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Ultrastructural and Morphometric Study of Enterochromaffin Cells from the Gastrointestinal Tract

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Serotonin (5-hydroxytryptamine, 5-HT) as a neurotransmitter and gut hormone is an important element of the not yet completely examined brain — gut axis which is part of the diffuse neural-endocrine system. Being one of the regulators of the gut motility, secretion and visceral sensitivity serotonin is the trigger of pathophysiological mechanisms of some of the symptoms of the gastro-intestinal disorders. The aim of this study is ultrastructural and morphometric characterization of the enterochromaffin (EC) cells of the gastrointestinal mucosa, one of the basic source of the serotonin in the human body. The EC cells are examined by means of electron microscope and morphometric methods in material of the biopsy specimens from stomach and duodenum of human. Character istics of the different types of serotonin receptors located in the structures – smooth muscle cells, neural fibers and neurons of the intestinal wall are also performed based on literature data.

Key words: enterochromaffin cells, serotonin, serotonin receptors, gastrointestinal tract.

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) can be found in great amounts in plants and animals. It was discovered in 1930 by V. E r s p a n e r et al. [11]. They extracted a substance that can contract the smooth muscle cells of the uterus and the intestines from enterochromaffin cells of the gastrointestinal mucosa. They called it enteramin. In 1948 J. Green and J. Page extracted vasocontractive substance from blood serum. After the molecular structure of serotonin was discovered enteramin turned out to be its analog. In 1951 they synthesized serotonin chemically [7]. After that the study of its functions began. The total amount of serotonin in the human body is about 10 μg. 95% of it is located in the gastrointestinal tract. 90% of the gastrointestinal serotonin is located in the enterochromaffine (EC) cells of the covering epithelium and the glands. 10% are located in the mast cells of the wall of the gastrointestinal tract. The aim of this study is to carry out the making of ultrastructural and morphometric characteristics of the enterochromaffin (EC) cells of the gastro-intestinal mucosa.

Materials and Methods

The materials about the morphologic research of the EC cells were obtained by fibrogastroscopy on 6 female patients, 45 — 72 years old from the MBAL "St. George" Clinic of Gastroenterology in Plovdiv. The biopsy specimens were taken from the body and antrum pylori of the stomach and superior portion of the duodenum.The biopsy specimens were removed and prepared for TEM according to the routine protocol. Examination and microphotographs were done with TEM "Philips CM 12". Morphometric study: A) Determining the saturation index of the secretory granules in the endocrine cells by the method of W. C r e u t z f e l d [1]. The granules are determined as 1 — empty; 2 — half-empty (with small quantity of material); 3 — half-full, and 4 — full. The saturation index of the granules of each endocrine cell is determined by multiplying the number of granules at each level of saturation by the corresponding coefficient (1, 2, 3, 4) and dividing the obtained value by the sum of the granules in the cell. B) Determining the cytoplasmic distribution coefficient of the secretory granules by the method by C h o m e r i k i and M o r o z o v [13].The microphotographs of the different endocrine cells done with the same magnification are divided into control areas of 0,5-0,25 cm^2 each. The number of the areas with high granule concentration (2 and more granules) — A2 and the number of the areas with low granule concentration (1 or no granules) — A1 are determined. The ratio between A2 and A1 represents the coefficient of granule distribution.

