Serotonin-Producing Cells in Duodenum of Siberian Chipmunk under Various Seasonal Conditions

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We studied species-specific characteristics of enterochromaffin cells in the duodenum in Siberian chipmunk in various physiological states. Predominance of synthetic or secretory phase in enterochromaffin cell activity depends on body temperature, metabolism, and feeding behavior.

Key Words: *enterochromaffin cells; ultrastructure; hibernation; chipmunk*

Biogenic amine serotonin (5-hydroxytryptamine, 5-HT) possesses a wide spectrum of activities. It affects digestion [8,10], regulates blood circulation, decelerates metabolism and heat production by suppressing shivering thermogenesis. Apart from these activities 5-HT belongs to hibernation regulators [3,4,11]. 5-HT is primarily (~90%) produced by gastric and intestinal enterochromaffin cells (ECC) [4].

The purpose of the present study was to examine morphofunctional features of duodenal ECC in Siberian chipmunks in summer and winter (in torpid and spontaneously awakening animals) and to compare them with previously studied ECC of red-cheeked ground squirrels [1,5,6].

MATERIALS AND METHODS

Siberian chipmunks (*Eutamias sibiricus*), small hibernating animals were used in the study. Chipmunk hibernation is characterized by alteration of hypothermia (7-8 days) and normothermia (up to 2 days). Specimens were obtained in summer (body temperature 37-39°C), in winter during torpid state (6-11°C), and during spontaneous awakening (33-35°C). Duodenal specimens were fixed in 3% glutaraldehyde, postfixed in 1% OsO_4, and embedded in epon-araldite mixture. Sections were contrasted by the method of Reinolds and examined under a JEM-100CX electron microscope (×61,600). The number of ECC per 1000 epithelial cells was counted on garnet fast-stained sections.

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RESULTS

Most ECC are located in the lower part of crypts, few ECC can be found in intestinal villi, solitary ECC are present in the duodenal glands.

The number of ECC is maximum in summer. These cells have cone shape and large round nucleus with perimembrane heterochromatin and 1-2 nucleoli; transparent cytoplasm contains heteromorphic electron dense secretory granules and numerous ribosomes. Rough endoplasmic reticulum (EPR) is presented by elongated narrow profiles. Golgi complex located in the perinuclear zone is presented by numerous rough and smooth vesicles indicating intense granule formation (Fig. 1, *a*). In summer, volume density of secretory granules is maximum. The formation and accumulation of secretory product are accompanied by its active release. In summer, the secretory product is released not only by diffusion after disintegration of secretory granules (45% secretory granules are disintegrated), but also via large cisterns formed by fused granules (Fig. 1, *b*).

In red-cheeked ground squirrel 5-HT is primarily accumulated in summer (only 29% disintegrated

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granules). The secretory product is released only via diffusion without exocytosis.

Torpid hibernating animals are characterized by hypothermia. In chipmunks, the number of histochemically visualized ECC was significantly decreased. The cytoplasm of these cells became more transparent, the nuclei had uneven contours, and nucleoli were only occasionally seen. The total number of ribosomes decreased 2.1-fold, the number of monosomes and attached ribosomes decreased significantly (Table 1),

