SEROTONIN-PRODUCING CELLS IN HUMAN GASTRIC MUCOSA - IMMUNOHISTOCHEMICAL AND ELECTRON MICROSCOPIC STUDY

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ABSTRACT
The great many hormones released by the endocrine cells of the glands and lining epithelium of gastric mucosa determine its significance for the processes in the gastrointestinal tract. One of these hormones, serotonin, plays an important role in the regulation of the motility, secretion and sensation in the gastrointestinal tract.

The AIM of the present study was to conduct immunohistochemical and electron microscopic studies of serotonin-producing EC cell of gastric mucosa.

MATERIAL AND METHODS: Gastric mucosa biopsies were obtained and studied immunohistochemically for serotonin expression in the mucosa endocrine cells. Electron microscopic study was performed to specify the processes of synthesis, accumulation and release of secretory product by those cells.

RESULTS: The immunohistochemical study revealed a considerable number of serotonin-containing EC cells scattered in the lining epithelium and between the glands in the corpus and pyloric region of the stomach. The electron microscopic study followed the stages of formation of the secretory granules from the initial accumulation of granular substance, its membrane packing and formation of mature granules to their disintegration in the secretory process.

CONCLUSIONS: Serotonin as a neurotransmitter and gastrointestinal hormone appears to be a key to understanding a number of symptoms of gastrointestinal disorders like nausea, vomiting, pain, diarrhea and constipation. A detailed study of serotonin functions in the gastrointestinal tract realised through different types of receptors, and of the development of specific antagonists and agonists to these receptors would open up new opportunities for a more efficient treatment of gastrointestinal disorders.

Key words: serotonin, EC cells, gastric mucosa, gastrointestinal tract

INTRODUCTION
Serotonin (5-hydroxytriptamin, 5-HT) is found in a broad range of plants and animals. It was found by V. Erspamer et al. in 1930. They extracted a substance from enterochromaffin cells in the stomach and intestinal mucosa that was able to contract gut and uterus smooth muscles and called it enteramine. In 1948 J. Green and J. Page isolated a vasoconstrictor substance in the blood serum, which they called serotonin. After its chemical formula was confirmed it proved to be analogous to enteramine.

Serotonin started to be extensively researched after it was synthesized in 1951.¹ Serotonin is an indole derivative belonging to the group of biogenic amines. It is synthesized from the essential amino acid tryptophan. The reaction is an enzyme-mediated pathway consisting of two main steps. At the first step 5-hydroxytryptophan is formed mediated by tryptophan-5-hydroxylase. At the second step tryptophan is decarboxylated by 5-hydroxytryptophan decarboxylase into 5-hydroxytryptamine or serotonin. The total amount

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of serotonin in human organism is about 10 mg. Five percents are found in the platelets and CNS. Ninety-five percents of the serotonin is in the gastrointestinal tract: 90% of this amount is in the enterochromaffin EC cells in the lining epithelium and glands and 10% in the mast cells in the wall of the digestive tube. Serotonin-producing EC cell are found throughout the entire gastrointestinal tract but mostly in its proximal regions – stomach, duodenum, jejunum.

**AIM**

The aim of the present study was to make an immunohistochemical and electron microscopic characteristics of serotonin-producing EC cell from gastric mucosa.

**MATERIAL AND METHODS**

Morphological study of EC cell was made on biopsy samples from the corpus and antrum of the stomach obtained during fibrogastroscopic examination of six female patients aged 45–72 years treated in the Clinic of Gastroenterology of St. George University Hospital in Plovdiv. Endoscopic examination revealed no pathological changes of the gastric mucosa.

Later on (2006 - 2008) the 2002 electron microscopic examination was supplemented by immunohistochemical verification of the EC cells. The material for immunohistochemical examination was fixed in Bouin’s solution for 24 hours and embedded in paraffin wax. The paraffin sections were treated by avidin–biotin-peroxidase complex (ABC) method. Primary antibody for detection of MAB352 serotonin (rabbit polyclonal antibody – Chemicon USA) was applied in 1:200 dilution at 4°C for 12 hours. Serotonin was detected by the presence of brown stained granules. The specificity of immunostaining for serotonin was confirmed by negative controls in which the specific antiserotonin antibody was replaced by buffer (PBS) or normal non-immune serum. For the electron microscopic examination biopsy pieces were fixed with 2.5% glutaraldehyde and 0.1M cacodilate buffer (pH 7.4) with subsequent postfixation in 1% osmium tetraoxide and embedding in durecopan. The examination and microphotographs were made with TEM “PhilipsCM 12”.