Results

Enterochromaffine (EC) cells can be found in all parts of the gastrointestinal tract. But the greatest amount of EC cells is in its proximal parts — stomach, duodenum, jejunum. In the stomach they are located in the body and the antrum. EC cells are located in the whole length of the stomach glands but mostly in their basal parts. They are cone shaped with a narrow apical part turned towards the lumen of the gland and a wide basal part that lays on the basal lamina and often forms tentacles. The secretory granules of the EC cells are polymorphic with rod-like or biconcave shape and a narrow light halo. They have high electron density (Fig. 1). There are 3 types of EC cells based on the ultrascructural characteristic of the granules and the types of the secretory products — EC1, EC2 and ECn. EC1 cells are located mainly in the stomach. Their granules are polymorphic, mostly prolonged or oval. Their size is 200-300 nm. They contain serotonin and substance P (Fig. 2). EC2 cells are located in the small intestine and the colon. There are mainly oval shaped granules in the their cytoplasm sized 200-400 nm, that contain serotonin and motilin (Fig. 3). The ECn type cells are mostly located in the duodenum. They have small and middle sized granules with moderate electron density that contain serotonin. Based on their ultrastructucal characteristic and the coefficients discovered by morphometric researches we found out that EC cells show different morpho-functional stages. There are: 1. Stage of increased synthesis; 2. Stage of relative secretory rest; 3. Stage of increased secretion. In the **stage of increased synthesis** the EC1 cells have a big loose young granules that are detatched from the trans-surface of the dictiosomes. The young granules of the EC2 cells are numerous and filled a substance with low electron density. They increase their sizes and form a gritted progranule with osmiffil covering membrane. During the next ripening the core of the granules becomes homogeneous with high electron density (Fig. 4). The saturation index of the granules is 3.23 ± 0.3. During the **stage of relative secretory rest** the cytoplasm of the EC cells is filled with ripe granules that are full of secretion (Fig. 5). The saturation index of the granules is 3.45 ± 0.3. In the **stage of increased secretion** the disintegration of the granules begins. Their diameter increases, the electron density of the core

decreases, the halo of the membrane disappears. Remnants of a dispersed substance can be found later in the core of the granule. In the end there is only a membrane skeleton left (Fig. 6). The saturation index of the granules is 2.95 ± 0.2.

