>
<th>Group</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>VI</td>
<td>8</td>
<td>F</td>
<td>6</td>
<td>V2</td>
<td>7</td>
<td>S</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.31 ± 0.11</td>
<td>1.27 ± 0.12</td>
<td>1.38 ± 0.09</td>
<td>1.34 ± 0.11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

J Appl Physiol • doi:10.1152/japplphysiol.00134.2014 • www.jappl.org
DISCUSSION

The results obtained may be indicative of skeletal mass reduction in mice after completion of the 30-day space flight without concomitant statistically significant changes in the myocardium weight. Such observations are in line with the available data in the literature (9, 39). Such loss of skeletal muscle mass may be caused by atrophic changes of muscle fibers, which have been reported after space flights of different duration in different species of rodents (39, 7, 5).

The absence of significant changes in adrenal weights may be indicative of either the full avoidance of stress impact, or the development of initial stages of the body’s response to stress, when no noticeable morphological changes develop in tissues. Taking into consideration the fact that dissection was performed within 13–16.5 h after landing, it can be hypothesized that stress impact may occur during the landing, but this had not altered the investigated parameters at the time of sample collection.

Transversal stiffness of the cortical cytoskeleton and content of nonmuscle actin isoforms in skeletal muscle fibers and cardiomyocytes. The values of the transversal stiffness of the cortical cytoskeleton of cardiomyocytes and fibers of skeletal muscles almost did not differ across the study groups compared with the vivarium control group, but they were found to be slightly increased in fibers of the tibialis anterior muscle in group F. It should be noted that, according to Alford et al. (1), who measured the electromyographic activity of different skeletal muscles in rats under conditions of prolonged antorthostatic suspension, the activity of the tibialis anterior muscle increased during the first day of suspension and remained elevated (compared with the baseline level) during the entire period of suspension. On the one hand, we can assume that elevation of the stiffness in fibers of the tibialis anterior muscle after being exposed to terrestrial gravity even for 13–16.5 h can be regarded as a residual effect of the space flight.

On the other hand, it was demonstrated that readaptation of atrophied muscles to gravity resulted in deepening of the negative changes when compared with those which appeared under microgravity. This became clear after completion of the SLS-2 mission (1993), during which the tissue samples were drawn under conditions of weightlessness. No serious injuries were detected in the fibers of m. soleus and m. adductor longus, and also after the 3-h period of readaptation; they only became appreciable within 4.5 h of the landing (30). By analyzing the
results obtained in this experiment, the authors concluded that, during the early period of readaptation, the contractile activity was perceived by an atrophied muscle as eccentric loading, as alterations of sarcomere structure observed within 4.5 h of the restoration period were similar to those which appeared after eccentric contractions (28–32).

Furthermore, the sequence of data, which had been obtained earlier in our experiments, suggested that the transversal stiffness of the cortical cytoskeleton decreased in fibers of the skeletal muscles of the rear paws and elevated in cardiomyocytes under conditions of antiorthostatic suspension. However, the dynamics of the changes in transversal stiffness was opposite during the period of readaptation (19–24). Moreover, changes in the sarcomere cytoskeleton were appreciable much later than in the cortical cytoskeleton of muscle cells.

Therefore, the following assumption may seem to be the most likely: the time period between the biosatellite landing and dissection (13–16.5 h) can be regarded as the period of readaptation, and is accompanied by a decrease in mechanical load on fibers of the soleus muscle and the tibialis anterior muscle with corresponding increases in stiffness (up to the control level in the soleus muscle and exceeding the control level in fibers of the tibialis anterior muscle). This assumption is fully in line with the data obtained by Riley et al. (29), who reported extensive alterations of the sarcomere cytoskeleton in fibers of m. adductor longus within 8–11 h of returning to conditions of terrestrial gravity after completion of the space flight on board the Cosmos 2044 biosatellite.

It is possible that changes in the stiffness of the cortical cytoskeleton on their own are of no fundamental functional importance for muscle cells due to their insignificant contribution to mechanical features of this type of cells, which are mostly determined by their contractile apparatus. However, we have previously shown that the transversal stiffness of the cortical cytoskeleton of muscle cell reflected the state of its structure and directly correlated with the content of nonmuscle actin isoforms in the membranous fraction (21, 22, 24).

The content of beta-actin and gamma-actin within the membranous fraction of proteins of the left ventricular cardiomyocytes did not differ across the study groups; a similar situation was observed in fibers of the soleus muscle. However, an increase in beta-actin content within the membranous fraction of proteins was detected in group F in fibers of the tibialis anterior muscle, which was accompanied by corresponding elevation of the transversal stiffness of the cortical cytoskeleton. At the same time, in group F, the content of beta-actin was found to be decreased within the cytoplasmic fraction of proteins in all of the types of tissues studied compared with the...
control level, as well as expression rates of the gene encoding beta-actin.

