

Serotonin and its Functions as Gastrointestinal Hormone

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Abstract

Background: Serotonin plays the role of critical signal molecule in great number of physiologic processes and pathologic symptoms of gastrointestinal tract. As a gastrointestinal hormone serotonin takes place in the regulation of smooth muscle motor activity and glandular secretion. It affects the gastrointestinal sensation of pain, nausea and vomiting. The aim of our study is to determine the presence and localization of serotonin-producing EC cells and distribution of serotonin receptor 5-HTR3 in the human stomach.

Material and methods: Biopsy specimens from 25 patients aged 40-70 years: body and antral part of the stomach are studied by immunohistochemical reactions for serotonin, serotonin receptor 5-HTR3; and transmission electron microscopy.

Results: The serotonin-producing cells are found between columnar cells of gastric epithelium, in the fundic and pyloric glands of the gastric mucosa. Their ultrastructural characteristics show the presence of EC1 and EC2 enterochromaffin cells. The expression of serotonin receptor 5-HTR3 in covering epithelium, glandular and interstitial cells shows the possibility serotonin to affect gastric mucosa secretion. The intensive expression of 5-HTR3 receptors in gastric wall smooth muscle cells demonstrates the key role of serotonin in gastrointestinal motility.

Conclusion: In order to obtain a better understanding of the functional role of serotonin, we investigate the localization of serotonin-producing EC cells, their ultrastructural characteristics and the presence and distribution of serotonin receptor 5HTR3 in the stomach. Enzymes needed for serotonin secretion and degradation, its transporters, as it great numbers of receptors are the base new drugs to be synthesized aiding clinical practice in gastroenterology.

Keywords: Gastric mucosa secretion; Gastrointestinal motility; Enterochromaffin cells; Serotonin; Serotonin receptors

Introduction

The holistic vision on examining the human body in contemporary medicine is supported by regulatory factors with multidirectional action in different organs and systems. Serotonin is such a physiologically active substance. Serotonin, described as the hormone of happiness, is synthesized by the amino acid tryptophan via hydroxylation and decarboxylation. The diet is of major significance for the body serotonin level. Banana, kiwi, pineapple, plums, tomatoes, hazelnuts contain serotonin, while rich in tryptophan are almonds, banana, bean plants, cheese, chicken and duck meat, eggs, fish, milk, peanuts, soya products [1,2].

Serotonin synthesis is assisted by food rich in calcium, magnesium and vitamin B, omega-3, omega-6 and gamma linoleic acids [3]. The total amount of serotonin in the human body is about 10 µg. Only 5% of serotonin in human body is found in platelets and in the central nervous system (CNS). Serotonin plays the role of neurotransmitter in reticular formation nuclei, hypothalamus and limbic system [4,5]. The level of serotonin in CNS affects higher nerve functions as mood, sleep, memory, cognitive processes, sexual and behavioral reactions [6,7]. The sunlight triggers serotonin synthesis by epiphyseal pinealocytes

[8]. Almost 95% of it is found in the gastrointestinal tract (GIT), mainly in epithelial and glandular enterochromaffin (EC) cells.

Minimal serotonin is found in myenteric and submucosal plexus nerve fibers [9]. Gastrointestinal wall mast cells contain 10% of serotonin within the tube. As a gastrointestinal hormone, serotonin takes place in the regulation of smooth muscle motor activity [10,11] and glandular secretion [12]. It affects the gastrointestinal sensation of pain, nausea and vomiting [13]. Being part of platelets and mast cells specific granules, serotonin is a potent vasoconstrictor, an active factor in the processes of hemostasis, inflammation and allergic reactions [14,15]. Serotonin plays its role via specific receptors. They are identified and cloned in seven types and subtypes [16]. A great number of serotonin receptors is related to the long evolution history of serotonin intercellular signaling based on gene repetitions followed by mutations, coding different receptor subtypes [17].

Aim

The aim of our study is to determine the presence and localization of serotonin-producing EC cells and distribution of serotonin receptor 5-HTR3 in the human stomach, being the morphologic substrate of physiologically active serotonin in the gastro intestinal tract.

