Changes in Activity of Neutral Proteases in Tissues of Ground Squirrels in the Dynamics of Hibernation

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Total activities of neutral proteases in the cerebral, hepatic, and myocardial tissues of ground squirrel vary during hibernation: in autumn (before hibernation) activities of the enzymes in the brain and myocardium start increasing, while in the liver they do not change. A common feature for all tissues is minimum activity of active neutral proteases in the middle of hibernation month 1 bout, while the maximum activity is recorded before awakening.

Key Words: *hibernation; ground squirrels; neutral proteases*

Proteolytic enzymes play the key role in the realization of adaptive functions during changes of environmental conditions. Hibernation is a peculiar and interesting form of adaptation intrinsic of many mammalian species and aimed at survival under unfavorable conditions [8]. Biochemical processes and the work of organ and systems in hibernants are not inhibited, but are rearranged so that homeostasis is maintained at a certain level even at very low body temperature [3,5]. Neutral proteases can play an important role during transition to a new physiological level due to limited proteolysis reactions [4] triggering extracellular (matrix metalloproteinases) and intracellular (Ca^{2+}-dependent calpains) proteases.

We studied total activities of neutral proteases in the brain, liver, and myocardium of ground squirrel in the course of hibernation.

MATERIALS AND METHODS

Experiments were carried out on *Citellus pigmaeus Pallas* ground squirrels (200-250 g), typical representatives of hibernating animals. Ground squirrels were captured in the Buinak mountain pass region in winter-spring by flooding the holes; before cold weather the animals were kept in a vivarium on mixed plant and grain ration. In order to induce hibernation, the rodents were transferred into a dark cold room with a temperature corresponding to that outside and were kept on dry fodder. After temperature decreased to 8-6°C, the animals were placed into glass vessels with flooring. Animal activity was daily tested by the "sawdust test".

Total activity of neutral proteases was evaluated by modified Lowry's method with casein as the substrate. Optical density of trichloroacetic soluble filtrates was measured at 750 nm. Tyrosine solution (2 mM) served as the reference.

The data were processed using Student's *t* test.

RESULTS

Maximum activity of neutral proteases in hybernating ground squirrels was recorded in the liver (Table 1). In the brain it was 32.4%, in the myocardium 23.5% of enzyme activity in the liver. This difference is presumably explained by functional characteristics of these tissues. High activity of neutral proteases in the liver can be attrubuted to the fact that the liver works as a "biochemical laboratory", where all metabolic routes meet and where

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TABLE 1. Total Activity of Neutral Proteases (μmol tyrosine/g wet tissue) in Ground Squirrel Tissues during Hibernation (*M*±*m*; *n*=30)

Tissue	Active ground squirrels (July), normothermia 38°C	Before hibernation (October), 16-19°C	Hibernation month 1, 5-8°C	Hibernation month 2, 3-4°C	Hibernation month 3, 5-8°C	Before awakening, 6-7°C
Cerebral	3.3±0.5	4.6±0.2	None	0.70±0.02***	5.2±0.8	6.0±0.6*
Hepatic	10.2±1.0	10.9±0.9	6.9±0.4*	11.5±0.5	18.1±1.6*	18.7±1.0**
Myocardial	2.4±0.3	7.4±1.0***	3.0±0.4	8.1±1.0***	8.5±0.9***	9.5±0.5***

Note. *p<0.05, **p<0.01, ***p<0.001 compared to active animals.

protein degradation and synthesis processes are the most intense.

Total activity of neutral proteases does not change in the liver of ground squirrels preparing to hibernation. A drastic (almost 2-fold) drop of enzyme activity in the liver in comparison with the previous stages of the study was noted during month 1 of hibernation. During month 2, total activity of neutral proteases started increasing in comparison with month 1 (by 66.7%). By month 3 of hibernation, activity of neutral proteases in the liver increased significantly (almost 3-fold), and before awakening it peaked and was 171% increased in comparison with hibernation month 1.

Activity of neutral proteases in the brain of awaken ground squirrels (July) constitutes 1/3 of the enzyme activity in the liver. During preparation of animals to hibernation, enzyme activity was 39% higher, while during month 1 of hibernation dropped to the zero.

By month 2 of hibernation, activity of neutral proteases started increasing and was 20% of that in awaken animals. Month 3 of hibernation and awakening were characterized by an increase of total activity of neutral proteases in the brain in comparison with its level in active animals. Hence, total activity of neutral proteases in the brain before hibernation and by its end was similarly elevated (approximately 2-fold) in comparison with the active period.

In contrast to hepatic tissue, activity of neutral proteases in the myocardium was 2-fold elevated during the period of preparation to hibernation. This significant increase can be attributed to revision of the protein composition of tissues and appearance of new peptides [8]. Activity of neutral proteases remained high in the myocardium until month 1 of hibernation. Total high activity of neutral proteases was retained during 2- and 3-month hibernation and before awakening. Activities of the enzymes during these periods were virtually the same. On the other hand, activities of neutral proteases in the brain and liver increased drastically as the time of awakening was coming close. Presumably, total activity of neutral proteases is very important for the myocardium not only during preparation, but during all periods of winter hibernation.

Hence, reduction of total activity of neutral proteases in the middle of hibernation month 1 bout (presumably because of reduction of protein degradation and synthesis) and maximum increase of the enzymatic activity before awakening are characteristic of all tissues. Protein synthesis rate in the brain of hibernating ground squirrels is just 0.04% of its level in active animals [9]. The intensity of protein synthesis decreases not only because of reduced rate of the process, but also because of complete cessation of the synthesis of some proteins [9]. It is assumed that inhibition of protein synthesis during hibernation is caused by the need of energy resources economy during the period when blood flow rate, substrate content, and oxygen consumption are low [7,9]. Intensification of protein synthesis in tissues of awakening ground squirrels can be attributed to the need (common for all cells) to replenish functional and structural proteins lost and modified during hibernation or to replace some enzymes by specific isoforms needed at this temperature. Synthesis of new proteins or modification of the structure and function of proteins present in cells can play an important role in adaptation of the heterothermic animal cells to function at different temperatures.

During awakening of ground squirrels after hibernation, numerous proteins characteristic of active animals are synthesized in their tissues. Specific proteins absent in active or hibernating animals appear. It seems that the awakening process in ground squirrels requires the presence of specific proteins in the cells, which are actively produced during this period [1,2].

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