Specific Features in the Secretory Activity of Enterochromaffin Cells of the Duodenal Mucosa of Red-Cheeked Souslik under Different Physiological Conditions

L. V. Shestopalova and M. S. Vinogradova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 121, № 3, pp. 337-340, March, 1996
Original article submitted February 20, 1995

The ratio of the synthetic and secretory phases of the activity of duodenal enterochromaffin cells of the hibernating red-cheeked souslik under hypo- and normothermia is compatible with the functional significance of serotonin, one of their secretory products. The synthesis and accumulation of the secretion predominate during normothermia in summer and winter, whereas during deep torpor serotonin is mainly released from the cells.

Key Words: *hibernator; enterochromaffin cells; ultrastructure; secretory cycle*

A drastic change of elements of the gastrointestinal epithelial lining in winter in comparison with summer is a specific feature of the gastrointestinal secretory system of red-cheeked sousliks. As was previously shown, in summer all the secretory elements of the hibernator's duodenum are actively functioning. In winter the functional significance of the absorbing and of many secretory cells is reduced or completely precluded because of the abstention from feeding over the entire period of hibernation [1,2,4,9,11,12]. On the other hand, some elements of the endocrine system of the intestine continue to function, while other epitheliocytes remain in a state of relative rest. The secretory cycle of the endocrine cells is different under conditions of normo- and hypothermia, being determined by the demand for secretion products and their physiological fluctuations in summer and in different periods of hibernation [4,5].

Laboratory of Ecological Problems of Morphology, Institute of Regional Pathology and Pathomorphology, Siberian Division of the Russian Academy of Medical Sciences; Department of Physiology, Novosibirsk University (Presented by V. P. Kaznacheev, Member of the Russian Academy of Medical Sciences)

The secretory cycle of enterochromaffin cells (EC), whose main product, serotonin, is necessary to maintain hibernation, was examined in the study.

MATERIALS AND METHODS

Red-cheeked sousliks (*Citellus erythrogenys Brandt*) were used in the study. Their hibernation is characterized by a cyclic pattern and consists of periods of deep torpor concomitant with hypothermia and spontaneous awakenings during which normothermia is achieved. A total of 102 adult sousliks, mainly males weighing 250 to 300 g, were used.

Duodenal specimens were fixed in 2.5% glutaraldehyde solution. For electron microscopy the tissue was additionally fixed in 1% osmium tetroxide and after standard treatment embedded in an epon-araldite mixture in a 6:1 ratio. Ultrathin slices were contrast-stained in a saturated solution of uranyl acetate and lead citrate, after which they were examined under a JEM-100CX electron microscope at an accelerating voltage of 80 kV. For photooptic examination tissue specimens were embedded in paraffin. The slices were stained with fast garnet and the nuclei with Ehrlich's

