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Hormonal Production of the Developing Gastrointestinal Tract of Rat

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The aim of our study was to investigate the production of ghrelin and serotonin in the developing gastrointestinal tract of a rat and its paracrine role in the gastrointestinal wall. The earliest occurrence of ghrelin-producing cells we founded in the endoblastic epithelium of rat embryos on 12th day gestation. In the following periods this number increased. GHSR-1 was expressed during the same period in endoblast and myoblast cells of the developing digestive tube of embryos and fetuses as well as in the covering epithelium and glands of the stomach and small intestine of newborn rats. Serotonin-producing cells we found as late as one-day old rats. At that time there was presence of 5-HTR3 in smooth muscle cells. In conclusion, ghrelin-producing cells are among the earliest differentiating cells in the digestive tube and the presence of GHS-R1 reveals the ability of ghrelin to carry out paracrine regulation of organogenesis and histogenesis of the gastrointestinal tract. After birth serotonin-producing cells are already differentiated and the gastrointestinal wall is ready to respond to serotonin and ghrelin signals through the GHSR-1 and 5-HTR3 in the smooth muscle and glandular cells.

Key words: ghrelin, GHS-R1, serotonin, 5-HTR3, gastrointestinal tract, embryo

Introduction

Local regulation of digestion in the gastrointestinal tract is performed by a large number of hormones produced by the enteroendocrine cells in its mucosa. In their totality they form the gastroenteropancreatic endocrine system which is part of the diffuse neuroendocrine system. It includes more than 19 types of cells secreting more than 30 types of hormones. Some of the earliest described cells are the serotonin-producing cells [24,18]. As a gastrointestinal hormone serotonin participates in the regulation of motility, secretion of the glands, sensitivity to pain [21,11,5].

Ghrelin is a recently discovered hormone. It is an oligopeptide of 28 amino acid residues isolated for the first time from rat stomach. It was first identified by Masayasu Kodzima et al. in 1999, they ascertained that serum levels of ghrelin in slim rats are higher than those of fat ones [17]. Ghrelin participates in the formation of severe feeling

of hunger through its connection with specific receptors in the nuclei of the diencephalon. It releases growth hormone secretion through receptors in the adenohypophysis. Maturation of the digestive tube is carried out through complex intercellular signaling between the consecutively differentiating tissues which form it. The differentiating endoderm releases a number of signaling cells which influence the underlying mesenchyme and smooth muscle cells are differentiated in it. The signal pathways of the endodermal - mesenchymal interactions are conservative glycoprotein families with a long evolutionary history. These are growth factors which carry out cascade transductions between embryonic cells. Numerous signal pathways of this interaction are familiar: BMP, Hedgehog (Hh), Sonic hedgehog (Shh), PDGF, TGF-β, Wnt, TCP, Notch etc. Bidirectional intercellular interactions of the endoderm and mesenchyme of the developing digestive tube are carried out through these molecular mechanisms [3,8,22].

Some of the earliest differentiating cells in the wall of the future gastrointestinal tract are endocrine cells. In birds they have been found on 9th day of incubation [15]. In rats they are found on 18th day gestation [12], and in man in 8th day gestation [19].

The occurrence of enteroendocrine cells with signs of hormonal production prior to the definitive differentiation of tissues presupposes participation of the gastrointestinal hormones in the histogenetic processes in the digestive tube.

The aim of our study was to investigate the production of ghrelin and serotonin in the developing gastrointestinal tract of a rat and its paracrine role in the gastrointestinal wall.

Materials and methods

The material of study is white Wistar rats. We studied rat embryos, fetuses and GIT fragments of one-day-old rats. It is obtained from 24 male white rats inseminated through contact with male specimen fixed in time. The material is distributed in four age groups: 1st group of 8th-11th -day-old rat embryos; 2nd- group of 12-15th -day-old rat embyos; 3rd group of 16th-20th gestation day old rat fetuses; 4th group of one-day old newborn rats. We performed an immunohistochemical study of ghrelin, ghrelin receptor GHSR-1; serotonin and serotonin receptor 5-HTR3. Immunohistochemical reactions were performed according to the ABC method through rabbit ABC Staining System (Santa Cruz Biotechnology, USA) with the respective primary antibody&goat polyclonal ghrelin antibody: sc-10368; goat polyclonal antibody GHSR-1: sc-10351; goat polyclonal antibody, SR-3A: sc-19150 - Santa Cruz Biotechnology USA and rabbit polyclonal antibody, MAB352 serotonin - Chemicon USA.We used a semi-quantitative evaluation method for the obtained results. The specificity of immunohistochemical reactions for each studied antigen is confirmed by negative controls. Observation and photo documentation of microscopic preparations are performed with digital photo microscopic camera of a light microscope “Olympus BX51”.