**RESULTS**

The immunohistochemical examination of the biopsy samples from gastric mucosa revealed considerable number of serotonin-containing EC cells. Some of these were regularly scattered among the cells of the lining epithelium. Others were located in the neck and body of the corpus and pyloric glands (Fig. 1). The EC cells are cone like. With their narrow apical part they get as far as the glands’ lumen. Serotonin expression was evident in the distended basal part of the cells (Fig. 2).

The basic morphological characteristic of serotonin-producing EC cells is based on the electron microscopic description of the serotonin granules they contain. These are polymorphic stick-shaped or biconcave in form with narrow light halo and high electron density. According to the ultrastructural characteristic of the granules and character of
the secretory products three types of EC cells are distinguished – EC1, EC2 and ECn type. EC1 cells are found mainly in the stomach. Their granules are polymorphic, most often elongated or oval in shape and 200-300 nm in size. They contain serotonin and substance P (Fig. 3). EC2 cells are located mainly in the small and large intestine. Their cytoplasm is filled with oval or irregularly shaped granules, 200-400 nm in size, containing serotonin and motilin (Fig. 4). ECn are most numerous in the duodenum. Their granules are small to moderate in size, with moderate electron density.

Figure 3. Biopsy sample from antral mucosa. Fragment from EC1 endocrine cell. Elongated, stick-shaped secretory granules with high electron density. TEM ×25000.

Figure 4. Biopsy sample from antral mucosa. Fragment from basal part of EC2 endocrine cell with prevailing oval and irregularly-shaped granules with high electron density. TEM ×25000.

Figure 5. Fragment of EC cell from antral mucosa. Accumulation of granular substance with dense core. A large number of mitochondria with well developed cristae and matrix of high electron density are seen around it. TEM ×52500.

Figure 6. Fragment of EC cell from antral mucosa. Developing immature granule. A granular substance with irregular electron density seen in one pole of the granule and fine granular material delineated by membrane and narrow halo in the other. Canaliculi of RER and mitochondriae nearby. TEM ×52500.
and contain serotonin.

The electron microscopic study follows the stages of formation of the secretory granules. In a fragment of EC cell from antral mucosa we revealed initially a spheroid accumulation of granular substance with dense core. A number of mitochondria with well-expressed cristae and matrix with high electron density, tubes and vesicles of rough endoplasmic reticulum are found around (Fig. 5).

The process continues with formation of an immature granule. A granular substance with irregular electron density is found in one pole of the granule and fine granular material delineated by membrane and narrow halo in the other. Canaliculi of rough endoplasmic reticulum and mitochondria are found adjacent to the forming granule (Fig. 6).

Mature granules present with typical morphology. Their form is spheroid or ellipsoid, the secretory product with high electron density, finely granular and filling up evenly the granule. The granule has a delineating membrane and narrow halo (Fig. 7).

In the final stages disintegration of the granules sets off. Their diameter increases, the core electron density decreases, the halo beneath the membrane recedes. Part of the membrane breaks down and allows the granule secretion to pour into the cytosole (Fig. 8).

The completion of the secretory process presents with remnants of secretory granules: an empty granule with a segment of delineating membrane - membrane skeleton (Fig. 9).

DISCUSSION

The detailed study we performed on the formation of secretory granules of enteroendocrine EC cells in the gastric mucosa represents one aspect of the delineating membrane and narrow halo (Fig. 7).