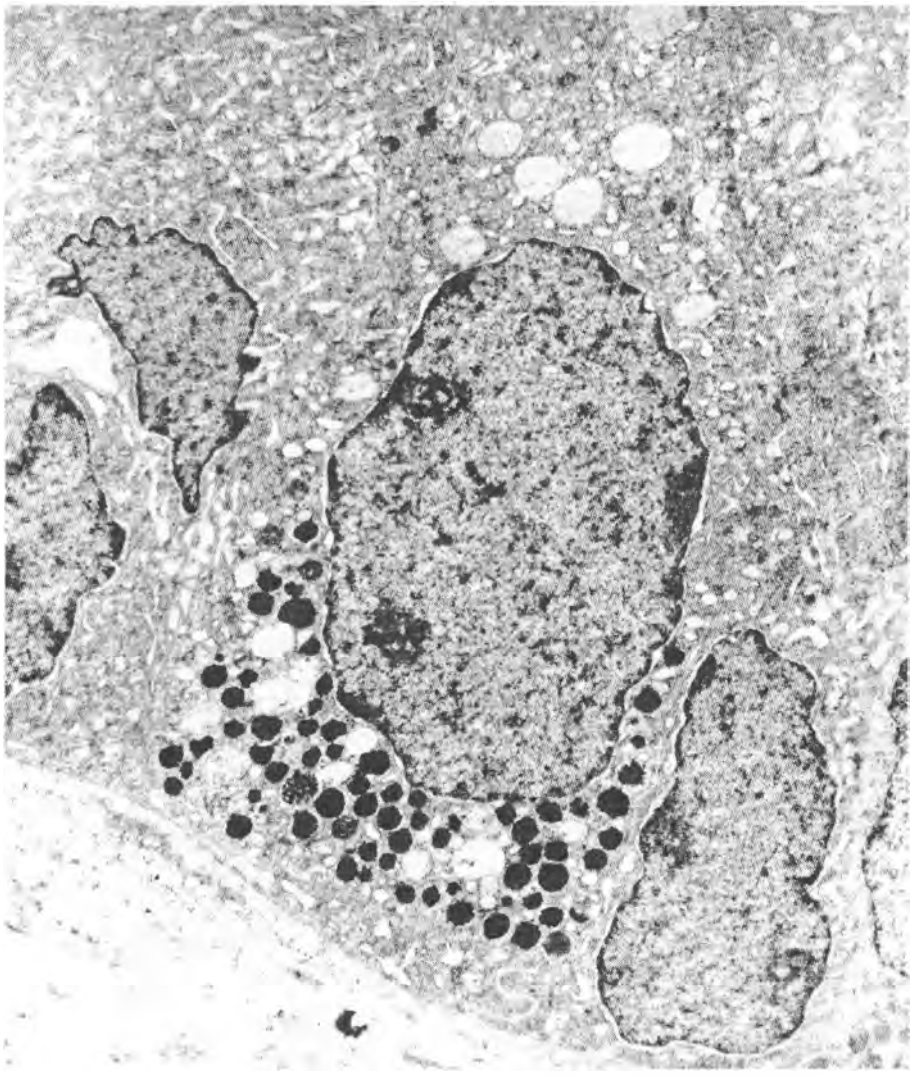

Fig. 1. Enterochromaffine cell EC2 type in duodenal gland. The basal part of the cell with polymorphic secretory granules with high electron density and narrow light halo.TEM (× 1500)

