SEASONAL TREND IN THE NUMBER OF GASTRIC APUDOCYTES DURING HIBERNATION

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The apudocytes (endocrine cells) of the mammalian gastrointestinal tract are fairly numerous. They are located in the epithelium of the glands and produce peptide hormones and biogenic amines, which have a local and general influence on the body [1,5,9,11-13,16]. Morphological features of the apudocytes in the gastric mucosa of a hibernating rodent, the red-cheeked suslik, in different seasons of the year have been described [3, 4]. During hibernation the digestive system of susliks is completely inactive and changes are observed in the structure and content of the apudocytes.

In the investigation the number of apudocytes in the gastric mucosa of the red-cheeked suslik was studied in the periods of summer activity, of hibernation, after awakening from hibernation, and also in animals active in the winter.

EXPERIMENTAL METHOD

Active animals (June), animals in a state of deep hibernation (December, January), 3-4 days after awakening in spring (April), and animals kept in a warm room in winter, and consequently active in winter (December, January) were investigated. The animals of the last group did not fall into hibernation and took food. Each group consisted of 5-7 susliks. All the active animals were deprived of food for 24 h before decapitation, but given water ad lib. Decapitation was carried out in the morning. Material for investigation was excised from the fundus, body, and pylorus of the stomach, and fixed in 2.5% glutaraldehyde solution in 0.1M phosphate buffer, pH 7.4. Paraffin sections were stained by the diazonium method with Fast Garnet GBS [8] and counter stained with lead-hematoxylin [2, 14]. This combined treatment enabled the serotonin-containing EC cells and all the other cells, not containing this biogenic amine, together, in one section. Apudocytes were counted at the level of the terminal portions of the gland, where they are concentrated in the greatest number, per 10,000 epithelial cells. The results were expressed in promille. All cells were counted by one investigator on coded specimens.

EXPERIMENATL RESULTS

Apudocytes of two main types were discovered in the sections. Cells of the first type stained brick red with Fast Garnet, and were serotonin-producting EC cells. Intensely stained cells with a dense distribution of granules (bright EC cells) and others containing only a few, palely stained granules (pale EC cells) were distinguished. In the pyloric part of the stomach a few EC cells whose granules were stained reddish-black, also were found. Evidently they contained less serotonin and (or) more of the protein components of the granules. The second type include cells taking up lead hematoxylin, which gave them a black-blue-azure hue. These were non-EC cells, a term which covers all cells producing various peptide hormones and biogenic amines, but not serotonin, in the mucosa of the gastrointestinal tract [10, 15]. In the red-cheeked suslik this category of non-EC cells in the mucosa of the gastric fundus contains A-like, D-, D_1- cells, whereas in the pyloric mucosa they include G-, D-, and D_1-cells [5]. Cells of the second type also could be subdivided into those containing many brightly stained and pale blue granules. It is considered that pale cells of both types are functionally active, whereas the bright cells contain more of the reagents stored in numerous granules, and their activity is correspondingly depressed. Besides EC- and nonEC-cells, endocrine cells not containing granules and not staining by the method used, were found in the pyloric region of the susliks — these were agranular cells. Apudocytes were unevenly distributed in different parts

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TABLE 1. Number of Apudocytes in Gastric Fundal Glands of Susliks

Type of cell	Number of endocrine cells, per cent			
	Summer activity (n = 6)	Hibernation (n = 5)	3rd-4th days after awakening (n = 5)	Winter activity (n = 5)
Total number of apudocytes	107,66±8,28	101,20±14,53	130,84±10,82	158,80±14,34
All EC-cells	39,66±2,96	62,64±7,97	57,36±5,35	67,94±8,23
Bright	12,73±2,76	12,16±4,83	4,46±1,30	8,32±2,12
Pale	26,93±1,43	50,48±11,42	52,90±4,47	59,62±7,33
All non-EC-cells	68,0±5,70	38,56±7,73	73,48±11,76	90,86±6,28
Bright	59,98±5,06	10,16±0,85	11,12±1,81	45,72±3,94
Pale	7,85±0,97	28,40±7,10	62,36±13,16	47,14±8,35

