ISSN 1607-6729, Doklady Biochemistry and Biophysics, 2018, Vol. 481, pp. 205–207. © Pleiades Publishing, Ltd., 2018.
Original Russian Text © I.O. Petrova, S.A. Tyganov, T.M. Mirzoev, A.K. Tsaturyan, I.B. Kozlovskaya, B.S. Shenkman, 2018, published in Doklady Akademii Nauk, 2018, Vol. 481, No. 3.

BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

Early Decline in Rat Soleus Passive Tension with Hindlimb Unloading: Inactivation of Cross-bridges or Activation of Calpains?

I. O. Petrova[a], S. A. Tyganov[a], T. M. Mirzoev[a], A. K. Tsaturyan[b], Corresponding Member of the RAS I. B. Kozlovskaya[a], and B. S. Shenkman[a, *]

Abstract—The study was aimed at testing the hypotheses about the role of cross-bridges and calpains in reduction of rat soleus passive tension under conditions of hindlimb unloading. For this purpose, we used an inhibitor of μ-calpain PD 150606 as well as a blocker of actomyosin interaction (blebbistatin). It was found for the first time that a decrease in passive tension of rat soleus after 3-day hindlimb unloading is associated with the activity of μ-calpain and does not depend on the processes of cross-bridges formation.

DOI: 10.1134/S1607672918040075

It is known that, in real and simulated (hindlimb unloading) microgravity, both passive and active as well as both transverse and longitudinal tension of muscles and muscle fibers is reduced [1–5]. Previously, it was found that passive and active muscle tension to a certain extent depends on the actomyosin bonds (the so-called cross-bridges). However, the nature of atony at different duration of hindlimb unloading and the cytoskeletal or other factors that determine the severity of atopic changes still remain obscure.

On the basis of the previously obtained data, two mutually nonexclusive hypotheses can be formulated, which make it possible to explain the reduced passive tension of postural muscles in hindlimb unloading. Firstly, this is the calpain hypothesis (i.e., the assumption that, in the early stages of unloading, the intensification of the calpain-dependent degradation of proteins of the sarcomeric cytoskeleton leads to a reduction in the passive muscle tension [6]. Another hypothesis is that, under unloading conditions, the distance between the thin and thick filaments in the sarcomeres increases, which leads to a decrease in the number of cross-bridges and, respectively, to a reduction in the passive tension of an isolated muscle [7].

The purpose of this study was to test these hypotheses. We measured the passive tension of isolated rat soleus 3 days after hindlimb unloading simulated by antiorthostatic suspension (i.e., removal of support of hindlimbs). To test the first hypothesis, we used daily administration of the specific μ-calpain inhibitor PD 150606 [8], and the second hypothesis was tested by treating an isolated muscle before testing with blebbistatin, a selective blocker of actomyosin interactions [9].

Male Wistar rats weighing 180–200 g were divided into three experimental groups (15 animals each): the control vivarium group (C), the group that was subjected to 3-day antiorthostatic hindlimb suspension (HS), and the group that was subjected to suspension in combination with daily injections of the calpain inhibitor PD 150606 (Tocris Bioscience, United Kingdom; dose, 3 mg/kg body weight) in 10% DMSO in both soleus muscles (PD). After the end of suspension, both soleus muscles were taken for analysis under anesthesia with a sublethal dose of avertin. Then, the animals were euthanized with a lethal dose of avertin. All manipulations with animals were approved by the Biomedical Ethics Board of the Institute of Biomedical Problems, Russian Academy of Sciences and were performed in compliance with the internationally accepted standards of work with experimental animals.

After extraction from animals, soleus muscles were incubated in Krebs–Ringer solution (25 mM NaCl, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 25 mM $NaHCO_3$, and 25 mM glucose), which was aerated with carbogen (95% O_2 + 5% CO_2) to maintain the physiological pH 7.4 and oxygen saturation. During the incubation at 37°C in the dark for 1 h, the incubation medium was supplemented with 75 μM (–)-blebbistatin (Apexbio Technology, United States). Then, the optimum length of the muscle (L_0) was determined. Thereafter, the muscle was subjected to a single direct stimulation with parallel platinum electrodes (0.5 ms, 10 V) to produce a maximum single contraction. Then, the muscle was stretched by

[a] *Institute of Biomedical Problems, Russian Academy of Sciences, Moscow, 123007 Russia*
[b] *Institute of Mechanics, Moscow State University, Moscow, 119192 Russia*
** e-mail: bshenkman@mail.ru*

