Conclusions. 1) Dispersions of usual phospholipids (PL) in any medium are over a long period of time non-equilibrium systems at any PL concentration higher than 10 pM. However reactions of association/dissociation are so slow that any such dispersion looks like a stationary system. 2) The fluorescence of NBD-PE dispersion in any medium fluctuates. 3) A model of PL dispersion is proposed which allows the interpretation of experimental data presented.

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SEASONAL CHANGES OF TITIN ISOFORM COMPOSITION IN SKELETAL MUSCLES OF HIBERNATING GROUND SQUIRRELS (*SPERMOPHILUS UNDULATUS*)

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It is known that the development of pathological (dilated cardiomyopathy, hypertrophy of the myocardium, myodystrophies, the Stiff-person syndrome) [1−6] and adaptational processes (adaptation to hibernation and

microgravity conditions [4, 7−9]) is accompanied by changes in the isoform composition of the giant sarcomere protein titin. In particular, it was shown that an increase in the content of the long N2BA isoform of titin in the myocardium of hibernating ground squirrels *Spermophilus undulatus* plays an adaptative role and consists in the enhancement of the contractile response of the myocardium aimed at the release of more viscous blood during hibernation [7]. Conversely, in the myocardium of the hibernating bear *Ursus arctos horribilis*, the content of the short N2B isoform of titin increases, which, in the opinion of the authors, is aimed at maintaining the systolic function of the myocardium during hibernation [10]. The studies of changes in the isoform composition of titin in slow (m. soleus) and fast (m. psoas) skeletal muscles of hibernating ground squirrels *Spermophilus undulatus* revealed a decrease in the content of the known N2A isoform of titin with the retention of the content of the higher-molecular isoform NT [11] of this protein during the winter hibernation [4, 7]. In the present work we studied changes in the isoform composition of titin in some skeletal muscles of the back and extremities of the hibernating ground squirrels *Spermophilus undulatus* in different periods of activity: summer activity, winter activity (5−6 h after the exit from hibernation), hibernation, and arousal to determine the contribution of these changes to adaptation of skeletal muscles to hibernation conditions.

The following skeletal muscles of the ground squirrel were used: *m. vastus lateralis, m. gastrocnemius, m. longissimus dorsi, m. psoas, m. soleus, m. triceps*. SDS PAGE was carried out by the method described in [11] with a content of agarose of 0.55% and polyacrylamide of 2.1−2.3%. The densitometry of gels was performed using the program Total Lab 1.11 [2]. The content of titin was estimated relative to the content of heavy myosin chains. The statistical processing was carried out using the nonparametric *U*-test of Mann−Whitney. Differences with a confidence level $p < 0.05$ were considered significant. The Western blotting of titin was carried out as described in [4] using monoclonal antibodies AB5 to the region of the titin molecule localized in the A disc of the sarcomere.

In all skeletal muscles of torpid ground squirrels, the relative content of the N2A isoform of titin was found to decrease ~1.3−1.5 times, whereas the relative content of the NT isoform of titin either remained unchanged or increased ~1.3−1.4 times. In this case, the content of the T2 fragment of titin decreased two times and more, and the total content of titin decreased by 20−30%.

During the exit of animals from hibernation, a high NT/N2A ratio of the titin isoforms was retained against the background of the decreased total content of titin. An increase in the content of titin, in particular N2A and T2 isoforms in skeletal muscles of winter-active ground squirrels was observed even two to three hours after the exit from hibernation. The content of titin was restored after 24 h of winter activity. In this case, the increased NT/N2A ratio of the titin isoforms was retained.

What are the mechanisms of the decrease in the content of titin, in particular N2A and T2 isoforms, in muscles of ground squirrels during hiberna-

tion? It is known that the translation process in different organs of the ground squirrel during hibernation is inhibited due to both the inactivation of initiation (eIF-2) and elongation (eEF-2) factors through reversible phosphorylation [12] and owing to a decrease in the fraction of polyribosomes [13]. These data agree with the results of in vivo study of the incorporation of labeled amino acids into proteins, which showed that the synthesis of the protein in the heart of ground squirrels during hibernation was strongly (more than 200 times) inhibited [14]. The proteolytic activity at the level of proteosomes is also inhibited during hibernation [15]. However, the development of atrophic processes in skeletal muscles of animals during hibernation [16] indicates that catabolic processes dominate in this period over anabolic processes. Considering that the half-life of titin is 72 h [17], it can be assumed that the ~1.3−1.5-fold decrease in the content of the N2A isoform of titin and the stronger decrease in the content of the T2 in muscles of hibernating ground squirrels result from the inhibited but not completely suppressed proteolysis of titin in the absence of the synthesis of this protein during the hibernation bout, which lasts for 7−14 days. Presumably, enzymes responsible for titin proteolysis are Ca^{2+}-dependent proteases calpaines [18].