However, changes in the cortical cytoskeleton stiffness may initiate a number of signaling pathways and regulate the activity of ion channels. By means of the patch clamp technique, it was shown that actin microfilaments, which formed the structure of the cortical cytoskeleton, participated in the regulation of chloride ion channels (38, 34), Na\(^+/K\(^+\)-ATPase (8), voltage-gated sodium channels in brain cells (35), and sodium channels in the cells of reabsorption epithelium (4). Disintegration of actin filaments with cytochalasin D resulted in activation of sodium channels in the K562 cell line; actin polymerization on the cytoplasmic side of the outer cell membrane induced their inactivation (18). Moreover, fragmentation of actin filaments (associated with plasmatic membrane) induced by cytosol actin-binding Ca\(^{2+}\)-sensitive protein (similar to endogenous gelsolin) may constitute the main factor enhancing the activity of sodium channels in response to an increase in intracellular calcium ion concentrations in the K562 cell line (15, 16). Furthermore, actin can be transferred from the membranous to the cytoplasmic fraction in the form of F-actin with plasmatic membrane bound Ca\(^{2+}\)-sensitive protein (similar to endogenous gelsolin) can mediate the activity of sodium channels in response to an increase in intracellular calcium ion concentrations in the K562 cell line (15, 16).

Thus the data obtained on the transversal stiffness of the cortical cytoskeleton and content of nonmuscle actin isoforms in the left ventricular cardiomyocytes and in fibers of the skeletal muscles of rear paws may be evidence of nearly full completion of the readaptation processes of the submembranous cytoskeleton of these cells within 13–16.5 h after the 30-day space flight.

However, it should be noted that triggers inducing adaptational changes at the cellular level must be specifically different for cardiomyocytes and for fibers of skeletal muscles, as a reduction of external tension is applied in the first case and its elevation in the second case, respectively.

### Content of Nonmuscle Alpha-Actinin Isoforms and Their Expression Pattern

Previously, we have suggested a hypothesis that the fundamental difference in cell responses to elevation/reduction of external mechanical tension might be mediated through the dissociation of different actin-binding proteins caused by different deformations of the cortical cytoskeleton, which is induced by changes in external mechanical tension (22).

As calcium is one of the key messengers, it is natural that calcium-sensitive isoforms of alpha-actinin have drawn our exclusive attention within the wide range of actin-binding proteins. Alpha-actinin-1 and alpha-actinin-4 are able to bind calcium ions in micromolar concentrations, while intracellular calcium concentrations exceeding 10\(^{-7}\)M completely inhibit alpha-actinin binding to actin (26), as well as tyrosine phosphorylation with focal adhesion kinases within actin-binding domains (46).

There are only very limited data on the role of nonmuscle alpha-actinin isoforms in skeletal muscle cells. It is well known that alpha-actinin-1 is expressed in cardiomyocytes (43), as well as in skeletal muscle cells, together with alpha-actinin-4 at different stages of differentiation (10). Being located along
The data obtained in group F and control groups (V1, V2, S); *P < 0.05 compared with the group V1.

**Conclusion.** Thus we can assume that the dissociation of alpha-actinin-1 and alpha-actinin-4 from the cortical cytoskeleton initiates various signaling pathways. While alpha-actinin-4 directly penetrates inside the nucleus and acts as the regulator of transcription, its dissociation from the cortical cytoskeleton initiates a shorter (or more sensitive) signaling pathway, resulting in the earlier manifestation of changes in the structure of cardiomyocytes. At the same time, the alpha-actinin-1-mediated signaling pathway is initiated in the soleus muscle fibers, which may be longer and results in later responses of muscle cells to changing external mechanical conditions.

In general, some experimental data have been obtained after the completion of the abovementioned studies, following the 30-day flight of the BION-M1 biosatellite (particularly, data concerning changes of the organization of the cortical cytoskeleton in different types of muscle cells under the conditions of a space flight and during the following 13–16.5-h period of readaptation). These data allowed the existence of various signaling pathways to be suggested, which may be integrated within the cortical cytoskeleton and could determine possible pathways of mechanoreception. The latter allows a solution of the fundamental problem of modern cell biology and biophysics, related to interactions between physical field and a cell, to be proposed, which in turn, will allow fundamentally new measures to be developed for the protection of different tissues of the body from the negative effects of space flights, as well as rehabilitation methods following long-term exposure to weightlessness.
Acknowledgments

The authors express gratitude to N.S. Biryukov for the assistance in carrying out the experiments.

Grants

The financial support of the Russian Foundation of the Basic Research (RFBR grant 13-04-00755-a) is greatly acknowledged.

Disclosure

No conflicts of interest, financial or otherwise, are declared by the author(s).

Author Contributions

Author contributions: I.V.O. conception and design of research; I.V.O. and M.V.M. performed experiments; I.V.O. analyzed data; I.V.O. and I.M.L. interpreted results of experiments; I.V.O. and M.V.M. prepared figures; I.V.O. drafted manuscript; I.V.O. edited and revised manuscript; I.V.O. approved final version of manuscript.

References