Material and Methods

The study is carried out on biopsy material from stomach-body and antrum of 25 patients aged 40-70 years from Clinic of Gastroenterology, UMHAT "St. George", Plovdiv after written informed consent from each of the patient. Our study was conducted over a period of six months in 2016. Biopsy specimens were collected from the 25 healthy subjects. In our study were included only cases with a typical characteristic of the gastric wall without endoscopic and histological data for a pathological process. Immunohistochemical (IHC) methods (IHC reactions for serotonin and serotonin receptor 5-HT₃) and transmission electron microscopic study (TEM) are used.

IHC reactions are based on ABC method via rabbit ABC Staining System (Santa Cruz Biotechnology, USA). Biopsy material for the IHC study are fixated in Buen solution for 24 hours and it included paraffin. Paraffin cuts with thickness of 5 µm are deparaffinated and incubated for 30 min. in 2% H₂O₂ methanol for inactivation of the endogenous peroxidase. The primary antibody for serotonin (rabbit polyclonal antibody, MAB352 serotonin- Chemicon USA is diluted in PBS in ratio 1:200. The primary antibody for serotonin receptor (goat polyclonal antibody, SR-3A: sc-19150, Santa Cruz Biotechnology USA is diluted in PBS in ratio 1:100.

The incubation of the cuts with the corresponding antibody is performed at 4°C for 12 hours in damp camera. What follows is an incubation with the biotinylated secondary antibody for 30 min. in ABC complex for 15 min. and visualization with DAB chromogen. Deparaffinated and included in Vecta mount cuts are observed under microscope and photographed. Semi-qualitative method is used for received results assessment. The specificity of IHC reactions is confirmed via negative controls with a buffer (PBS) or normal non-immune serum. Observation and photo documentation of the microscopic preparations are performed with digital photo microscopic camera of a light microscope "Olympus BX51".

The biopsy material for TEM is fixed in 4% glutaric aldehyde, NaPO₄ buffer 0.1 M, with followed post-fixation in 4% OsO₄ and 0.2 M S'collin buffer. It is dehydrated in ascending alcohol series and was included in durcopan. It is warmed in gelatin capsules at 56°C for 48 hour. Gelatin capsules are removed with warm water and the material is cut into thick slices 0.5 mm. Cuts are mounted on glass slides, stained with methylene blue, covered and observed with a light microscope to determine the area of cutting.

What follows is the cutting of ultrathin slices with thickness of 300 nm and their mounting on platinum nets. The mounted cuts are contrasted with 5% uranyl acetate, diluted 1:1 with 100% alcohol for 80 min. in dark. They are washed with 50% alcohol and Reinold's solution (water solution of Pb(NO₃)₂, Na(CoH₅O₇)₂H₂O for 20 min. and bid H₂O. Observation and microphotography we performed on TEM Philips CM 12.

Results

IHC study for serotonin

The biopsy material from the body and antrum of the gastric wall showed positive reaction for serotonin. Serotonin-positive cells are found to be located either in groups or as single cells in the fundic and in the pyloric glands. Some of the serotonin-positive cells are distributed between columnar cells of gastric epithelium. Serotonin-

positive cells are found in the base of the glands, rarely in the neck region (Figure 1).

All the serotonin-positive cells have a dark brown colour in the cell body and cytoplasmic processes. The nuclei with a spherical shape are located in the middle part of the cells. The intracellular localization of the serotonin production is observed in transverse sections of the glands. In some of the endocrine cells it is concentrated in the apical part while in others cells in the basal part. The most of the serotonin-positive cells appear as closed type, without a lumen contact, but some of the cells seem to reach the lumen opened type (Figures 2 and 3).