Results

In the developing gastroduodenal tract of rat embryos 12th-15th gestation day we established a positive immunohistochemical occurrence of ghrelin. Individual ghrelin-positive cells were localized among the endoderm of the digestive tube **(Fig. 1A).** Immunohistochemical reaction for ghrelin was positive in the stomach and small intestine of 16th-20th gestation day rat fetuses. Black granules were observed in a small number of stomach wall endoderm cells. **(Fig. 1B).** In one-day-old rats there was presence of positive expression of ghrelin in the stomach and small intestine. In the gastric cardia gherlin was expressed in single cells located in the base of cardiac glands **(Fig. 1C).**The reaction is positive also in the single cells from the small intestine epithelium **(Fig. 1D).**

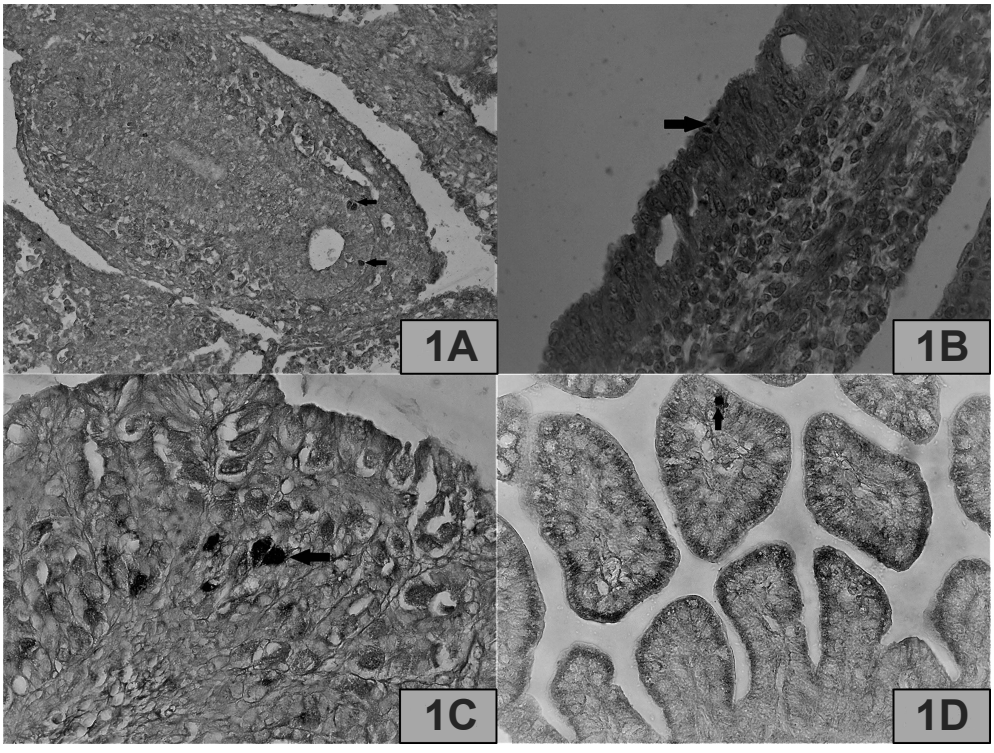

Fig 1. Ghrelin-producing cells in developing gastrointestinal tract of rat. IHC reaction for ghrelin. A. Digestive tube of rat embryos on 12th gestational day. Positive. x 100. B. Rat fetus stomach 16th gestation day. Single ghrelin positive cells in the multilayered endodermal epithelium. x 400. C. Stomach of a one-day-old rat - fundus. Positive ghrelin expression in a small number of cells at the base of the chief glands. x 400. D. Small intestine of a one-day-old rat - cross section. Single ghrelin positive cell in the covering epithelium of the villus. x 400.

