hibiting effect on vascularization at the early stages of regeneration is diminished), while in right-side injuries the drug should be applied as described above.

Our findings suggest that the principle of left-to-right gradient maturation established for the functions of contralateral regions in the brain is also valid for regeneration of the microcirculatory bed in peripheral paired organs and results in a more rapid vascularization of the left-sided organ. Moreover, any intervention into regenerative or pathological processes in microvessels is more effective when location of the injury is taken into account [5].

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Serotonin-Producing Cells in the Duodenum of Hibernants During Periods of Hypothermia in Midwinter and Before Awakening in the Spring

M. S. Vinogradova and L. V. Shestopalova

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Ultrastructural differences are demonstrated between the duodenal serotonin-producing cells of ground squirrel during periods of hypothermia in winter and before awakening in the spring. In spring, the free ribosomes/polysomes ratio changes in these cells, and serotonin is actively released from secretory granules. These changes are associated with the preparation of the digestive system to functioning after hibernation.

Key Words: *hibernation; hypothermia; hibernant; enterochromaffin cells; serotonin*

Duodenal serotonin-producing (EC) cells of the hibernants differ substantially in ultrastructure during normo- and hypothermia, i.e., in summer and winter [4]. These seasonal differences are due to those in their functional significance in different periods of the year. In summer, the secretory product accumulates in these cells, where it is stored as cytoplasmic granules and utilized for regulation of the digestive tract activity. In fall and winter, the major ingredient of secretory granules, serotonin, becomes necessary for hibernation [2].

Laboratory for Study of Ecology-Related Issues in Morphology, Institute of Regional Pathology and Pathomorphology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk; Department of Physiology, Novosibirsk University

In this study we examined duodenal serotonin-producing cells of ground squirrels during two different periods of hibernation, namely, in the middle of winter and before awakening in the spring, in order to reveal possible reorganizations in these cells before the active period of life begins.

MATERIALS AND METHODS

The objects of the study were red-cheek ground squirrels (*Citellus erythogenys* Brandt) living in Western Siberian steppes. They begin to hibernate late in September and come out of hibernation in April. The hibernation is cyclic, with periods of hypothermia interrupted by spontaneous awakenings, when the body temperature progressively rises to normal before de-

TABLE 1. Morphostereometric Parameters of EC Cells and Serotonin and 5-HIAA Levels in the Duodenum of Red-Cheek Ground Squirrel

Characteristic	State of animals	
	torpidity in winter, body temperature 3-5°C	torpidity in spring, body temperature 4-6°C
Proportion of soluble granules, %	57.6±2.9	73.3±1.5**
Numerical density (N_v), μ^{-3}:		
free ribosomes	994±7	206±16**
polysomes	91±16	263±28**
Surface density (S_v), μ^{-1}:		
lamellar complex	1.11±0.22	1.09±0.15
endoplasmic reticulum	2.01±0.24	1.91±0.18
Duodenal levels, μg/g:		
serotonin	5.58 (3.9—3.35)	9.27 (7.01—10.91)*
5-HIAA	21.25 (13.9—26.6)	8.42 (7.46—10.96)*

Note. *p<0.01, *p<0.001 relative to the state of profound hypothermia in winter months.

clining slowly to 3-5°C. In December and January, the periods of hypothermia range from 14 to 18 days, while those of spontaneous awakening are short (20-24 h). In March and April, the periods of hypothermia become shorter and those of spontaneous awakening longer, but the body temperature of sleeping animals in a torpid state in the spring does not differ from that in midwinter.

The material for study was taken and examined at the "height" of hibernation (in December and January) and before the animals came out of hibernation (late in March and early in April). Duodenal tissue specimens were fixed in 3.5% glutaraldehyde, postfixed in 1% osmium tetroxide, and embedded in Epon-Araldite. After treatment with uranyl acetate and lead citrate, ultrathin sections were studied under a JEM-100CX electron microscope.

Stereometric analysis was conducted with a Microfot 5PO-1 apparatus using an open test system with a 0.36 μ diameter of the small step of the grid, the final magnification being 55,000.

The contents of serotonin and its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA), were measured by a modified method [1] based on previously reported data [7,9,10].

The results were statistically analyzed by Student's *t* test and the nonparametric Mann—Whitney *U* test.

RESULTS

Ultrastructural analysis of EC cells showed their similarity in hypothermic animals in winter and spring months. The nuclei of most cells were of irregular shape with diffusely arranged heterochromatin and with sometimes widened perinuclear space. The endoplasmic reticulum was represented by short profiles with a moderate electron density contents and occasional ribosomes. The lamellar complex was vacuolized and contained smooth vesicles varying in size, small numbers of "coated" vesicles, large vacuoles, and individual granules whose formation had begun during the period of normothermia but was inhibited with the drop of body temperature when the animal fell asleep again. Occasional lysosomes were seen.

Stereometrically, the surface density of the endoplasmic reticulum and lamellar complex in spring was the same as in winter; in summer, the parameters of these organelles did not differ significantly from the "winter" values (Table 1). In contrast, highly significant seasonal differences were recorded in the relative proportions of ribosomal structures. The state of torpidity in winter was associated with the presence of numerous free ribosomes and occasional polysomes, which indicates a decrease in protein production.

Before awakening, the number of free ribosomes in EC cells decreased considerably, which was accompanied by almost equal increase in the number of polysomes. These changes in the free ribosomes/polysomes ratio are typical of the initial stages of cell activation at the end of hibernation, presumably as a result of a long-lasting normothermia during the periods of spontaneous awakening. Previously, we reported a similar although less pronounced reorganization of the ribosomal apparatus in December and January [3]. We believe that the greater changes observed in the spring are associated with the preparation of cells for active functioning of the digestive tract immediately after awakening.

During the "spring hibernation", the level of serotonin in the duodenum rises, while that of its metabolite 5-HIAA falls. These changes correspond to the increase in the proportion of dissolving secretory granules, indicating that their contents are released from the cells (Table 1). These findings are consistent with the current concept that serotonin is involved in the regulation of digestion [5,6,8,14] and in cell proliferation [12]. Intense division of digestive tract cells was observed immediately at the end of torpid state [11,13].

From our results it can be concluded that although body temperature during hypothermia in midwinter does not differ from that just before spring, there are differences between these two periods in the respect of morphofunctional changes occurring in serotonin-producing cells. These changes concern the activity of ribosomal apparatus and the release of serotonin from secretory granules.

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