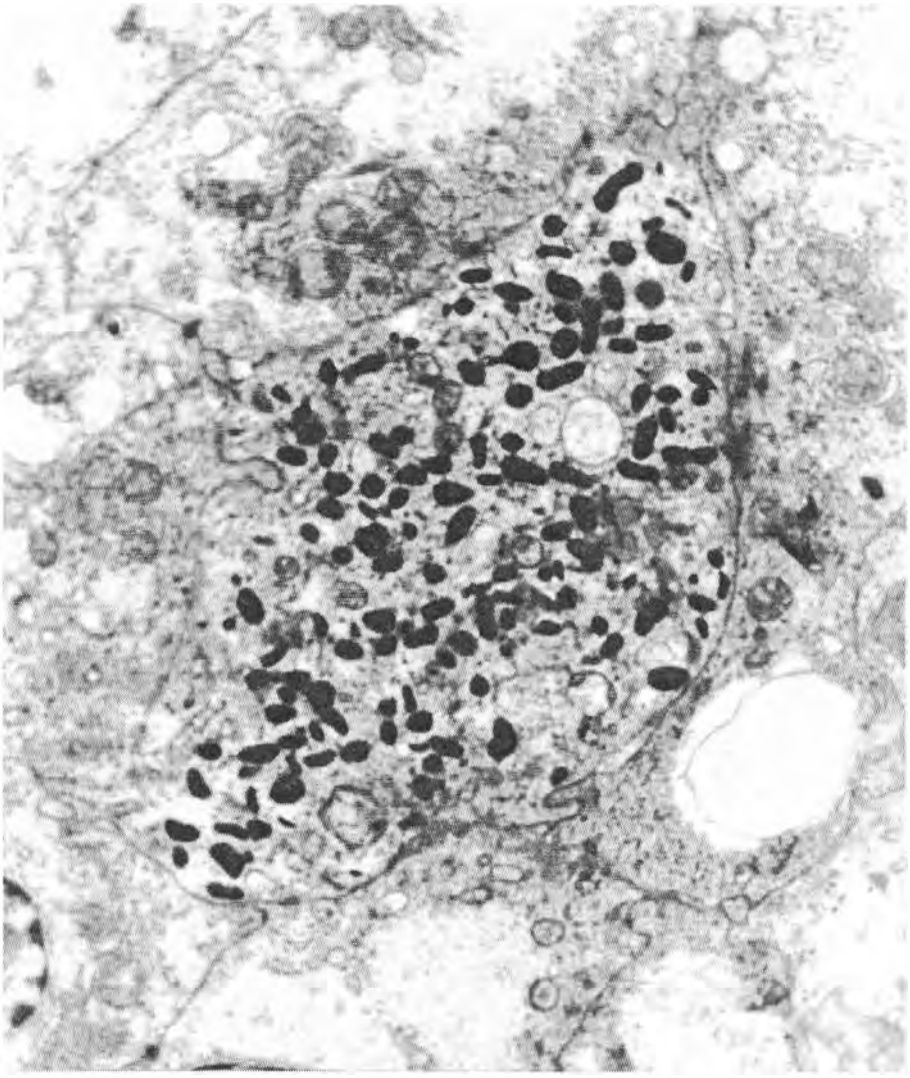

Fig. 2. Fragment from an EC1 cell in stomach gland. Polymorphic, mostly prolonged, rode-like or oval secretory granules, size 200 - 300 nm. TEM (× 2350)

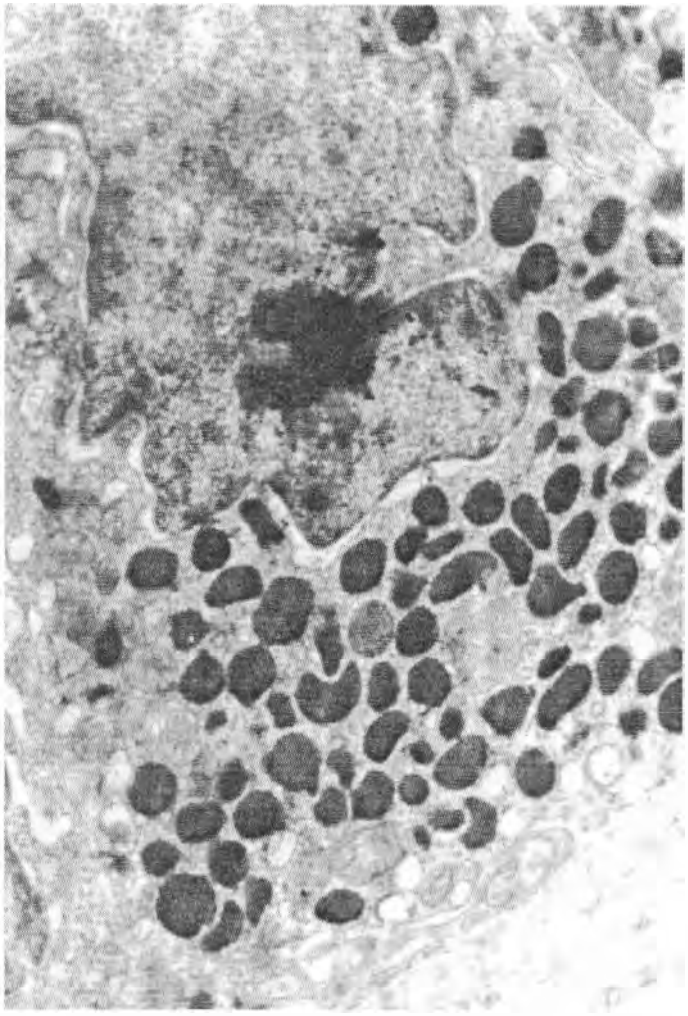

Fig. 3. Fragment from an EC2 cell in duodenal gland. Secretory granules with oval or biconcave shape, size 200 - 400 nm. TEM (× 2350)