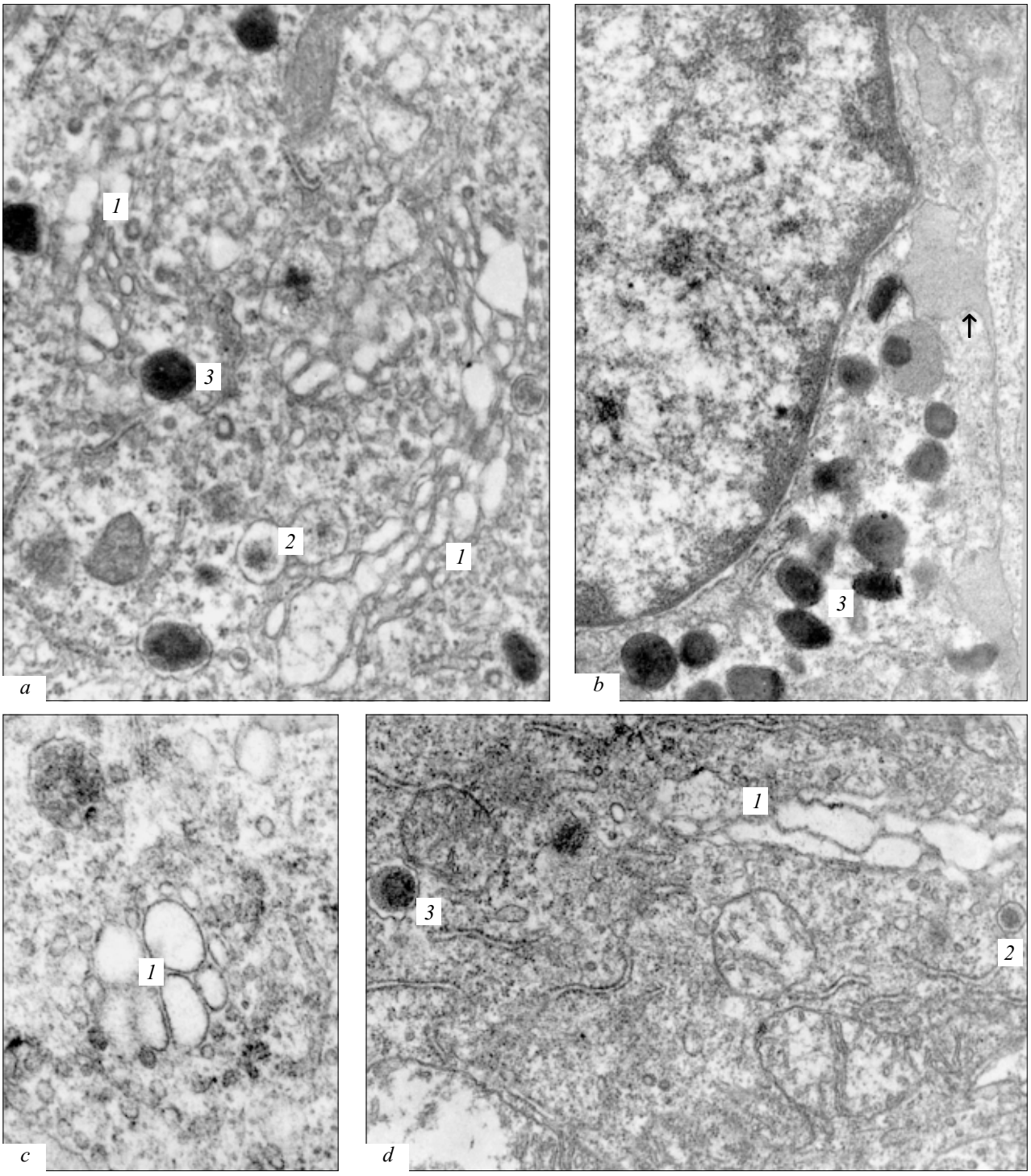

Fig. 1. Fragments of duodenal enterochromaffin cells from Siberian chipmunk in summer (*a* and *b*, ×29,700 and 22,400, respectively) and winter during torpid state (*c*, ×38,000) and spontaneous wakening (*d*, ×18,200). Golgi complex (*1*), immature secretory granules (*2*), mature secretory granules (*3*), arrows indicate secretion release.

TABLE 1. Characteristics of Duodenal ECC in Siberian Chipmunks in Different Seasons ($M \pm m$, n=5)

Parameter	Summer activity, 37-39°C	Deep torpor, 6-11°C	Normothermia, spontaneous wakening, 33-35°C
Histochemically visualized ECC, ‰	50.0±4.3	33.4±3.6**	36.6±4.0***
Number density of ribosomal structures in 1 μ³			
free ribosomes	435.5±55.0	117.9±17.7*	289.7±26.1*+
free polysomes	477.8±56.9	355.1±43.4	512.6±39.7++
attached ribosomes	791.2±98.6	353.3±36.3*	603.2±76.2++
Disintegrated granules, %	44.68±2.06	52.87±5.66	38.53±1.86***++
Volume density of granules	0.226±0.012	0.143±0.010*	0.164±0.015*

Note. *p<0.001, **p<0.01, ***p<0.05 compared to summer; +p<0.001, ++p<0.01 compared to deep torpid state.

while the number of polysomes remained at the summer level, *i.e.* synthetic processes can be resumed immediately after termination of hypothermia. Rough EPR was fragmented and Golgi complex was vacuolated and presented by vesicles of various sizes (Fig. 1, *c*). Similar phenomenon was described by S. Hurtley [9] after inhibition of transport between EPR and Golgi complex. The number of disintegrated granules did not differ significantly from the summer level. Thus, active utilization of the secretory product was not compensated by its synthesis and accumulation.

In ground squirrels, ECC changes during torpid state differ from those in chipmunks [5,6]. The number of monosomes increases several times, while the content of polysomes and attached ribosomes decreases, which corresponds to long-term inhibition of the digestive function. The portion of disintegrated granules (58%) increases 2-fold compared to the summer level, which could be explained by intensive 5-HT utilization during hypothermia lasting twice longer than in chipmunks.

Awakening, a necessary component of hibernation [2,7], was associated with activation of physiological functions [12]. During spontaneous awakening (normothermia), the number of ECC in chipmunks did not differ from that during hypothermia. ECC had large transparent nuclei with diffuse heterochromatin and distinct nucleoli. Activation of the synthetic apparatus manifested in a significant increase in the number of monosomes, polysomes, and attached ribosomes compared to the torpid period (Table 1). EPR was presented by long narrow profiles with multiple attached ribosomes. Elongated and flattened Golgi complex cisterns (Fig. 1, *d*) and numerous coated vesicles are characteristic signs of active membrane transport from EPR to Golgi complex. Active synthesis of secretory products and its packing into granules occurred. The portion of disintegrated granules decreased to a minimum. The secretory product was not released via fusion of secretory granules.

In ground squirrels, spontaneous awakening, warming, and normothermia were also associated with activation of synthetic functions in ECC, while cooling was accompanied by inhibition of synthetic processes and enhanced release of the secretory product [5,6]. Ribosome redistribution during normothermia in ground squirrels differed from that in chipmunks: numerical density of monosomes decreased, while the content of polysomes and attached ribosomes increased. The number of histochemically visualized ECC significantly increased compared to the torpid state. The content of disintegrated granules decreased 2-fold (37%). Hence, the state of intestinal cells including ECC in these animals in winter was determined by the absence of exogenous feeding.

Thus, predominance of secretory and synthetic phases in activity of duodenal ECC in Siberian chipmunks depends on the season, hibernation period, and feeding behavior. Synthesis and accumulation of the secretory product prevail in summer, while during torpid state it is primarily released from cells; winter awakenings are characterized by active production and utilization of the secretory product. It can be hypothesized that during spontaneous awakenings the ECC secretory products in chipmunks is utilized mainly for digestion.

Different activities of ECC in examined hibernating mammals depend on peculiarities of hibernation. In ground squirrels, the duodenum in the absence of digestion (in winter) functions as an endocrine organ. In chipmunks, frequent and prolonged winter awakenings and food consumption associated with temperature rises were accompanied by rapid activation and the corresponding ultrastructural changes in intestinal cells.

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