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BIOLOGICAL MOTILITY

New facts and hypotheses

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This volume contains the presentations that were made during the International Symposium "Biological motility: new facts and hypotheses". It took place in Pushchino, Moscow region and was devoted to new ashievements and perspectives in this area of knowledge.
Materials of the Symposium are of interest for biologists, medical and other specialists.

Responsible for the issue S.N. Udaltsov

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we looked after changes of intracellular H_2O_2 level in PDGF-stimulated fibroblasts. Obtained experimental data showed that PDGF activates long-term production of endogenous H_2O_2 and NOX inhibitor apocynin suppresses this effect. At the same time during NIH-3T3 activation EGF does not trigger off H_2O_2 synthesis and PI3K pathway. To understand better NOX/ H_2O_2 relation with PDGF we researched PDGF-stimulated increased production of H_2O_2 influence on PI3K and Ras/Erk1/2 MAP-kinase pathways. Obtained data showed that NOX/ H_2O_2 system activation leads to increase of PI3K-pathway signaling amplitude and duration of its activation. PDGF and EGF activate Ras/Erk1/2 MAP-kinase pathway by means of redox-independent mechanism. PI3K redox-dependent activation takes part in PDGF-mediated regulation of migration and proliferation in NIH-3T3 fibroblasts.

SEASONAL CHANGES IN THE ISOFORM COMPOSITION OF TITIN AND MYOSIN HEAVY CHAINS IN STRIATED MUSCLES OF BEARS

N.N. Salmov[1], A.D. Ulanova[1], I.M. Vikhlyantsev[1], A.G. Bobylev[1], D.S. Kozhevnikova[2], A.P. Saveljev[3], V.V. Makariushchenko[3], G.Yu. Maksudov[4], S.N. Udaltsov[1,5], Z.A. Podlubnaya[1,6]

[1]Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Moscow region, 142290, Russia;
[2]Samara State University, Samara, Russia;
[3]Russian Research Institute of Game Management and Fur Farming, Russian Academy of Sciences, Kirov, Russia;
[4]Moscow Zoo, Moscow, Russia;
[5]Institute of Physicochemical and Biological Problems of Soil Science, Russian Academy of Sciences, ul. Institutskaya 2, Pushchino, Moscow region, 142290, Russia
[6]Pushchino State Institute of Natural Science, Pushchino, Moscow region, 142290, Russia

Hibernation (winter torpor) is evolutionary capability of some mammals representing a temporal reduction in metabolism in order to survive at low temperatures and under conditions of seasonal food shortage. In true (obligate) hibernators (ground squirrels, woodchucks) the dormancy lasts 5-8 months and includes the 2-3 week cycles (torpor bouts) with short-term (several hours) arousal. During torpor bout the animal body temperature falls to 2-4°C, and the heart rate drops to 4-20 beats per minute (bpm) [1]. Bears entering winter sleep are the hibernating animals. The temperature of the bear's body decreases only by 5-7°C and animals easily come out of torpor into active state, but changes in metabolism are similar to those observed in the true hibernators during winter torpor.

Previously we revealed adaptation changes in the isoform composition of giant protein titin and myosin in striated muscles of an obligate hibernator, the long-tailed ground squirrel *Spermophilus undulatus* during winter dormancy. For instance, in cardiac muscle of ground squirrels during hibernation the increased content of the long (more elastic) N2BA titin isoform and the lowered content of the short N2B-isoform were observed [2]. Similar changes in titin content, the increased content of the long NT-isoform and lowered content of the short N2A-isoform, were recorded in skeletal muscles of these animals during hibernation [2]. Adaptation to these changes contributes to the maintenance of a highly-ordered sarcomeric structure and the necessary level of a contractile muscle activity for different periods of a hibernating cycle in the ground squirrel (entering hibernation, hibernation, arousal, winter interbout activity) [2]. It should be noted that during winter hibernation of the ground squirrel in atrophied skeletal muscles the increased content of the "slow" I isoform and the lowered content of "fast" IIa and IIx/d isoforms of myosin heavy chains were observed [3]. These changes point to the increase in the content of the slow, more endurable fibers with characteristic protein isoforms in muscles of the hibernating animals. Similar changes are adaptive response allowing considerable reduction in energy expenditure during hibernation period. It is necessary for the animal in order to survive under severe conditions of this period.

In this work we explored changes in isoform composition of titin and myosin heavy chains in cardiac and skeletal muscles of Brown (*Ursus arctos*) and Asian Black (*Ursus thibetanus*) bears with the aim of elucidating the role of these changes in adaptation of muscle system to the conditions of winter sleep.

In this study we used following striated muscles of the bears: (1) cardiac and skeletal muscles (*m. longissimus dorsi, m. biceps*) of the hibernating Asian black bear (adult male) trapped in the den on 28 January 2013 in Obluchenski district, the Jewish Autonomous Region in the Upper Ditur River watershed, 90 km west of Birobidzhan; (2) cardiac and skeletal muscles (*m. gastrocnemius, m. triceps, m. biceps, m. longissimus dorsi*) of the hibernating brown bear (adult female) bagged in the den in Yukamensk district, Udmurt Republic; (3) cardiac and skeletal muscles (*m. gastrocnemius*) of active brown bear (six-year old female) shooted down on 20 April 2013 at biostation of VNIIOZ (Kirov); (4) skeletal muscles (*m. gastrocnemius, m. triceps, m. longissimus dorsi)* of active brown bear (adult male) shooted on 9 September 2013 in vicinity of the village of Katny, Kotelnich district, Kirov region. All animals were legally gained in accord with special permits issued at regional offices of animal health and usage of the animal world objects.