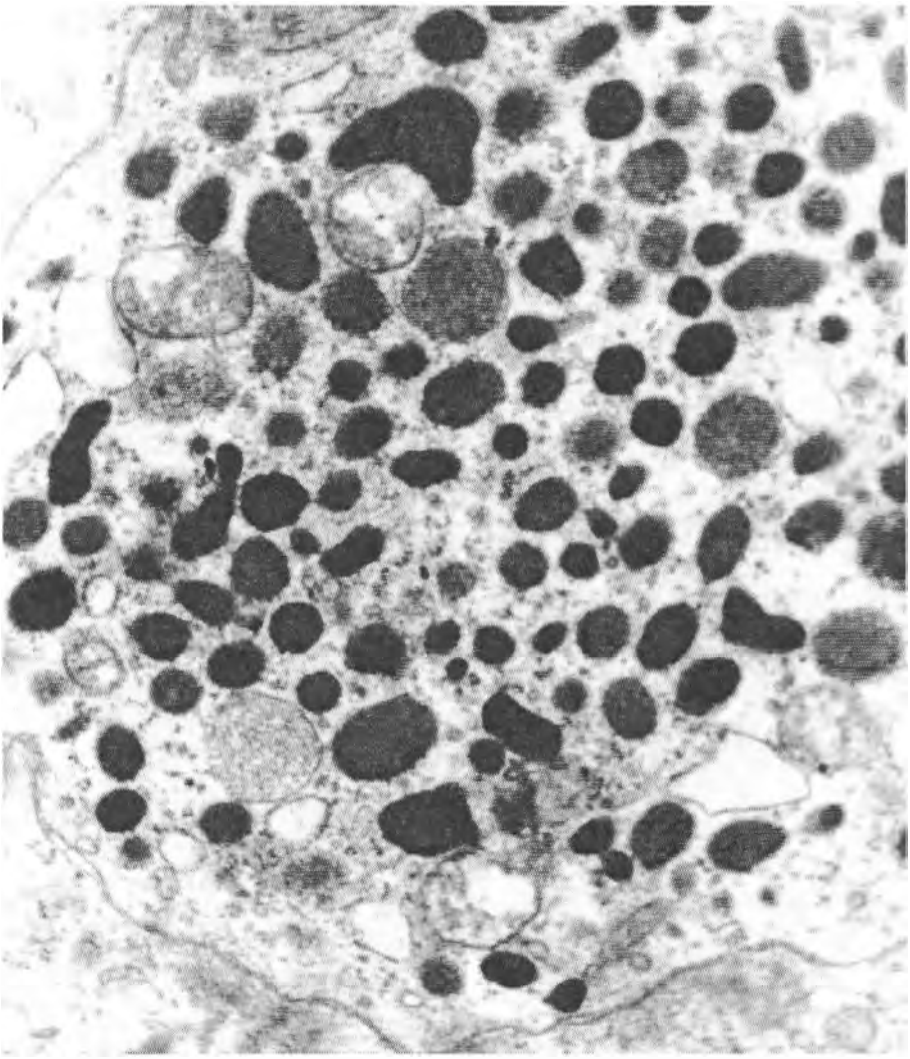

Fig. 4. Fragment from an EC2 cell in duodenal gland. Stage of increased synthesis. Young gritty progranules with low electron density. TEM (× 5000)