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DISCUSSION

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morphological studies of endocrine cells from gastro-enteropancreatic endocrine system. Some researchers (Korostysheskaya IM), explore the formation of the secretory granules of the endocrine glands in gastrointestinal tract. This takes place in the Golgi complex and different stages of the secretory conveyor can be observed with electron microscope. The mechanism of formation of the mature granules is different in the different endocrine cell types. Unlike our object of study - the secretory granules, Kuramoto et al. in an immunohistochemical and electron microscopic study of the EC cells of the glands in the distal colon of rats focus on the morphology of the cells in terms of their form. The authors reveal presence of cytoplasmic processes filled with specific granules. EC cells with bipolar processes supplied with terminal expansions are described. One process reaches the lumen of the gland and the other the basal lamina. Processes of EC cells protrude in the adjacent enterocytes and goblet cells, contact nerve processes. That morphology of the enteroendocrine EC cells suggests paracrine action, as well as detection of luminal signals and participation in sensory processes.

The secretion of serotonin from the EC cells is a response to a number of factors: luminal – acidification of duodenal content, presence of a hypertonic glucose solution; circulatory and nervous factors – intestinal ischemia, sensory and vagal stimuli; mechanical distension – increase of the intraluminal pressure, mechanical obstruction; infectious factors - viruses, bacterial toxins. Recent studies reveal complex systems of receptors and ion canals that regulate serotonin secretion. Release of serotonin from the EC cells is a Ca$^{2+}$-dependent process determined by receptor-mediated or voltage-dependent Ca$^{2+}$-canals. EC cells express broad spectrum of receptors by which their activity is stimulated or inhibited. Adrenergic receptors (a2A, a2B, b1 и b2), muscarinergic and GABAergic receptors, nicotinic, acetylcholine and serotonin receptors (realizing autocrine and paracrine effect) are recognized. An interesting finding is the establishment of olfactory and vomeronasal receptors like OL1, QTL, HGL, EVA and SCR, as well as pheromone receptors (putative pheromone receptor). This suggests that EC cells can analyze chymus signals like the intraepithelial receptors in the airways and circulatory system. These data indicate the important role of EC cells and serotonin in the recognized brain–gut axis.

Serotonin produces its effects by binding specific receptors. In 1957 Gadum suggested that serotonin binds two different receptors in isolated tissues, one in smooth muscle cells and another one in nervous tissue. These receptors were called D and M-receptors referring to dibenzyl-selective antagonist in smooth muscle cells and morphine-selective antagonist in nervous tissue, respectively. Nowadays, serotonin (5HT) receptors are recognized and cloned into seven groups from 5HT1 to 5HT7 with various subtype numbers. Most of these receptors are coupled with G-protein, which activates membrane-gated adenylylase or phospholipase. 5HT3 receptors are ion channels. Some of the 5HT receptors are presynaptic and others postsynaptic. Cloning of new types of 5HT receptors is ongoing.

Serotonin receptors determine its crucial role in the regulation of different processes in the gastrointestinal tract – resorption of nutrients, secretion of the glands, motility and sensation. In the smooth muscle cells of the gastrointestinal tract 5HT3 and 5HT4 receptors have been established. In the smooth muscle cells of stratum circulare of the colon are found 5HT4 receptors and in stratum longitudinale 5HT7 receptors. 5HT1p receptors are verified in afferent somatic nerve fibers from plexus submucosus. 5HT3 receptors are found in afferent somatic fibers of plexus myentericus. Detailed study of the serotonin functions in the gastrointestinal tract, mediated by different types of receptors, reveals new opportunities in the therapy of the gastrointestinal functional disorders. The incidence of these disorders is considerably high. Almost 7 of 10 adults suffer from irritable colon syndrome, gastroesophageal reflux disease or functional dyspepsia. The uncertain etiology and lack of specific diagnostic criteria for elucidation of these functional disorders require profound knowledge of the gastroenteropancreatic system and particularly disturbances in the serotonin level regulated by its synthesis and secretion. In this relation Voutilainen et al. examine the role of serotonin released by the cardiac EC cells in the control of the lower esophageal sphincter. Motility disorders as major symptom of functional gastrointestinal disorders is a subject of different therapeutic approaches. Antagonists of 5HT3 receptors have an effect on the diarrheic syndrome in irritable colon. Besides blocking the afferent nerve impulses, 5HT3 antagonists decrease motility and slow down passage through the small and large intestine. Agonists of 5HT4 receptors affect constipation by increasing the motility and accelerating the gut passage.
tract are crucial in the diagnosis of neoplastic processes. Gastric mucosa is a site of development of endocrine tumors and carcinoids. Of all carcinoid tumors in the gastrointestinal tract 3.3% are localized in the stomach. The role of serotonin in the carcinoid syndrome is so pronounced that it was called initially “a serotonin syndrome”.1 The phenotype characteristics of the endocrine cells in them exhibits deviation from the normal morphology.12 The secretory granules characteristic found also in our study including oval, elongated or biconcave form; evenly distributed and with high electron density secretory product is not observed in the neoplastic cells. The neoplastic EC cells present with big lobulated nucleus and numerous mitochondria; mixture of small vesicles, empty vesicles and secretory granules with irregular electron density.13 The excessive release of serotonin from EC cells carcinoids determines the clinical picture of the carcinoid syndrome - diarrhea, constipation, abdominal pain. The release of serotonin from the neoplastic EC cells is triggered by the same luminal factors that activate also the normal enteroendocrine EC cells.14