The isoform composition of myosin heavy chains (MHC) was studied by 7% SDS-PAGE as described in [4]. SDS-PAGE of titin isoform was carried out by the method described in [5] with a content of agarose of 0.55% and polyacrylamide of 2.1-2.3%. The densitometry was performed using the program Total Lab 1.11 [2]. The content of titin was estimated relative to the content of MHCs. 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.

In skeletal muscles of the hibernating bears the changes in the isoform composition of titin and myosin heavy chains similar to those recorded in the muscles of the hibernating long-tailed ground squirrels were found. For instance, the content of the longer NT-titin isoform increases and the content of the shorter N2A-isoform of this protein declines (fig. 1). In this case in *m. gastrocnemius* and *m. longissimus dorsi* of the hibernating brown bear the content of the "slow" I isoform was higher than that of the "fast" IIa isoform of myosin heavy chains (fig. 2). In *m. longissimus dorsi* and *m. biceps* of the hibernating Asian black bear the contents of "slow" I and "fast" IIa isoforms of myosin heavy chains were nearly equal (~50% each) (data not shown).

It is interesting to note that the observed changes in titin and myosin heavy chains in muscles of the hibernating animals are opposite to those during the development of a series of pathological processes. For

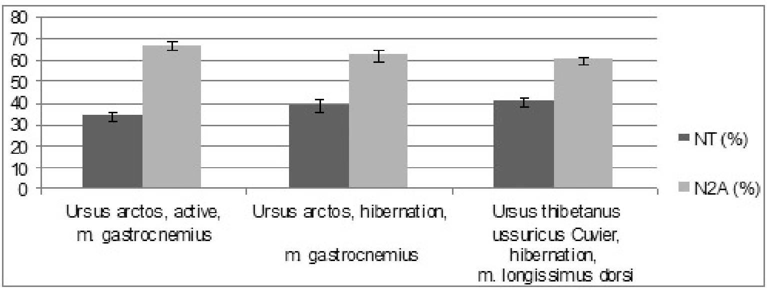

Fig. 1. Seasonal changes in titin isoforms composition in skeletal muscles of the brown bears.

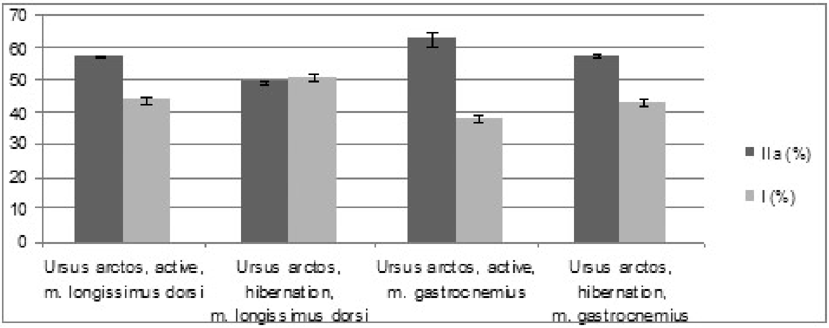

Fig. 2. Seasonal changes in the isoform composition of myosin heavy chains in skeletal muscles of the brown bears.

instance, during pathological and atrophied changes in skeletal muscles of humans and animals the content of NT- and N2A titin isoform decreases, the content of proteolytic T2-fragments of this protein increases and myosin phenotype shifts towards the augmentation of the content of "fast" isoforms of myosin heavy chains [2]. These alterations lead to disruption of a sarcomeric structure and a deterioration of the capacity of muscle contraction [2]. Based on the results obtained we believe that the increase in the content of NT-titin isoform in muscles of the true hibernators (ground squirrels) and in muscles of the bears is adaptive contributing to the maintenance of a highly-ordered sarcomeric structure and the necessary level of a contractile muscle activity in winter period. The decrease in the content of proteolytic T2-titin fragments in bear muscles during hibernation may also be adaptation. It is known that the products of protein catabolism are substrates of gluconeogenesis. Most likely that during hibernation the processes of gluconeogenesis in the liver occur due to the products of protein degradation, in particular, degradation of T2-titin fragments, the decreased content of which we observed in skeletal muscles of the true hibernator [2] and bears as well. The changes in myosin phenotype in skeletal muscles of the winter hibernating animals that directed to the increase in the content of "slow" isoforms of this protein have also a great physiological importance. Taking into account that the main strategy of hibernation is a strict economy of energy resources, it can be stated that transformation of skeletal muscles of the winter hibernating animals towards the increase in "slow" fibers with characteristic isoforms of myosin heavy chains is also an adaptation to hibernation conditions. These changes allow considerable decrease in energy ex-