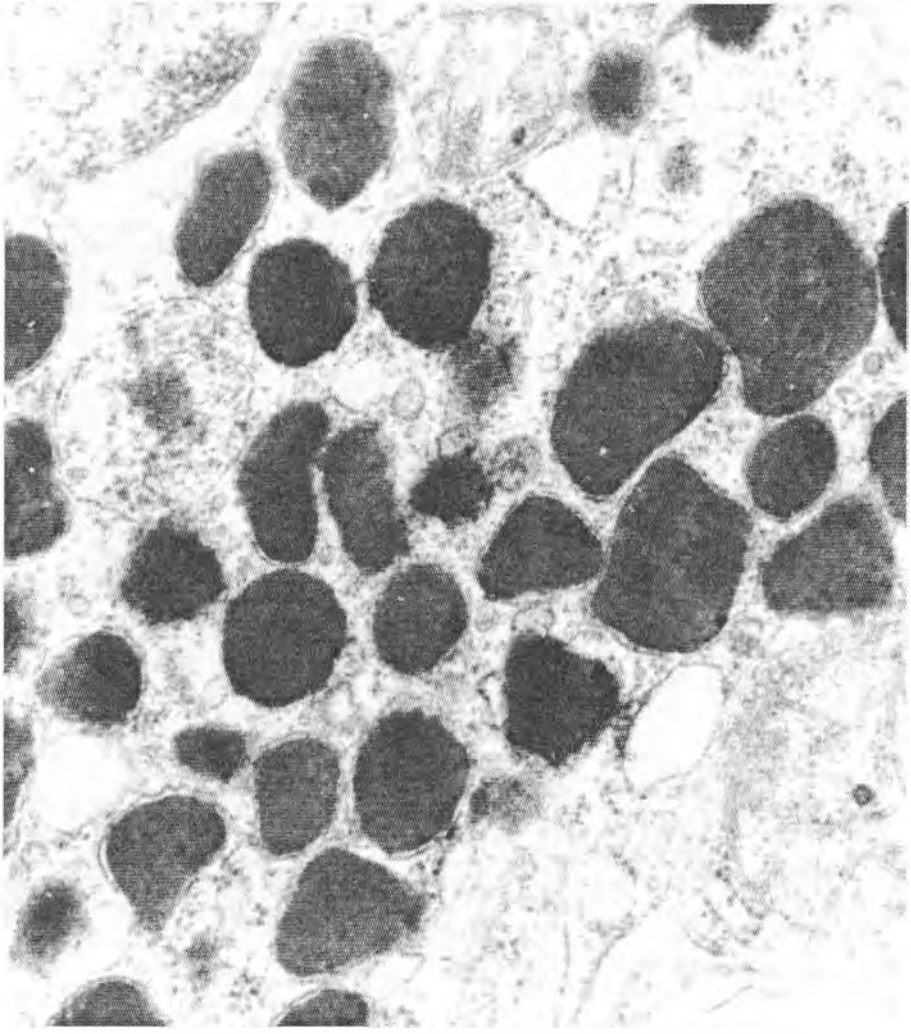

Fig. 5. Fragment from an EC2 cell in duodenal gland. Stage of relative secretory rest. Ripe secretory granules with high electron density, narrow light halo and covering membrane. TEM (× 10 500)