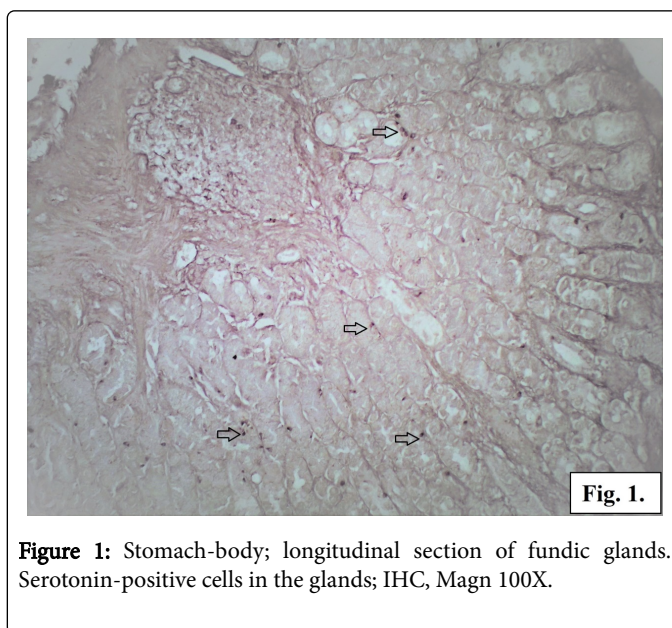


Figure 1: Stomach-body; longitudinal section of fundic glands. Serotonin-positive cells in the glands; IHC, Magn 100X.

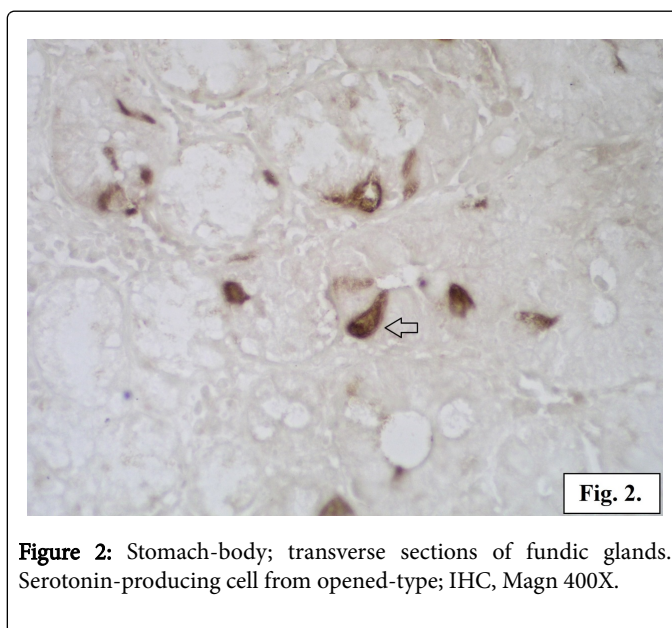


Figure 2: Stomach-body; transverse sections of fundic glands. Serotonin-producing cell from opened-type; IHC, Magn 400X.

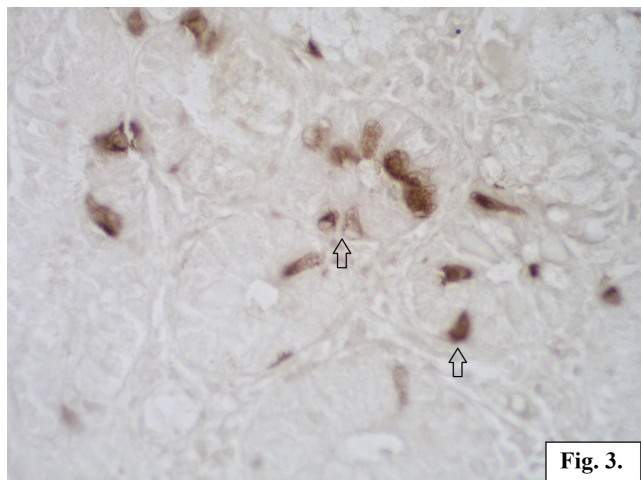


Fig. 3.

Figure 3: Stomach-body; transverse sections of fundic glands. Serotonin-producing cell from closed-type; IHC, Magn 400X.