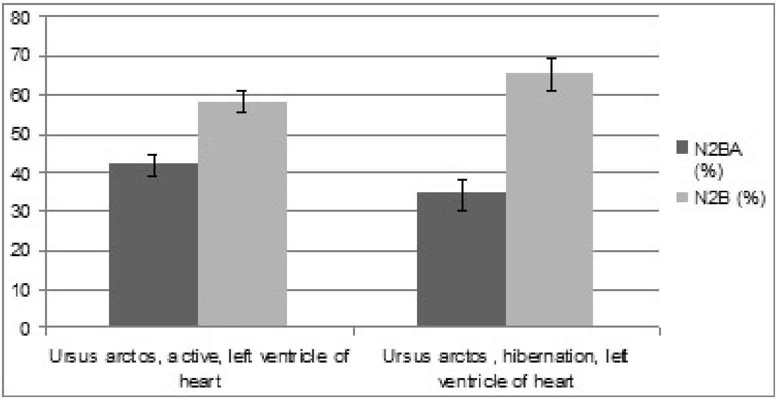

Fig. 3. Seasonal changes in titin isoforms composition in the left ventricle of the brown bear's heart.

penditure during winter dormancy that is necessary for the animal to survive under severe conditions of this period. It should be also noted that "slow" muscle fibers as opposed to "fast" ones are not only energetically more advantageous but more endurable. This property of muscles is vital to keep the postural pose of the animal during hibernation.

In the bear's cardiac muscle the changes in titin isoform composition were opposite to those in the cardiac muscle of the winter hibernating ground squirrel. For instance, as opposed to the increased content of the long N2BA-titin isoform in the cardiac muscle of the hibernating ground squirrel the content of this isoform in the cardiac muscle of the hibernating brown bear was lower than that in the active animal (fig. 3). In the cardiac muscle of the hibernating Himalayan black bear the N2BA to N2B ratio was similar to that one observed in the cardiac muscle of the hibernating brown bear (data not shown). Therefore, it can be stated that seasonal changes in titin isoform composition in the cardiac muscle of bears are directed to the increase in the content of the short N2B-isoforms of this protein while hibernating. The results obtained in our study are in accord with [6] – the increase in the relative content of the short N2B-titin isoform in the cardiac muscle of the hibernating grizzly bears (*U.a.horribilis*). Adaptive response of the changes revealed is presumably in preventing chamber dilation in heart of hibernating bears.

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References

1. Hochachka P., Somero J. (1988) Biochemical adaptation 568 p.
2. Vikhlyantsev IM, Podlubnaya ZA. New titin (connectin) isoforms and their functional role in striated muscles of mammals: facts and suppositions // Biochemistry (Mosc). 2012; 77(13): 1515-35. doi: 10.1134/S0006297912130093.
3. Lazareva MV, Trapeznikova KO, Vikhliantsev IM, Bobylev AG, Klimov AA, Podlubnaia ZA. Seasonal changes in the isoform composition of the myosin heavy chains in skeletal muscles of hibernating ground squirrels Spermophilus undulatus // Biofizika. 2012; 57(6):982-7.
4. Tikunov BA, Sweeney HL, Rome LC // J Appl. Physiol. 2001. V. 90(5). P. 1927-1935.
5. Vikhlyantsev IM, Podlubnaya ZA, Kozlovskaya IB. New titin isoforms in skeletal muscles of mammals // Dokl. Biochem. Biophys. 2004; 395: 111-3.
6. Nelson O.L., Robbins C.T., Wu Y., Granzier H. Titin isoform switching is a major cardiac adaptive response in hibernating grizzly bears // Am. J. Physiol. Heart Circ. Physiol. 2008; 295(1): H366-71. doi: 10.1152/ajpheart.00234.2008. Epub 2008 May 23.

DESIGN OF CULTURED CELL-BASED MODELS FOR ELUCIDATION OF THE 210 KDA MYOSIN LIGHT CHAIN KINASE ROLE IN MECHANOPHENOTYPE AND PERMEABILITY OF MICROVASCULAR ENDOTHELIUM

M.V. Samsonov[1], M.M. Khalisov[2], V.A. Penniyaynen[2], O.A. Kazakova[1], A.Y. Khapchaev[1], E.L. Vilitkevich[1], A.V. Nikashin1, A.V. Ankudinov[3], B.V. Krylov[2], V.P. Shirinsky[1]

[1]Institute of Experimental Cardiology, Russian Cardiology Research and Production Center, Moscow, 121552, Russia;
[2]Pavlov Institute of Physiology RAS, Saint-Petersburg, 199034, Russia;
[3]Ioffe Physical Technical Institute, Saint-Petersburg 194021, Russia

Microvascular endothelium participates in the formation of barrier between blood and tissues and in exchange of nutrients, gases and metabolic products between these compartments. High molecular weight myosin light chain kinase (MLCK210) is the key regulator of endothelial barrier. MLCK210 activation leads to attenuation of microvascular barrier and increased permeability to macromolecules. Acute increase in permeability of microvessels due to insufficiency of their endothelial lining is observed during physiological defence reaction of inflammation as well