EXPERIMENTAL BIOLOGY

Seasonal Patterns of Epithelium Mitotic Activity in the Duodenum of two Representatives of the Sciuridae with Different Ecological Specialization

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A marked seasonal dynamics of the mitotic activity of duodenal epithelium is shown in hibernating red-cheek sousliks (*Citellus erythrogenys*). These seasonal variations are absent in *Sciurus carolinensis*, a closely related nonhibernating species. During hibernation, the mitotic index of souslik epitheliocytes is lower than during the summer period; however, during periodic spontaneous awakenings, a considerable rise of this index takes place, due to the activation of proliferative processes.

Key Words: *duodenum; mitotic index; hibernation*

Hibernation of mammals is attended by a marked drop of body temperature and metabolic level. Under these conditions an important role in homeostasis is assigned to the processes maintaining the structure of tissues, particularly of rapidly self-renewing ones. Inhibition of proliferative activity in various self-renewing tissues of hibernating animals during the torpid period has been unequivocally shown in many studies [3,4,12,13,15]. Periodic activation of proliferative processes during spontaneous awakenings has also been described [5,14,15].

Thus, spontaneous awakenings play an important role in the physiological regeneration of a hibernant during the long period of torpor. It should also be noted that the specific features of DNA precursor metabolism in the souslik make it practically impossible to study DNA synthesis [7,15].

The goal of this work was to study the comparative intensity of the seasonal variations of the mitotic index (MI) in the duodenum of the hibernating souslik (*Citellus erythrogenys*) and of a closely related nonhibernating representative of the same family (Sciuridae), *Sciurus carolinensis*. We also undertook a detailed analysis of the dynamics of MI in the duodenal epithelium of the souslik during a hibernation cycle, which consists of a period of hypothermia (lasting for around 2 weeks) and a short (up to 20-24 hours) spontaneous awakening, during the course of which the animal reaches normothermal status and then gets cooled again.

MATERIALS AND METHODS

The work was carried out on 8 squirrels (*Sciurus carolinensis*) and 47 sousliks (*Citellus erythrogenys*).

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Table 1. MI Levels in the Duodenal Epithelium of the Souslik during Hibernation

State	Number of animals	Body temperature, °C	MI, ‰
Hypothermia	5	6	1.22±0.37
Warming	6	9–17	2.65±1.14
	7	26–34	4.35±1.35
Normothermia	6	37	13.09±0.72
Cooling	7	32–24	6.77±2.10
	6	16–8	2.61±0.54

The specimens were taken between June and January. The specimens from sousliks were also taken at different stages of the natural torpid cycle. The samples of duodenal mucosa were fixed with glutaraldehyde and osmium tetroxide, and embedded in an Araldite-Epon mixture. Semithin sections were stained with methylene blue. Mitoses were counted in the region of crypts; 3000 epithelial cells per sample were scored. The frequency of mitoses was expressed in promille. The reliability of differences was assessed using Student's test.

RESULTS

The duodenal MI of squirrels showed no seasonal variations and was on average 21.17±2.66 ‰ . However, in sousliks this index was a function of the season, owing to the different physiological states; thus, during the active summer period, MI was reliably higher (9.55±0.25‰) than in the period of deep hibernation (1.22±0.37‰; $p<0.001$). A similar drop in the duodenal MI during deep hibernation was described earlier in hibernating sousliks, dormice, and hedgehogs [6,7,13,14].

Simultaneously with the drop in the duodenal MI, DNA synthesis in enteric epitheliocytes also falls [9]. Probably, at this time the length of intestinal villi diminishes due to the excess of the rate of cell shedding over the rate of repopulation [8]. Taken as a whole, these data attest to a reduction of mitotic activity in the small intestine of hibernating animals in the period of deep torpor. It is worth noting that analogous changes can be observed in the intestinal mucosa of long-fasting rats [2]. However, hibernation-associated alterations are obviously of a different physiological nature than in the case of "pathological" starvation.

Torpor-associated scarce mitoses are atypical; chromosomes form lumpy masses. Hypothermia directly influences the course of mitoses, i.e., it induces intramitotic blocking [1].

Periodic spontaneous awakenings that interrupt the deep sleep induce cyclic restructurings of the proliferative processes in the duodenal mucosa of the souslik.

In parallel with the change in the body temperature (Table 1), MI changes occur, reaching the maximal level (13.09±0.72‰) at the time of normothermia and dropping to the minimal level (1.22±0.37‰; $p<0.001$) during hypothermia. In the phase of normothermia the level of MI exceeds the "summer" index (9.55±0.25‰; $p<0.001$), while under analogous conditions the level of MI in the hedgehog duodenum reaches only half of the summer level [14].