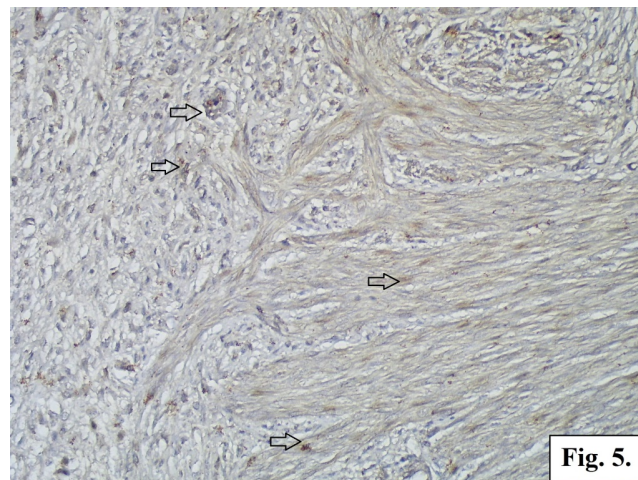


Fig. 5.

Figure 5: Stomach-antrum; positive expression of 5-HTR3 in smooth muscle cells from the muscularis externa. IHC, Magn 200X.

IHC study for serotonin receptor 5-HTR3

The IHC reaction for serotonin receptor 5-HTR3 is positive in the gastric mucosa and in the smooth muscle fibers from the muscularis externa of gastric wall. In the gastric mucosa 5-HTR3 is expressed in small number of columnar epithelial cells, fundic and pyloric glandular cells, interstitial cells from the loose connective tissue of lamina propria and submucosa (Figure 4). The reaction for serotonin receptor 5-HTR3 is positive in a great number of smooth muscle cells in stomach wall of different layers longitudinal, circular and oblique. The cytoplasm of the leiomyocytes is full of fine brown granulation visualizing the serotonin receptor 5-HTR3 (Figures 5 and 6).

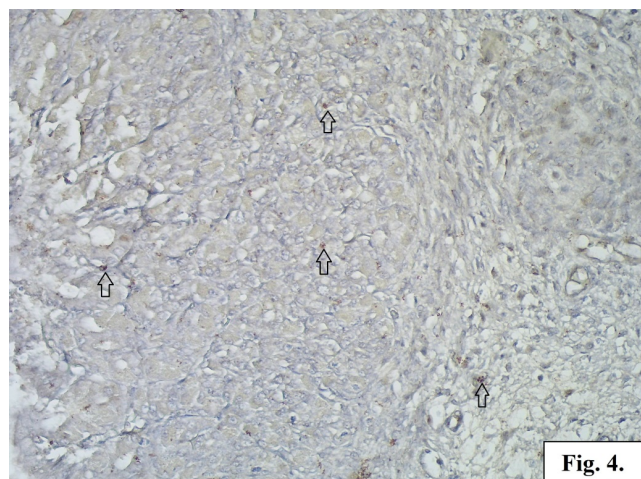


Fig. 4.

Figure 4: Stomach-body; expression of 5-HTR3 in single cells distributed in covering epithelium, fundic glands and submucosa. IHC, Magn 100X.

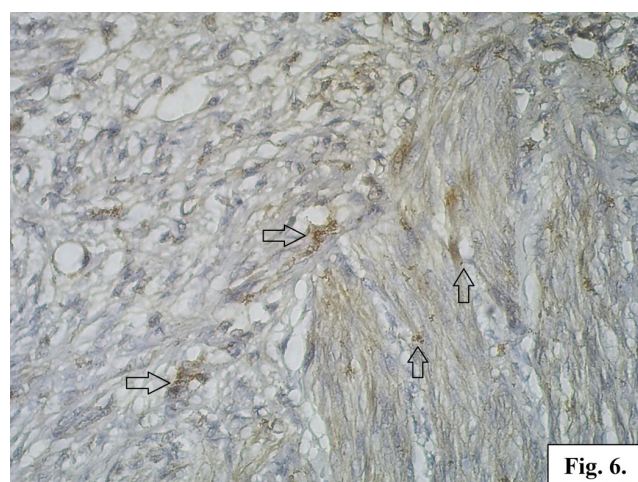


Fig. 6.

Figure 6: Stomach-antrum; expression of 5-HTR3 in smooth muscle cells in longitudinal and circular layers. IHC, Magn 400X.