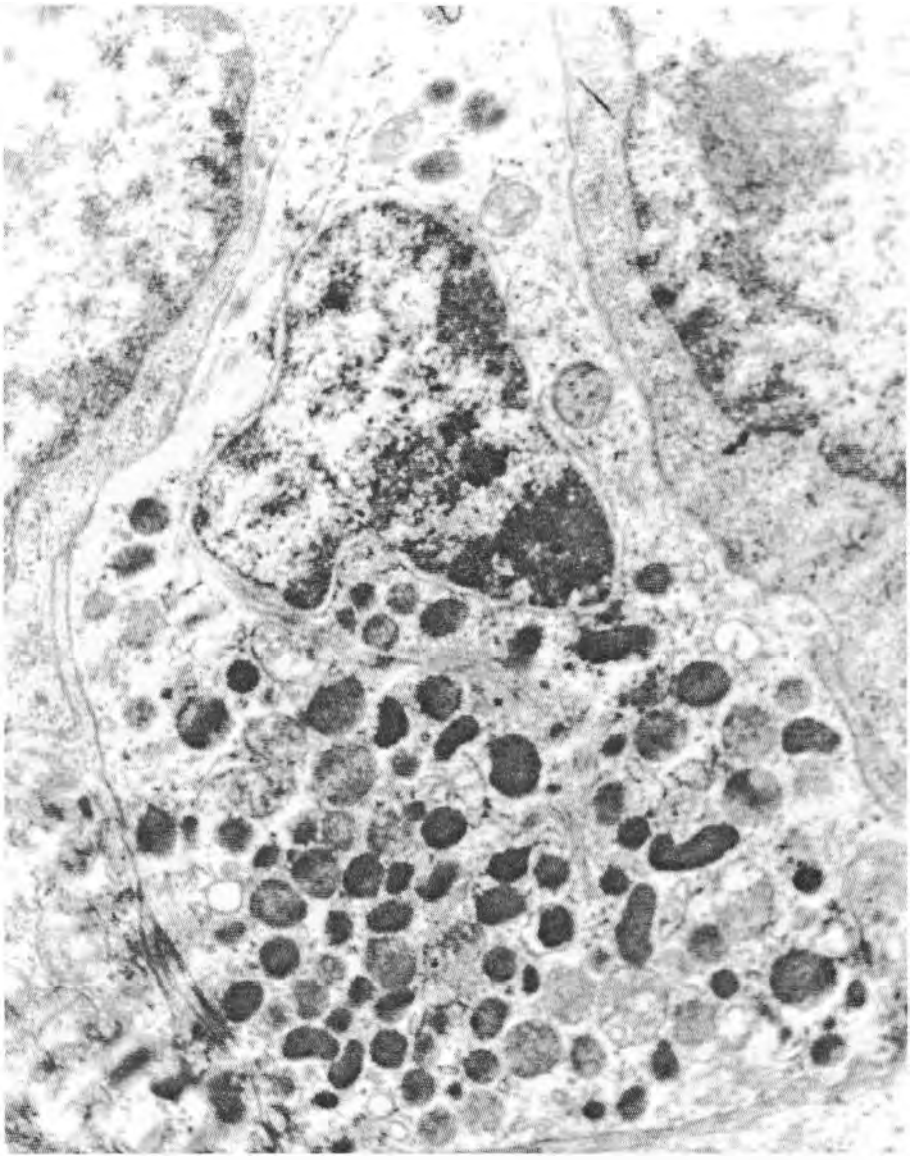

Fig. 6. Fragment from an EC2 cell in duodenal gland. Stage of increased secretion. Secretory granules with decreased electron density. Half-empty granules with remnants of a dispersed substance. TEM (× 3900)

The changes in the distribution index in the granules in the cytoplasm show an activation of the secretory processes. In the **stage of increased synthesis** the granules are uniformly dispersed in the whole cytoplasm. The number of the A1 fields with low density of the granules is lower than the number of A2 fields with high density of the granules. The distribution index of the granules is 0.70 ± 0.3. In the **stage of relative secretory rest** the granules occupy mainly the basal part of the cell. The number of the A1 fields with low density of the granules increases and the distribution index starts to decrease — 0.38 ± 0.3.

In the **stage of increased secretion** the granules are concentrated to the highest degree. There are mostly A1 fields with low electronic density. The distribution index of the granules decreases to 0.36±0.2.

Discussions

Our results of the granular contents in the 1-4 scale (empty — full) are identical with the results of other authors. According to T z a n e v a [9] the saturation index of the granules of the EC cells is 2.8-3.7±0.3. The secretion of serotonin from the EC cells is an answer to many different factors: A) lumenal — the increased amount of acid in the duodenal content or the presence of hypertonic glucose solution; B) blood and nervous factors — intestinal ishaemia, sensory and vagal stimuli; C) mechanic factors — the increase of the intralumenal pressure mechanical obstruction; D) infectious factors — viruses, bacterial toxins. The release of serotonin is thought to be calcium-dependant or voltage dependant calcium channels. Many receptors are present on the EC cells that stimulates or inhibits their activity. There are: adrenoreceptors (a2a, a2b, b1 and b2), muscarinic M3, GABA-A, nicotinic, acetylcholine, histamine-2 and serotonin (auto and paracrine) receptors. Unexpected receptors are olfactory (SCR, HGL, HFL, QIL, and EVA), vomeromasal and pheromone receptors (putative pheromone receptor) [10]. It appears that EC cells may analyse chyme, similar to other intraepithelial sensors in the airway or blood system. This data shows the important role of the EC cells and serotonin in the already discovered brain — gut axis. The function of serotonin is exerted upon its interaction with specific receptors. In 1957, Gaddum suggested that 5-HT interacted on two different receptors in isolated tissues, one on smooth muscle and one on nervous tissue. Since dibenzyline selectively