0007-4888/96/0003-0308$15.00

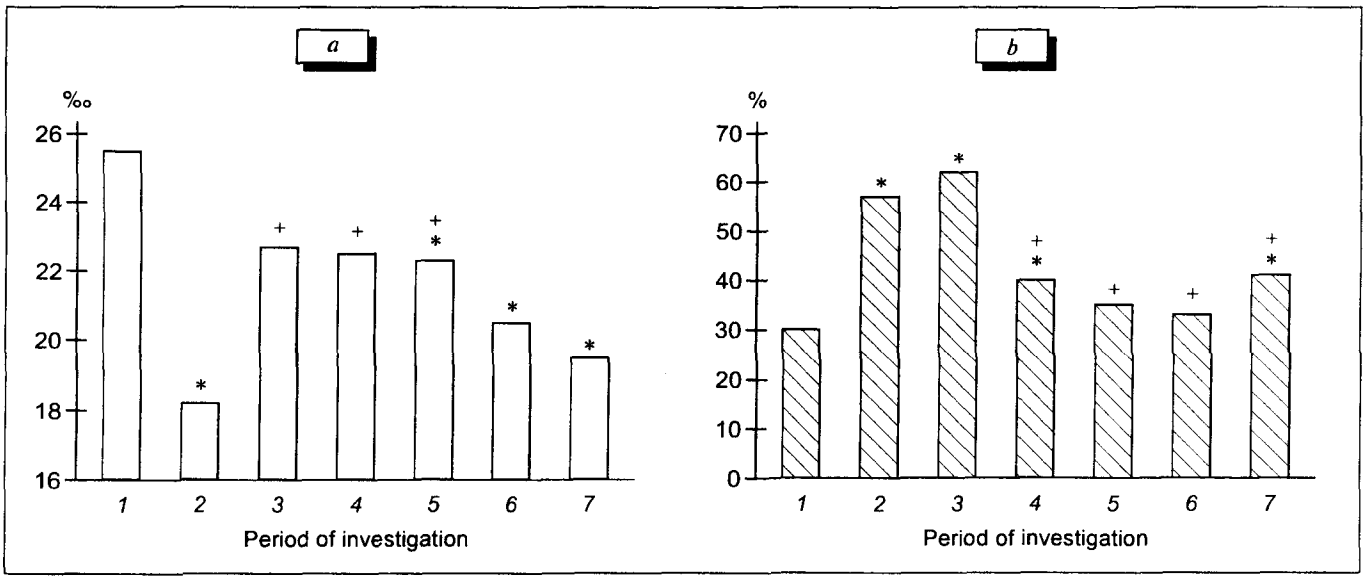

Fig. 1. Share of histochemically detected enterochromaffin cells of the duodenal mucosa of a hibernating souslik (*a*) and their dissolving granules (*b*) under different seasonal conditions. *1*) summer activity; *2*) deep hibernation; *3*) beginning of spontaneous awakening (8-10°C); *4*) beginning of spontaneous awakening (16-22°C); *5*) spontaneous awakening (normothermia); *6*) resumption of hibernation (16-22°C); *7*) resumption of hibernation (8-10°C). $p<0.05$: *reliable differences in comparison with summer; +in comparison with the period of deep hibernation.

hematoxylin. Histochemically detected EC were counted in at least 5000 crypt and villous epitheliocytes and the results expressed in promille.

RESULTS

The cells of the APUD system, including EC, are capable of capturing and decarboxylating the precursors of biogenic amines [10]. As a result of decarboxylation, 5-hydroxytryptamine (serotonin, 5-HT) forms from 5-hydroxytryptophan in EC. It is not yet quite clear in which organelles this occurs. The secretory product form granules in the Golgi apparatus, after which the granules accumulate in the basal part of the cell. There is an obvious relationship between the content of 5-HT in EC and of the acid proteins chromogranins (CG), which promote monoamine accumulation in the granules [6,7]. CG are matrix proteins of secretory granules promoting the accumulation of calcium ions which bind to the secretion and thus act as agents involved in the accumulation and release of amines and peptides [8]. CG are produced in the structures of rough endoplasmic reticulum and are further modified in the Golgi apparatus.

Hence, the status of the synthetic EC apparatus is a measure of its activity

Morphologically, only two stages can be clearly distinguished in the activity of an endocrinocyte: the synthesis of secretory material and its secretion. The parameters of synthetic activity of the cell are as follows: a high density of ribosomal material in the form of polysomes and attached ribosomes; long, narrow cisternae of the endoplasmic reticulum, flattened cisternae of the Golgi apparatus with granule formation; mitochondria with densely packed cristae; numerous "sunken" and smooth vesicles participating in the transport of the secretory material; and accumulation of secretory granules. The main criterion of secretion is the release of product from the cells and (or) the presence of many disintegrating granules in it. The ratio of these two parameters gives an idea of the level of functioning of the endocrinocytes.

These data indicate that during the periods examined, the ratio of synthetic to secretory activity of EC depends directly on the functional status of the hibernating animal, which is probably associated with the changes in the significance of 5-HT.

In summer the effect of EC secretion is aimed both at performing local regulation as a tissue hormone and at regulating some other physiological systems [3]. In winter, when feeding ceases completely, the digestive effects of endocrinocytes are switched off [12] and EC are involved in mechanisms regulating hibernation [4].

The EC secretion requirements of the organism change, and, accordingly, so does the morphological picture of the synthetic apparatus. The "summer" cells are characterized by an active synthetic apparatus with all the signs of intensive production of secretory material without any appreciable release of it outside the cell. The number of histochemically detected EC in the mucosa of the duodenal villi and crypts is maximal in summer: 25.6‰ (Fig. 1, *a*). The share of dissolving granules is minimal in comparison with other periods of the year: 29.6% (Fig. 1, *b*).