Electron microscopic study

It was established that there are two types of serotonin-producing enterochromaffin (EC) cells EC1 and EC2 based on TEM of corporal and antral gastric biopsy specimens. The ultrastructural characteristics of the two types of serotonin-producing EC cells granules showed presence of membrane and narrow halo. The EC1 cells granules are with lower dimensions, polymorphic, some being prolonged or rod-shaped and containing secretor product with different electronic density-low, medium or high. It is homogenic with low electron density in the greater number of the secretory granules, while other granules contain material either with fine grit, or with high electron density (Figure 7). The granules of EC2 type cells are with bigger dimensions, mainly oval in shape and with high electron density (Figures 8 and 9).

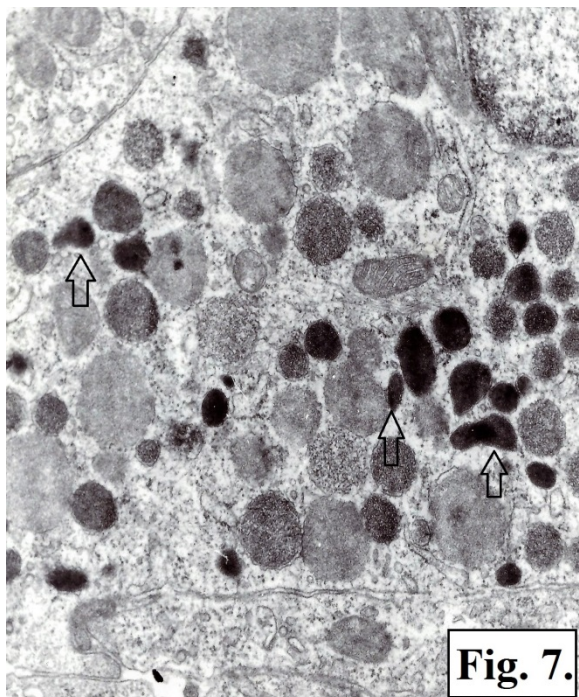


Fig. 7.

Figure 7: Stomach-antrum; EC1 cell. Polymorphic prolonged granules. Secretory product with different electron density, TEM.

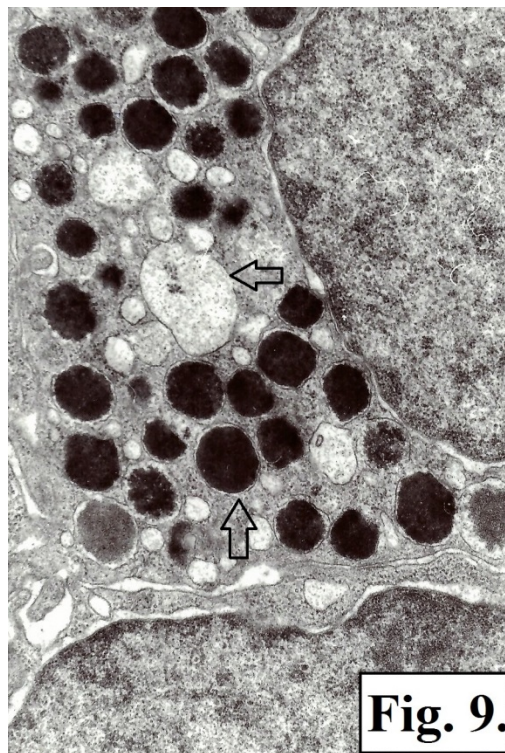


Fig. 9.

Figure 9: Stomach-body, EC2 cell. Oval granules, secretory product with high electron density and narrow halo; semi-empty granules.