It has been shown that in the torpid period the proliferating cells of the souslik duodenum accumulate in the G_2 phase [10], the duration of which is equal to 3 min to 1 hour for the gastrointestinal cells of rodents [11]. Taking into account that the period of warming in the spontaneous awakening lasts about 1.5-2 hours, we may consider the MI increase as a rise in mitotic activity; in other words, an increased frequency of mitoses results from the transition of "resting" cells from the G_2 phase to mitosis.

Thus, the mitotic index yields very early results on the proliferative activity of self-renewing tissues during spontaneous awakening.

During the spontaneous awakenings within the period of hibernation a partial renewal of the intestinal epithelial layer takes place thanks to coordinated proliferation, migration, and shedding [2]. This adaptive reaction is necessary for maintaining the structural integrity of the duodenal epithelium, since, although it does not fulfill its main function during hibernation (sousliks fast during the 7-8 months of hibernation), this epithelium continues to play a barrier role and is an important organ of the diffuse endocrine system.

REFERENCES

1. I. A. Alov, *Uspekhi Sovr. Biol.*, № 1, 64-72 (1982).
2. T. B. Timashkevich, *Pathways and Mechanisms of Gastrointestinal Regener. in Vertebrates* [in Russian], Moscow (1978).
3. T. M. Shivacheva and A. I. Khadzhiolov, *Arkhiv Anat.*, № 5, 48-54 (1987).
4. V. M. Yunker, *Seasonal Changes of Leukocyte Composition of Blood and Hemopoietic Organs in the Red-Cheek Souslik, a Representative of Hibernating Mammals* [in Russian], Ph. D. Thesis, Tomsk (1975).

5. V. M. Yunker and G. V. Alekseeva, *Zh. Evolyuts. Biokhim. Fiziol.*, № 2, 193-195 (1974).
6. S. J. Adelstein, C. P. Lyman, and R. C. O'Brien, *Mammalian Hibernation*, III, New York (1967), pp. 396-408.
7. S. J. Adelstein and C. P. Lyman, *Exp. Cell Res.*, **50**, 104-116 (1968).
8. H. V. Carey, *Amer. J. Physiol.*, **259**, № 2, R385-R392 (1990).
9. I. I. Kruman, S. G. Kolaeva, S. A. Rudchenko, and Z. S. Khurkhulu, *Comp. Biochem. Physiol. [B]*, **89**, № 2, 271-273 (1988).
10. I. I. Kruman, E. N. Iliysova, S. A. Rudchenko, and Z. S. Khurkhulu, *Ibid.*, **90**, № 2, 233-236 (1988).
11. M. Lipkin, *Physiol. Rev.*, **53**, № 4, 891-915 (1973).
12. F. J. Manasek, S. J. Adelstein, and C. P. Lyman, *J. Cell Comp. Physiol.*, **65**, № 3, 319-324 (1965).
13. W. V. Mayer and S. Bernick, *Anat. Rec.*, **130**, № 4, 747-758 (1958).
14. P. Suomalainen and H. Oja, *Comment. Biol. Soc. Scient. Fennica*, **30**, № 4, 1-6 (1967).
15. M. S. Vinogradova, *Comp. Biochem. Physiol. [A]*, **91**, № 2, 235-239 (1988).

GABAergic Basket-Pyramidal and Basket-Granular Systems of the Hippocampal Formation in the Cat

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The localization of GABA transaminase, the marker enzyme of axon terminals of GABAergic neurons, was studied in slices of cat hippocampus by the histological method. By its specific staining, the reaction product was consistently localized in synaptic basket terminals on granular cells of the dentate fascia and of pyramidal neurons of hippocampal areas CA1-CA3.

Key Words: *hippocampal formation; GABA transaminase; epilepsy*

A nerve impulse reaching the entorhinal cortex is capable of activating four excitation routes in succession: the perforant (from the entorhinal cortex to the dentate fascia), mossy fibers (from the dentate fascia to area CA3), Schaffer's collaterals (from the CA3 to the CA1 area), and, finally, the fibers passing from area CA1 to the entorinal cortex through the subiculum [3]. Granular cells of the dentate fascia and pyramids of the CA1-CA3 areas are the main effectors in this trisynaptic circulation; they synthesize glutamate and utilize it in the transmission of a nerve impulse, thus maintaining a high neurotransmitter balance of one of the main stimulating transmitters in the central nervous system [2,6].

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The hippocampal formation possesses an inhibitory apparatus which is one of the most potent in the brain [5,12] and which realizes through GABAergic axoaxonal and axosomatic terminals a retrogressive inhibition of the pyramidal and granular cells and selective depression of the presynaptic release of glutamate in them.

Some authorities [12] believe that impaired functioning of the inhibitory GABAergic mechanisms in the hippocampal formation is the key cause of epilepsy.

In this connection, mapping of GABAergic systems in this region of the brain is vital for the creation of histochemical models to investigate the effects of various drugs in studies of not only convulsive states, but the mechanisms of spatial memory, which is believed to be the principal function of the hippocampus [3].

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