For the first time the reaction for ghrelin and its receptor GHS-R1 was positive on 12th gestation day. Expression of GHS-R was found in individual endodermal cells from the covering epithelium as well as in myoblast cells from the surrounding mesenchyme **(Fig. 2A).** Immunohistochemical reaction for GHS-R1 was positive in the wall of the developing stomach of 16th-20th gestation day rat fetuses. There was presence of fine brown granulation in groups of endodermic cells. Expression of GHS-R1 was also found in a thin layer of smooth muscle cells in the periphery of the gastric wall **(Fig. 2B)**. Ghrelin receptor GHS-R1 was expressed in the gastric wall of one-day-old rats. The reaction was positive in individual cells of the covering epithelium and the glands **(Fig. 2C)** and in the the covering epithelium of the intestinal villus **(Fig. 2D).**

Immmunohistochemical reaction for serotonin was positive in the small intestine of one-day-old rats.The reaction is positive for a small number of cells. They were located singly between the resorptive cells in the covering epithelium of the intestinal villus. They were found along the villi and in their peak area. **(Fig. 3)**. Serotonin receptor 5HTR3 was expressed in the gastric wall of one-day-old rats. The reaction was visualized through brown granulation which fills the cytoplasm of a large number of smooth muscle cells from the muscle lining of the stomach **(Fig. 4).**

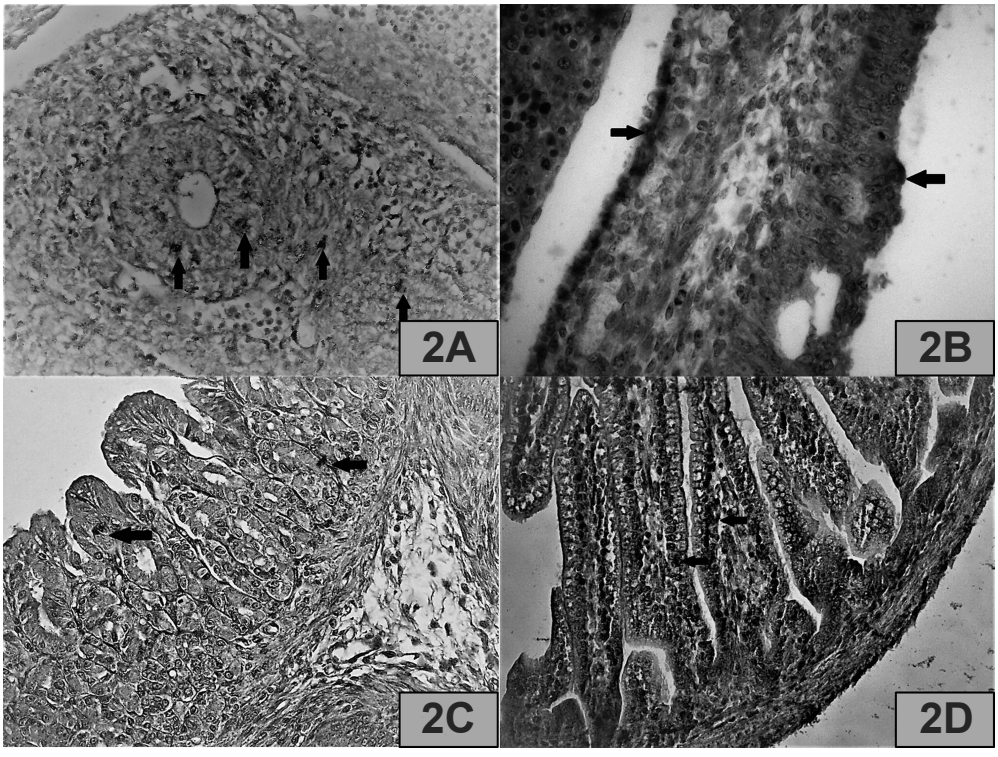

Fig. 2. Ghrelin receptor GHS-R1in developing gastrointestinal tract of rat.IHC reaction for GHS-R1. A. Digestive tube of rat embryos 12th gestation day. Positive GHS-R1 expression in endodermal and myoblast cells. x 200. B. Rat fetus stomach 16th gestation day. Positive GHS-R1 reaction in the multi-layered endodermal epithelium and peripheral smooth muscle layer. x 400. C. Stomach of a one-day-old rat. Positive GHS-R1 expression in single cells from the glands of the gastric mucosa. x 200. D. Small intestine villi of one-day-old rat - longitudinal section. Positive GHS-R1 expression in the epithelial cells. x 200.

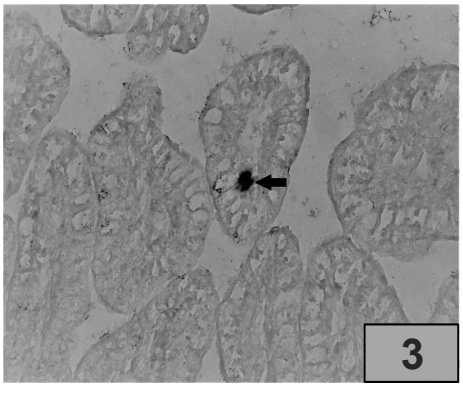

Fig. 3. Small intestine villi of one-day-old rat-cross section. Positive expression of serotonin in the basal part of epithelial cells. IHC. x 200.