TABLE 2. Number of Apudocytes in Gastric Pyloric Glands of Susliks

Type of cell	Number of endocrine cells, per cent			
	Summer activity (n = 5)	Hibernation (n = 5)	3rd-4th days after awakening (n = 5)	Winter activity (n = 7)
Total number of apudocytes	37,60±2,84	53,25±2,86	74,86±5,02	73,48±6,13
All EC-cells	15,24±1,22	21,38±1,64	22,04±1,41	30,02±3,80
Bright	5,16±0,59	9,28±1,59	3,52±0,56	3,35±0,61
Pale	8,20±0,72	10,07±1,50	17,02±1,40	25,55±3,23
With reddish-black granules	1,86±0,29	2,01±0,81	1,10±0,25	1,11±0,26
All non-EC-cells	18,70±2,20	16,38±2,23	29,02±5,42	20,90±2,54
Bright	8,46±1,35	3,37±0,56	4,44±1,25	3,31±0,83
Pale	10,24±1,36	10,85±3,21	24,38±4,49	17,58±2,68
Agranular cells	3,68±0,70	15,50±2,72	23,8±4,11	22,55±2,33

of the stomach. They were most numerous in the mucosa of the fundus, where the short gastric glands are situated, and significantly fewer in the pyloric portion and in the body of the stomach, the site of the long gastric glands, they are found extremely rarely, and for that reason they were not counted in this situation.

Analysis of the results of counting apudocytes in the gastric fundus revealed (Table 1) no significant difference in their total number during hibernation ($P>0.05$). After awakening from hibernation their number increased: this correlated competely with activation of proliferative processes and intensive functioning of the digestive system. The largest number of apudocytes was found in animals active in winter; it differed significantly from the number in summer ($P<0.03$) and during hibernation ($P<0.03$).

The number of serotonin-producing EC-cells increased significantly during hibernation ($P<0.03$), due to an increase in the number of most active, pale cells. This is logical because we know that serotonin takes part in the maintenance of the state of hibernation [6, 7]. The number of EC-cells in animals awake during the winter did not change significantly compared with their number during hibernation ($P>0.1$), but it was significantly higher than their number in summer ($P = 0.005$). On the 3rd-4th day after awakening a tendency was observed for the number of EC-cells to fall, mainly due to a decrease in the number of bright EC-cells.

The number of non-EC-cells fell sharply and significantly ($P<0.01$) during hibernation. This group consisted mainly of cells involved in digestion, which does not take place during hibernation. The increase in the number of pale non-EC-cells ($P<0.05$) was possibly connected with functioning of the D-cells, which are members of this group and produce somatostatin, which inhibits activity of many cells in the gastric mucosa. The total number of non-EC-cells 3-4 days after hibernation was significantly increased ($P<0.05$) compared with the period of hibernation, and corresponded to their number in summer. After awakening, susliks eat actively and digestion takes place completely; the number of endocrine cells concerned in its regulation therefore increases. The increase in the number of non-EC-cells during this period was associated with the more active pale non-EC-cells. The total number of non-EC-cells in susliks active in winter was greater than their number during hibernation ($P<0.001$) and during summer activity ($P<0.03$). This is logical, because the animals are taking food. The total number of apudocytes in the pyloric part of the stomach was only about one-third of their number in the fundus of the stomach. The number of apudocytes in the fundus (Table 2) increased during hibernation ($P<0.01$), increased even more on awakening ($P<0.001$) and was at a high level in animals active in winter. Just as in the fundal mucosa, the increase in the number

of cells in the pyloric portion was due to an increase in the number of serotonin-producing EC-cells, among which both the pale active forms and the bright cells storing granules become more numerous in winter. However, in relation to the total number of EC-cells, the mucosa of the gastric fundus must evidently be considered to be the main supplier of serotonin. The number of EC-cells on awakening from hibernation was the same as their number in winter. In animals active in winter, the number of pale EC-cells increased distinctly compared with their number during hibernation ($P<0.01$).

The level of all non-EC-cells during hibernation did not change significantly compared with their number in summer ($P>0.01$), but increased after awakening, when it actually exceeded the summer level.

Agranular cells deserve particular attention. There were very few of these cells in summer but their number increased significantly during winter ($P = 0.003$); their number increased after awakening, but not significantly, compared with the period of hibernation. Undifferentiated, emptied, and dying cells were included in this category; the increase in their number in winter and after awakening is fully substantiated.

Apudocytes of the gastric mucosa and the red-cheeked suslik thus undergo marked numerical changes in the different seasons of the year, in close correlation with their functional features. The increase in the number of apudocytes in animals active in winter by comparison with hibernating animals is evidently associated with the unusual nature of the state of normally hibernating animals at this time of year and the increased intensity of functioning of the digestive system.

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