T a b l e 1. Localization and distribution of serotonin receptors in the body

Type	Subtype	Localisation	
5-HT1	1A	CNS	
	1B	only in rats	
	1C	Pl. choroideus	
	1D	CNS smooth muscle cells in blood vessels	
	1F	no data	
	1P	gastrointestinal tract — afferent somatic and vagal neurons in submucosal plexus	
5-HT2	2A	platelets	
	2B	smooth muscle cells in blood vessels	
	2C	only in rats	
5-HT3		gastrointestinal tract	smooth muscle cells
			afferent somatic and vagal neurons in myenteric plexus
5-HT4		CNS	
		heart	
		gastrointestinal tract	smooth muscle cells
			nociceptive nerve
5-HT5	5A	no data	
	5B	no data	
5-HT6		CNS, limbic system	
5-HT7		CNS, limbic system	
		gastrointestinal tract	smooth muscle cells

antagonized smooth muscle, and morphine was selective for nervous tissue, these receptors were named "D"; and "M" receptors respectively [8]. Nowadays several serotonin receptors have been cloned and are identified in seven groups. Group one (5-HT1) includes the 6 subtypes. Group two includes 3 subtypes. Group five includes 2 subtypes (Table 1).

Most of those receptors are coupled to G-proteins that affect the activities of either adenilate cyclase or phospholipase Cγ. The 5-HT3 class of receptors are ion channels. Some of serotonin receptors are presynaptic and others are postsynaptic. The cloning of new types of 5-HT receptors continues. Serotonin and its receptors play an important role in the regulation of different processes in the gastrointestinal tract — resorption of nutrients, gland secretion, motility and sensitivity. 5-HT3 and 5-HT4 receptors are discovered in the smooth muscle cells of the gastrointestinal tract [5]. 5-HT1 receptors are located in the smooth muscle cells of the circular muscle of the colon and 5-HT7 — in the longitudinal muscle [3]. 5-HT1p receptors are found in the afferent somatic nerve fibers of the submucosal plexus [4]. 5-HT3 receptors are located in the somatic and vagal neurons and nerve fibers of the myenteric plexus [12]. The detailed study of the functions of serotonin in the gastrointestinal tract that are carried out through different types of receptors as well as the creation of specific antagonists and agonists for these receptors reveals new trends in the therapy of the functional gastrointestinal disorders. Nearly 7 of 10 adults are affected by one or more functional gastrointestinal disorders, such as irritable bowel syndrome (IBS), gastroesophageal reflux disease and functional dyspepsia. Serotonin plays a key role in the pathogenesis of these disorders. Serotonin is released from the EC cells, psychosomatic mechanisms or from the mast cells during inflammatory processes. 5-HT3 receptors take part in the complicated vomit reflexes during functional gastrointestinal disorders as well as in therapy of cancer diseases. The blocking of these receptors by 5-HT3 antagonists interrupts the afferent impulses to the vestibular system, the cerebral cortex and the chemoreceptor trigger zone located in floor of the fourth ventricle that regulates the physiological emetic centre. 5-HT3 and 5-HT4 receptors take part in the gastrointestinal sensitivity. They are located in vagal and visceral nociceptive neurons that activate different pain systems. The fibers of those neurons transmit signal from the viscera to the specific lamine of the dorsal horn, nuclei of the thalamus and cerebral cortex [2]. Using distal colonic stimulation, several studies have demonstrates alterations in regional brain activation in patients with IBS compared with healthy control subjects.There is such a difference in the activity of the visceral nociceptive stimuli in the anterior midcingulate cortex. insula and dorsal pons (in the region of the periaqueductal grey) [6].

Conclusion

Being one of the regulators of the gastrointestinal motility, secretion and sensitivity serotonin plays a key role in many symptoms of gastrointestinal diseases. EC cells that secrete serotonin are filled in different degrees with secretory granules. The granules are polymorph, rod-, biconcave or oval shaped with a light halo and high electronic density. By the means of a morphometric study and ultrastructural characteristic we defined coefficients that show 3 different morphofunctional states of the EC cells: stage of increased synthesis, stage of relative secretory rest and stage of increased secretion.

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