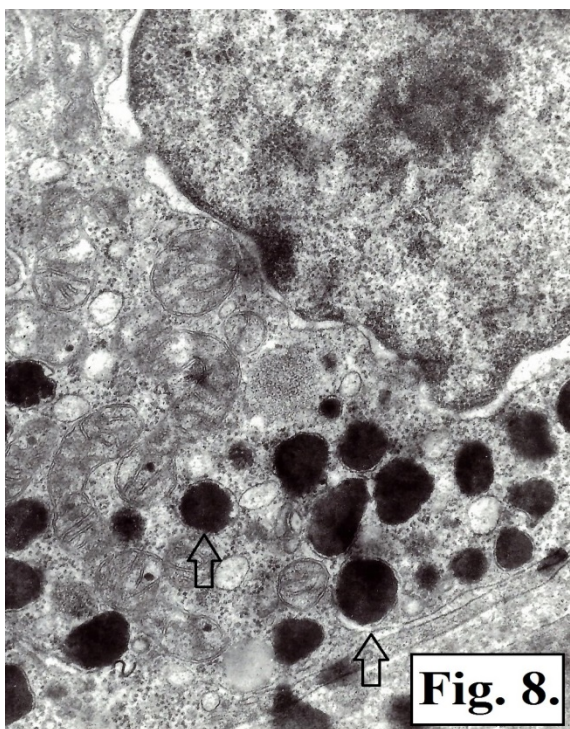


Fig. 8.

Figure 8: Stomach-body; EC2 cell. Large oval granules, secretory product with high electron density. TEM Micr Magn 5000X.

Discussion

In our IHC study of human stomach we registered serotonin-producing cells, scattered in the covering epithelium, fungic and antral glands. The granules electronic microscopic characteristics show two types of serotonin-secreting enterohromaffin cells EC1 and EC2. The enterochromaffin EC cells secreting serotonin are found in all GIT sections, being the most rich population [18]. In the stomach they are located mainly in the antral and not so often in the corporal mucosa [19,20]. On the base of their granular ultrastructural morphology enterohromaffin EC cells are divided into EC1, EC2 and ECn type [21]. EC1-type cells are mainly found in the small and large intestine. EC2-type are greatest in number in the duodenum. ECn are mostly in the stomach. Enteroendocrine cells of EC type are differentiated not only in terms of their granular morphology but according to the secreted hormones, too. Immunohistochemical studies show that all EC cells produce serotonin [22]. EC1 cells secrete serotonin and substance P [23], while EC2 serotonin and motilin [24,25]. Up to now only serotonin is known as secretory product of ECn cells.

The serotonin-producing cells we registered in the gastric mucosa are of two structural types: opened and closed. The opened-type cells show serotonin expression concentrated in the apical part. These cells possess an apical surface with microvilli reaching the lumen and have the ability to analyze chemically the food. The serotonin-producing cells of the so called closed-type do not reach the lumen. Their serotonin secretion is concentrated in the basal part of the cell. These cells are triggered by mechanical distension and stimuli coming from

blood. Both types of serotonin secreting cells respond to different irritants by hormones synthesis, storage and release [26].

As biogenic amine serotonin is synthesized in EC cells cytosol. The serotonin molecules are packed as secretory product in EC granules via vesicular monoamine transporter VMAT1. Proton gradient, generated by ATPase is needed for this process [27]. Secreted by EC cells serotonin is an electrically loaded molecule. As its action is intracellular, it has to be transported through the target cell membrane. Highly effective transport molecule is needed. Such transporter, 5-HTT or SERT in CNS is produced by serotonergic neurons, not available in GIT mucosa. As far as GIT is concerned, this molecule is secreted by enterocytes [28]. SERT is localized on the intestinal epithelial cells apical and basal membrane [29]. Some scientists determine serotonin-producing EC cells as sensors reacting to mechanical tension and substances like glucose short- and long chained fatty acids, peptides [30].

The strategic localization of EC cells within the gastrointestinal covering epithelium gives them a chance to serve as visceral stimuli sensory transducers [31]. One of the first steps in this process is the release of serotonin from EC cells [32]. Serotonin, secreted by EC cells and serotonergic neurons within myenteric and submucosal plexus is the main molecule transmitter synchronizing gastrointestinal reflexes and transmitting information to CNS [33]. Under physiological conditions the serotonin release leads to reflex coordination responsible for the transition of food with velocity suitable for digestion and absorption [34].