CONCLUSIONS

Serotonin-producing cells are found in considerable number in the gastric mucosa, scattered in the lining epithelium, corpus and pyloric glands. The electron microscopic study followed the stages of formation of the secretory granules from the initial accumulation of granular substance, its membrane packing and formation of mature granules to their disintegration in the secretory process.

As one of the regulators of the gastrointestinal motility, secretion and sensation serotonin plays a crucial role in the pathogenesis of many disorders of the gastrointestinal tract. The data of the localization, distribution and morphology of EC cells in the different sections of the gastrointestinal tract would contribute to the clinical interpretation of certain pathological processes such as gastric and duodenal ulcer, gastroesophageal reflux disease, neoplasms.

The detailed study of the serotonin functions in the gastrointestinal tract realized via different receptors, as well as development of specific antagonists and agonists to these receptors holds out new opportunities in the therapy of gastrointestinal diseases.

REFERENCES

СЕРОТОНИН-ПРОДУЦИРУЮЩИЕ КЛЕТКИ, ВЗЯТЫЕ С СЛИЗИСТОЙ ОБОЛОЧКИ ЧЕЛОВЕКА - ИММУНОГИСТОХИМИЧЕСКОЕ И ЭЛЕКТРОННОМИКРОСКОПИЧЕСКОЕ ИССЛЕДОВАНИЯ

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РЕЗЮМЕ
Участие слизистой оболочки в координации процессов, протекающих в гастроинтестинальном тракте, определяется большим числом гормонов, секретирующихся эндокринными клетками в ее железах и в покровном эпителии. Один из этих гормонов, а именно серотонин, играет важную роль в регуляции мотилизации, секреции и чувственных восприятий в гастроинтестинальном тракте.

Цель: Настоящее исследование ставит себе целью провести иммунохимическое и электронномикроскопическое исследования серотонин-продуцирующих клеток, взятых с слизистой оболочки.

Материал и методы: Биопсийный материал с слизистой оболочки здоровых пациентов исследуется иммунохимически относительно экспрессии серотонина в ее эндокринных железах. Электронномикроскопическое исследование изучает в деталях процессы синтеза, накопления и секреции секреторного продукта в этих клетках.

Результаты: Иммунохимическое исследование устанавливает наличие значительного числа серотонин-содержащих EC клеток, разбросанных в покровном эпителии и в пилоре желудка. Электронномикроскопическое исследование прослеживает этапы при формировании секреторных гранул - от первоначального накопления зернистой субстанции, ее упаковки мембраной и формирования зрелых гранул до их дезинтеграции при секреторном процессе.

Выводы: Серотонин, как нейротрансмиттер и желудочно-кишечный гормон, оказывается ключом к ряду симптомов гастроинтестинальных заболеваний (тошнота, рвота, боль, диарея, запор). Детальное изучение функций серотонина в гастроинтестинальном тракте, осуществленных посредством различных типов рецепторов, как и создание специфических антагонистов и агонистов для этих рецепторов открывает новые направления в терапии гастроинтестинальных заболеваний.