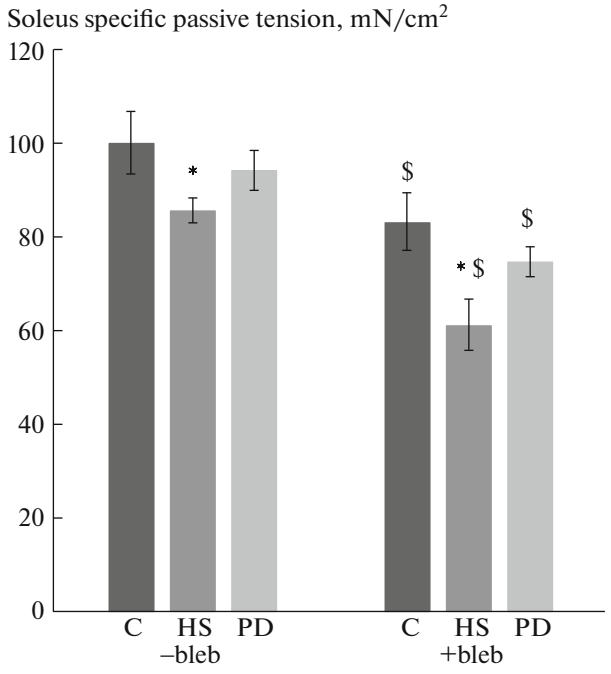

Fig. 1. Soleus specific passive tension (mN/cm^2). Designations (here and in Fig. 2): C—the control group rats; HS—the rats that were subjected to a 3-day hindlimb unloading; PD—the rats that were subjected to a 3-day hindlimb unloading combined with the calpain inhibitor PD 150606 injections; "–bleb" and "+bleb"—measurements in the absence and presence of blebbistatin, respectively. $^{\$}p < 0.05$ between the tension values at "–bleb" and "+bleb," $^{*}p < 0.05$ compared to the control. Here and in Fig. 2, data are represented as the median and quartile range (0.25–0.75), $n = 7$ for each group.

15% L_0 for 50 ms. This length was maintained for 100 ms; during this time, the resistance voltage was measured. After the measurement, the muscle was returned to the optimal length for 50 ms. All measurements were performed using an Aurora Scientific 305C-LR instrument (Aurora Scientific, Canada). Data were fixed at a frequency of 1 kHz, and the obtained results were processed using the Aurora Scientific Model 610A Dynamic Muscle Control software (Aurora Scientific).

The content of cytoskeletal proteins (myosin-binding C-protein MyBPC1, α-actinin-2, α-actinin-3, and teletonin) was determined by Western blot hybridization. The polyclonal antibodies against the above-mentioned proteins were from Abcam (United States) and ProteinTech Group, Inc. (United States). GAPDH was used as a reference protein (monoclonal antibodies from Cell Signaling Technology, United States). Data were statistically processed using the nonparametric Wilcoxon–Mann–Whitney U test.

It is known that hindlimb unloading is accompanied by a significant decrease in both passive and active tension of muscles and individual muscle fibers. For example, experiments [1] showed that the rat soleus passive tension after a 3–4-week suspension significantly decreased. However, it remains unclear which molecular mechanisms underlie this decrease in tension.

In the first series of experiments we found that, in group HS (suspension), the soleus passive tension decreased by 20% compared to the control (Fig. 1). The passive tension in the presence blebbistatin was significantly lower than in its absence both in the control and after a 3-day suspension. This fact indicates that the actomyosin interaction contributes to the passive tension of the muscle both in the control and after hindlimb unloading. However, the absolute values of the decrease in the passive tension in the presence of blebbistatin did not differ in the control and HS groups. Therefore, it cannot be concluded that the reduction in the passive tension under the hindlimb unloading conditions was caused by a reduction in the actomyosin interactions.

It is known that the passive tension of muscles under stretching is determined both by cross-bridges [10–12] and by cytoskeletal proteins (titin, nebulin, obscurin, and myosin-binding C-protein) [13, 14]. These proteins are degraded primarily by using the calcium-dependent cysteine protease calpain [6]. Therefore, the use of the specific calpain-1 inhibitor PD 150606 makes it possible to determine the role of the cytoskeleton degradation in the muscle tension decrease.

We found that the passive tension in group PD (3-day suspension combined with PD 150606 injections) did not differ from that in the control (Fig. 1). Thus, the inhibition of calpains prevented the decrease in the passive tension at the early (3-day) stage of hindlimb unloading. This fact may support the first hypothesis, according to which the decrease in muscle tension at this stage is determined by the calpain-dependent degradation of cytoskeletal proteins.

In the final series of experiments, we determined the content of cytoskeletal proteins by using Western blot hybridization. The content of MyBPC1, α-actinin-2, and teletonin did not differ between the groups. The content of α-actinin-3 in group HS was significantly (by 20%) reduced compared to the control, whereas in group PD it did not differ from the control (Fig. 2).

This fact consistent with the previously published results [4], which indicated a greater vulnerability of the Z-disc degradation zone during hindlimb unloading and a possible involvement of this process in the transverse tension reduction. Recently [15], weak mechanical bonds between α-actinin and titin molecules in the Z-disc zone in intact muscle were revealed. Possibly, the calpain-dependent degradation of α-actinin-3 and, respectively, the break of bonds with titin might facilitate the decrease in the soleus passive tension under hindlimb unloading.