Serotonin plays role in motor, secretory and sensory functions of GIT acting via specific receptors. On the base of their pharmaceutical profile and DNA sequence the serotonin receptors are identified and cloned in seven groups [35]. According to NC-IUPHAR they are: 5-HT1, 5-HT2, 5-HT3, 5-HT4, 5-HT5, 5-HT6 and 5-HT7. All the seven main types serotonin receptors are identified in different cell populations in GIT. Glandular and resorptive cells, smooth muscle cells, enteroendocrine cells and enteric neurons express receptors for serotonin [36-38]. Not like all other receptors for serotonin coupled to G-protein activating adenylate cyclase or phospholipase, 5-HTR3 receptors are ion channels.

In our IHC study we establish the presence and distribution of serotonin receptor 5-HTR3. Its expression is positive in the covering epithelium and glands and in some mucosal interstitial cells, but is mainly localized in gastric smooth muscle cells. In regard to motor function serotonin has precise effects on each GIT segment. Some authors report on the specific role of the serotonin-producing cells in the base of the foveolae of the gastric cardia mucosa – this narrow band of tissue between the oesophagus and the stomach. The authors suggest that serotonin secreted in this region is involved in the regulation of the lower esophageal sphincter [39].

Various types and subtypes of serotonin receptors in the smooth muscle cells of the circular and longitudinal layers of the wall of the small and large intestine are found [40,41]. The fine coordination of their contractions and relaxation determines the normal peristalsis of the GIT. 5-HTR3 receptor mediates ascending and descending peristalsis reflex components thus determining the transit velocity in GIT [42,43]. Unfortunately the great expectations for serotonin receptor agonists and antagonists in the treatment of diarrhea and constipation are not confirmed. The reason probably is the great number of types and sub-types serotonin receptors and lack of ligands for studies *in vivo* [33].

The secretory effect of serotonin is mediated by epithelial 5-HT2 and neuronal 5-HT1P, 5-HT3 и 5-HT4 receptors [12,44]. Serotonin increases the serosal secretion of salivary glands, activates the gastric principal cells directly or indirectly via gastrin but inhibits the secretion of HCl by wall cells [45]. In the small intestine it stimulates the secretion of mucus and fluid as mucosa protector reaction or in pathologic reactions like carcinoid syndrome. Serotonin activates the secretion of bile contracting the smooth muscle cells within the extra hepatic bile pathways and gall bladder. The secretion of pancreatic juice is not affected significantly [46].

In regard to the sensory role of serotonin within the GIT, it is studied in terms of inflammatory and functional diseases as duodenal ulcer, irritated bowel syndrome (IBS), gastrointestinal reflux disease, conditions after radiotherapy [47]. Symptoms like abdominal pain, nausea and vomiting in IBS are related to the increased secretion of serotonin by mast cells and EC cells within GIT [48]. It is established that in mucosal biopsy there is correlation between the expression of IBS, EC cell number in the large intestine mucosa and the level of serotonin [49,50].

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 laminae of the dorsal horn, nuclei of the thalamus and cerebral cortex [51].

Serotonin plays the role of critical signal molecule in great number of physiologic processes and pathologic symptoms of GIT [11,52,53]. Enzymes needed for serotonin secretion and degradation: tryptophan-5-hydroxylase (TpH1) and monoaminoxidase (MAO); its transporters: VMAT1 and SERT; as its great number of receptors is going to be the morphologic substrate determining the physiologic functions of serotonin in GIT. They are the base new drugs to be synthesized aiding clinical practice in gastroenterology.

Conclusion

In order to obtain a better understanding of the functional role of serotonin, we investigate the serotonin-producing EC cells in human stomach. In our study the serotonin-producing cells localized in the covering epithelium, fundic and pyloric glands are found in the gastric mucosa of adult individuals. Their ultrastructural characteristics show the presence of EC1 and EC2 types. The expression of 5-HTR3 serotonin receptor in epithelial, glandular and interstitial cells shows the possibility serotonin to affect gastric mucosa secretion. The intensive expression of 5-HTR3 receptors in gastric wall smooth muscle cells demonstrates the key role of serotonin in GIT motility.

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