Studies of EC granules of the summer period enabled us to single out several modes of their dissolving,

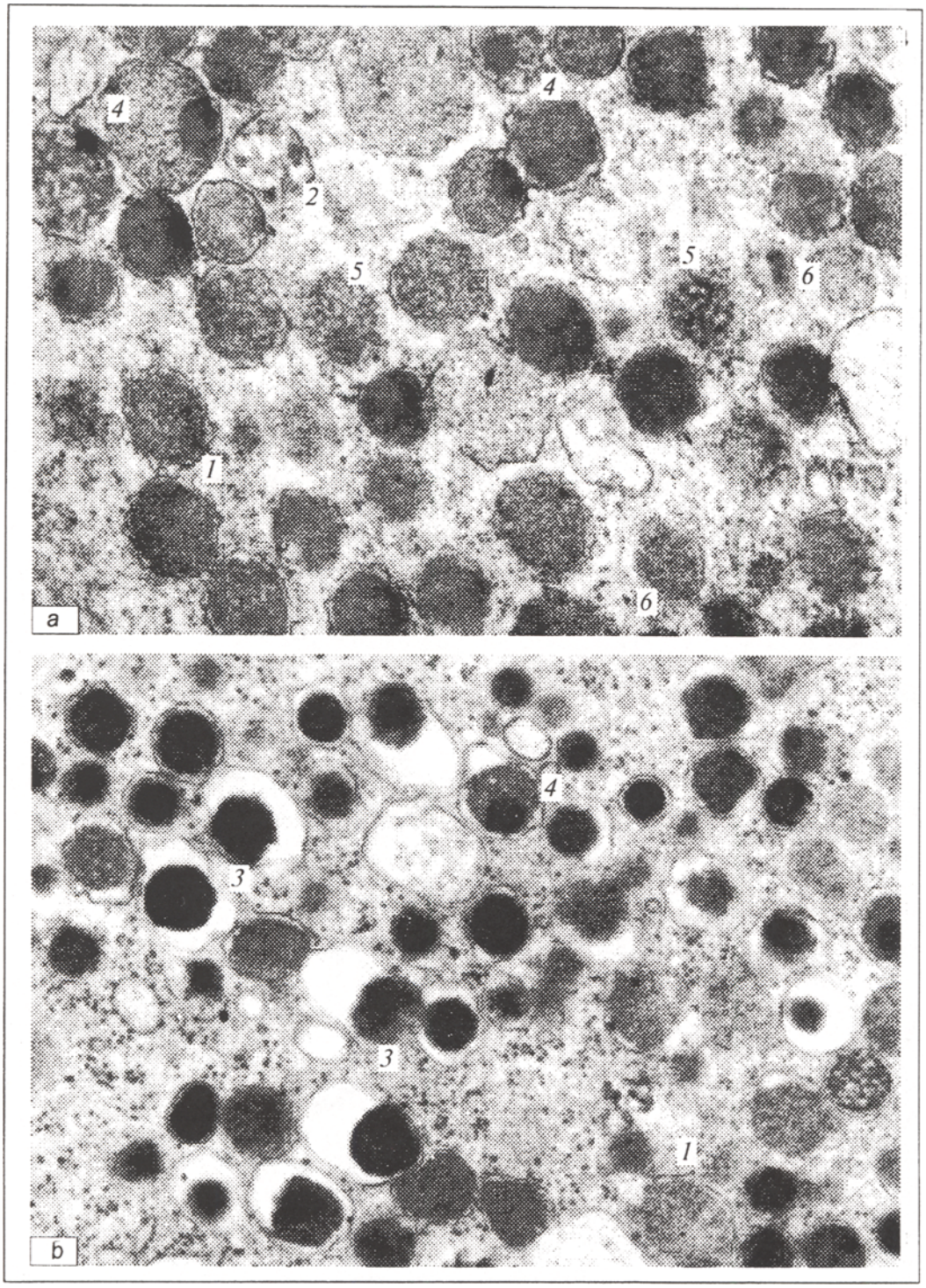

Fig. 2. Disintegration of granules in the basal part of enterochromaffin cells of the duodenal mucosa of a hibernating souslik (*1-6*: variants of granule dissolving). ×45,000. *a*) summer activity, normothermia; *b*) hibernation, hypothermia.

differing in the degree of granular membrane intactness, consistency, and electron density of the contents. Different variants were detected for one and the same cell (Fig. 2, *a*). The secretion is released by diffusion through the cell membrane. No exocytosis was observed.