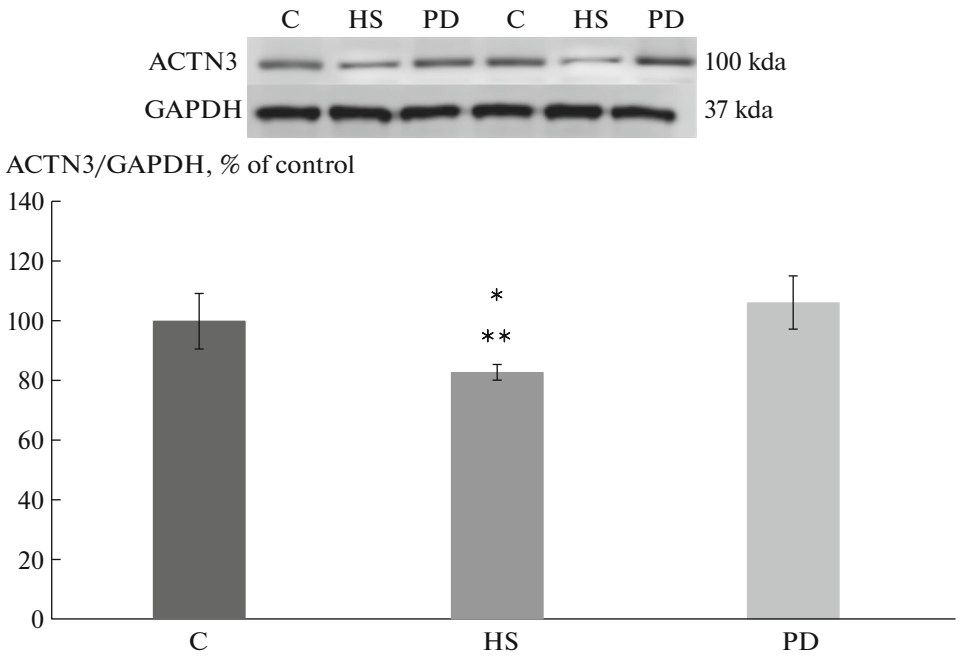

Fig. 2. Content of α-actinin-3 in rat soleus expressed in percent relative to the control group; *$p < 0.05$ compared to the control, **$p < 0.05$ compared to group PD.

Thus, in this study we for the first time established that the reduction in the passive tension as a result of a 3-day hindlimb unloading is due to the μ-calpain activity and does not depend on the processes of formation of cross-bridges (actomyosin bonds).

ACKNOWLEDGMENTS

This study was supported by the Russian Foundation for Basic Research (project no. 16-04-00529) and the Basic Research Program of the Institute of Biomedical Problems, Russian Academy of Sciences (subject nos. 65.3 and 63.1).

REFERENCES

1. Canon, F. and Goubel, E., *Pflugers Arch.*, 1995, vol. 429, pp. 332–337.
2. McDonald, K.S. and Fitts, R.H., *J. Appl. Physiol.*, 1995, vol. 79, pp. 1796–1802.
3. Toursel, T., Stevens, L., Granzier, H., and Mounier, Y., *J. Appl. Physiol.*, 2002, vol. 92, pp. 1465–1472.
4. Ogneva, I., *J. Appl. Physiol.*, 2010, vol. 109, pp. 1702–1709.
5. Ogneva, I.V., Ponomareva, E.V., Altaeva, E.G., Fokina, N.M., Kurushin, V.A., Kozlovskaya, I.B., and Shenkman, B.S., *Acta Astronaut.*, 2011, vol. 68, pp. 1478–1485.
6. Salazar, J.J., Michele, D.E., and Brooks, S.V., *J. Appl. Physiol.*, 2011, vol. 108, pp. 120–127.
7. Riley, D.A., Bain, J.L.W., Romatowski, J.G., and Fitts, R.H., *Am. J. Physiol. Cell. Physiol.*, 2005, vol. 288, pp. C360–C365.
8. Shenkman, B.S., Belova, S.P., Lomonosova, Y.N., Kostrominova, T.Y., and Nemirovskaya, T.L., *Arch. Biochem. Biophys.*, 2015, vol. 584, pp. 36–41.
9. Kovacs, M., Toth, J., Hetenyi, C., Malnasi-Csizmadia, A., and Sellers, J.R., *J. Biol. Chem.*, 2004, vol. 279, no. 34, pp. 35557–35563.
10. Granzier, H.L. and Wang, K., *Biophys. J.*, 1993, vol. 65, pp. 2141–2159.
11. Campbell, K.S. and Lakie, M., *J. Physiol.*, 1998, vol. 510, pp. 941–962.
12. Whitehead, N.P., Gregory, J.E., Morgan, D.L., and Proske, U., *J. Physiol.*, 2001, vol. 536, pp. 893–903.
13. Irving, T., Wu, Y., Bekyarova, T., Farman, G.P., Fukuda, N., and Granzier, H., *Biophys. J.*, 2011, vol. 100, pp. 1499–1508.
14. Muhle-Goll, C., Habeck, M., Cazorla, O., Nilges, M., Labeit, S., and Granzier, H., *J. Mol. Biol.*, 2001, vol. 313, pp. 431–447.
15. Grison, M., Merkel, U., Kostan, J., Djinović-Carugo, K., and Rief, M., *Proc. Natl. Acad. Sci. U. S. A.*, 2017, vol. 114, pp. 1015–1020.

Translated by M. Batrukova