What are possible consequences of the decrease in the content of titin in muscles of hibernating ground squirrels? It is known that a decrease in the content of the N2A isoforms of titin (T1) in fibers of rabbit skeletal muscles by the action of ionizing radiation led to disturbances of the ordered structure of sarcomeres (in particular, a displacement of A-discs to the Z line, the appearance of broader A-zones of the sarcomere with irregular edges) and a decrease in the force of muscle contraction [19]. Similar disturbances of the structure and functional properties were observed in glycerinated fibers of skeletal muscles of the frog after the proteolytic degradation of connectin (titin) [20]. There is evidence indicating that a twofold decrease in the content of the N2A isoform of titin in m. soleus of the rat after the 6-week immobilization of the hind limb was accompanied by abnormalities of sarcomeric ultrastructure and a decrease in the Ca^{2+}-sensitivity of muscle filaments during contraction [8]. Considering these data, a disturbance of the ordered sarcomere structure and a reduction in the contractile ability of skeletal muscles of hibernating ground squirrels due to a decrease in the content of the N2A isoforms of titin could be expected. However, the available literature data and the results of our electron microscopic examination of the sarcomeric structure do not confirm the occurrence of these disturbances in skeletal muscles of ground squirrels during hibernation. It should be noted that there is evidence indicating that, in the myocardium of torpid ground squirrels, too, no disturbances in the structural and functional characteristics occur ([23] and Yu. M. Kokoz, personal communication) despite an about 1.5-fold decrease in the content of the N2BA and N2B isoforms of titin with the retention in the content of the NT isoforms of this protein [7]. These results indicate that the main role in maintaining the structural and functional characteristics of striated muscles is played by higher-molecular-weight NT isoforms of titin

rather than its N2A, N2BA, and N2B isoforms the decrease in the content of which is not accompanied by the impairment of the above characteristics in cardiac and skeletal muscles of ground squirrels. This conclusion is confirmed by the results of our studies indicating that the decrease first of all in the content of the NT isoform of titin in human and rat m. soleus atrophied under microgravitation conditions is accompanied by considerable disturbances of the contractile characteristics of this muscle [4, 24].

Interestingly the increase in the relative content of the NT isoform of titin in skeletal muscles of ground squirrels was observed even in the period of autumn activity during the preparation of animals for the hibernation. These changes correlated with the increase in the content of the slow isoforms of MHC in skeletal muscles of ground squirrels in this period [25]. The results obtained indicate that the NT isoform of titin is predominantly synthesized in slow fibers of skeletal muscles. It should be noted that our results on the transformation of the phenotype of muscle fibers of skeletal muscles of ground squirrels during the autumn preparation for hibernation agree with the data of other authors indicating early adaptation changes in different systems of the organism of hibernators in this period [26, 27].

Thus, seasonal changes in the isoform composition of titin in skeletal muscles of ground squirrels *Spermophilus undulatus* are directed towards an increase in the content of the slow NT isoforms of titin during the autumn preparation for hibernation and the retention of their high content throughout the hibernation season, which is a necessary condition for the maintenance of the highly ordered sarcomere structure and the required level of contractile activity of skeletal muscles in all periods of hibernation.

This work was supported by the Russian Foundation for Basic Research (grants No. 10-04-00141, 11-04-01026) and a grant of the Federal Targeted Program “Scientific and Scientific-Pedagogical Personal of Innovative Russia”, State Contract No. 02.740.11.0710.

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NEUROPHYSIOLOGICAL MECHANISM OF MOTOR AND MENTAL FUNCTIONS CORRECTION BY BIOFEEDBACK OF POSTURE STABILITY IN CHILDREN WITH ADHD

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Background and Aims: Attention deficit and hyperactivity disorder (ADHD) is the common syndrome affecting 3-20% of children. In previous researches it was revealed, that electric brain activity increased at realization