During deep torpor under conditions of reduced metabolism the synthetic apparatus of the cell is at rest. This is indicated by fragmentation of the endoplasmic reticulum, vacuolization of the cisternae of the Golgi apparatus, the absence of transport vesicles and young secretory granules in it, an appreciable reduction in the number of polysomes, and an increased number of free ribosomes. The secretion is actively released from the cell. On days 5-6 of the torpid pe-

riod the share of dissolving granules appreciably increased to reach 57.6%. The number of modes of dissolving decreased (Fig. 2, *b*). Owing to the active release of secretory material, the number of histochemically detected cells reliably decreased: by 31% in comparison with the summer period.

During short spontaneous awakenings, the body temperature reaches 36°C in 1.5-2 h. It is during this period of normothermia that the lost reserves of necessary bioactive substances, including serotonin, are replenished. The beginning of warming, complete warming (winter normothermia), and gradual cooling during the onset of the new hibernation cycle were characterized by appreciable changes in the secretory apparatus and EC. The structures of the endoplasmic reticulum and Golgi apparatus rapidly became activated, and the number of active ribosomes increased. When the body temperature had risen to just 20°C, the morphological picture of the cells already signaled the beginning of the synthetic process. When normothermia was attained, active formation of granules was at its peak. As cooling occurred and the animals started a new cycle of hibernation, the synthesis of secretory material tapered off. Four to six hours after the beginning of hibernation the cells appeared to be "sleeping".

As a result of the synthesis and accumulation of 5-HT due to warming and normothermia in spontaneous awakening, the share of disintegrated granules decreased (from 62.3 to 34.2%, respectively) (Fig. 1, *b*). The count of histochemically detected cells increased by 15% on average in comparison with the period of deep torpor (Fig. 1, *a*). Later, during cooling, we observed a tendency for the share of dissolving granules to increase; by days 5-6 of torpor this tendency transformed into a reliable 52% increase in comparison with the period of normothermia during spontaneous awakening. These changes were due to the need for 5-HT for the development of hypothermia and for its maintenance during the new cycle of hibernation.

Hence, synthetic processes and the accumulation of secretory product in the form of basally disposed granules predominate in the duodenal serotonin-producing cells during normothermia in summer and in winter. The release of secretion product from the cell increases during the development and course of deep hypothermia.

REFERENCES

1. M. S. Vinogradova, in: *Mechanisms of Hibernation* [in Russian], Pushchino (1987), pp. 160-168.
2. T. V. Guvakova, I. I. Buzueva, and M. S. Vinogradova, *Zh. Obshch. Biol.*, **36**, № 6, 951-956 (1975).
3. N. K. Popova, E. I. Naumenko, and V. G. Kolpakov, *Serotonin and Behavior* [in Russian], Novosibirsk (1978).
4. L. V. Shestopalova, *Serotonin-Producing Cells of the Duodenum under Different Seasonal Conditions and during Hibernation* [in Russian], Author's synopsis of Cand. Biol. Sci. Dissertation, Novosibirsk (1994).
5. L. V. Shestopalova, M. S. Vinogradova, O. N. Ponomareva, and E. V. Dubinin, *Byull. Eksp. Biol. Med.*, **115**, № 2, 119-122 (1993).
6. Y. Cetin, *Histochemistry*, **93**, № 6, 601-606 (1990).
7. Y. Cetin and D. Grube, *Ibid.*, **94**, № 5, 479-484.
8. D. Grube, *Anat. Embryol. (Berl.)*, **175**, 151-162 (1986).
9. W. V. Mayer, in: *Mammalian Hibernation*, C. P. Lyman *et al.* (Eds.), New York (1960), pp. 131-148.
10. A. G. E. Pearse, *Vet. Res.*, **79**, 587-593 (1966).
11. L. V. Shestopalova and S. V. Aidagulova, in: *Mechanisms of Natural Hypometabolic States* [in Russian], Pushchino (1992), pp. 177-189.
12. M. S. Vinogradova, *Ibid.*, pp. 169-177.