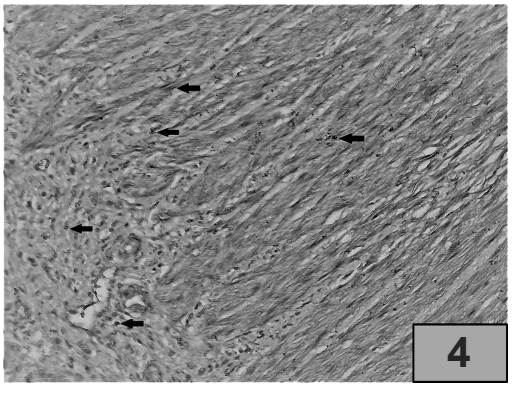

Fig. 4. Stomach of a one-day-old rat. Positive expression for serotonin receptor 5-HTR3 in smooth muscle cells. IHC. x 400.

Discussion

The earliest presence of differentiated ghrelin-producing cells we found in rat embryos on 12th gestation day. They are single cells dispersed among the endodermal epithelial cells of the esophageal tube. Our results show that in 16th gestation day rat fetuses and in one-day-old rats ghrelin-producing cells are already localized in the stomach as well as in the small intestine. The immunohistochemical expression of ghrelin receptor GHS-R1 is parallel with that of the hormone itself.The earliest occurrence of ghrelin-producing cells in rat is reported in fetal stomach on 18th gestation day [6,2]. However, some authors do not ascertain presence of positive ghrelin expression in gastric cells of rat fetuses although they found high plasma levels of ghrelin in the fetus [1,2,10]. Sakata et al., while studying ghrelin production in rat from 1st to 8th week of the postnatal period and in adult rats, ascertain that initially ghrelin-producing cells occur in the base of the chief glands of the stomach. Around the 3rd week their number increases and they spread to the corpus and neck of the glands [16].The presence of ghrelin receptor GHS-R in the prenatal period of rat is found as early as 20th day gestation namely in the pancreas. Wierup et al., study the expression of ghrelin and ghrelin receptor GHS-R in islets of Langerhans of fetal and neonatal pancreas of rat. They prove the presence of ghrelin receptor in the islet cells of 20th gestation day rat fetuses. It is through these receptors that ghrelin exerts a paracrine effect directly on β-cells [23]. Kitazawa et al. report of presence of GHS-R1 in gastrointestinal tract of rat and guinea pig [7]. There is immunohistochemical evidence for an endocrine/paracrine role for ghrelin in the reproductive tissues [9].

In our study we find serotonin expression in one-day-old rats. The small number of serotonin-producing cells is probably due to the unfinished processed of maturation in the small intestine wall. The localization of these cells, found by us, in the peak areas of the small intestine villi and not in their typical location- crypts of Lieberkuhn may be explained with the intensive processes of proliferation of stem cells in the developing crypts and epithelial cell migration along the radiant crypt-villus axis of the small intestine wall. The migration processes are performed also in the mature small intestine. At the bottom of the intestine glands a small group of stem cells, which provide several cellular phenotypes- resorptive, cup-like, endocrine. These cells constantly migrate to the adjacent villi [13, 14].Presence of serotonin receptor 5-HTR3 we found in the gastric wall of one-day-old rats. It is localized in a large number of smooth muscle cells both in the transverse and longitudinal layers of the muscle sheath of the stomach. Glatzle et al. ascertain 5-HTR3- immunoreactivity both in the gastric wall and in the duodenal wall but in adult rats [4]. The effects of serotonin on motility are achieved by 5-HT3R, localized on cholinergic neurons which stimulate smooth muscle cells [20].

Conclusion

Ghrelin-producing cells are among the earliest differentiating cells in the digestive tube and the presence of ghrelin receptor GHS-R1 reveals the ability of ghrelin to perform paracrine regulation in the earliest stages of organogenesis and histogenesis of the gastrointestinal tract. Serotonin regulates the motility, secretion and sensory function of the gastrointestinal tract. Immediately after birth serotonin-producing cells have already been differentiated and the wall of the gastrointestinal wall is ready to respond to serotonin and ghrelin signals through the presence of receptors for serotonin and ghrelin in smooth muscle and glandular cells.

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