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Ghrelin receptors in human gastrointestinal tract during prenatal and early postnatal development

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ABSTRACT

The aim of our study was to investigate the appearance, density and distribution of ghrelin cells and GHS-R1a and GHS-R1b in the human stomach and duodenum during prenatal and early postnatal development. We examined chromogranin-A and ghrelin cells in duodenum, and GHS-R1a and GHS-R1b expression in stomach and duodenum by immunohistochemistry in embryos, fetuses, and infants. Chromogranin-A and ghrelin cells were identified in the duodenum at weeks 10 and 11 of gestation. Ghrelin cells were detected individually or clustered within the base of duodenal crypts and villi during the first trimester, while they were presented separately within the basal and apical parts of crypts and villi during the second and third trimesters. Ghrelin cells were the most numerous during the first (~11%) and third (~10%) trimesters of gestation development. GHS-R1a and GHS-R1b were detected at 11 and 16 weeks of gestation, showed the highest level of expression in Brunner's gland and in lower parts of duodenal crypts and villi during the second trimester in antrum, and during the third trimester in corpus and duodenum. Our findings demonstrated for the first time abundant duodenal expression of ghrelin cells and ghrelin receptors during human prenatal development indicating a role of ghrelin in the regulation of growth and differentiation of human gastrointestinal tract.

Introduction

Ghrelin (P/D1) endocrine cells in human stomach produce gastric peptide involved in regulation of food intake and growth hormone release [25] as well as in a control over gastric acid secretion and motility providing gastroprotective action [12]. Ghrelin performs its function by binding to active form of its receptors. The ghrelin receptors are the growth hormone secretagogue receptor type GHS-R1a (a seven transmembrane domain G protein coupled receptor (GPCR)), and GHS-R1b (a five transmembrane domain GPCR) [5]. GHS-R1a was primarily detected in the pituitary gland and the hypothalamus, but is also expressed in peripheral tissues including the stomach and intestine [6,10].

Ghrelin endocrine cells are widely distributed throughout gastrointestinal tract with the highest density of cell population in corpus, and smaller density in antrum and duodenum. Study conducted by Falls et al. [4], showed that acyl-ghrelin peptide form binds to active form of ghrelin receptors. Recent results have demonstrated that des-acylated-ghrelin has different physiological functions via unknown receptor form, including participation in regulation of feeding behavior, energy and glucose homeostasis [1,2,30,33]. There are no studies showing a ligand for inactive form of ghrelin receptors.

During human fetal development, the first ghrelin endocrine cells appeared from 11 gestational weeks in antrum and corpus stomach [16], and according to our knowledge, there is no studies regarding its appearance in human duodenum. Stein et al. showed that gastrin, somatostatin and serotonin endocrine cells were simultaneously identified in the stomach and small intestine [29]. One study has reported that endocrine cells were identified later in the stomach than in the duodenum [24]. Volante et al. [32] showed that ghrelin endocrine cell was observed in the lung as early as 7th week of gestation, while in the stomach, duodenum and pancreas they were identified in the human fetus at 10th week of gestation.

In the small intestine, ghrelin endocrine cells were scattered in the epithelia of crypts and villi [7,27], while in antrum and corpus mucosa, ghrelin endocrine cells were mainly located in the

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glandular base and only some of them in the glandular neck [16,17]. In antrum and duodenum, ghrelin endocrine cells were elongated and bottle shaped with an apical cytoplasmic process in contact with the lumen, while in the corpus they were circular or irregular shape without any connection with the lumen [15,27]. During the early period of fetal development in antrum and corpus, ghrelin endocrine cells were characterized by clustering of three to four cells, while in the latest period of fetal development ghrelin containing cells were observed as single cells in the stomach glands [17]. The density of ghrelin endocrine cells was radically decreased in the lower part of gastrointestinal tract [27]. Lee et al. showed that the number of ghrelin endocrine cells in gastrointestinal tract corresponded well with the amount of peptide and mRNA expression levels [13].

Dass et al. demonstrated that GHS-R1a and GHS-R1b were expressed in human and rat stomach and colonic tissue sections within neuronal cell bodies and fibers, and in the cells associated with corpus stomach glands and mast cells [3]. In the same study, it was shown that smooth muscle cells and epithelia did not express GHS-R, while only rat stomach and colon tissue samples expressed GHS-R on nerve fibers associated with the muscle layers [3].

Ghrelin open-type of endocrine cells in antrum and duodenum were functionally regulated by receiving luminal information such as nutrients and pH, while ghrelin endocrine cells in the corpus were closed-type of cells functionally modulated by hormones and neuronal stimulation [26]. Ghrelin endocrine cells in the adult human corpus mucosa were accounted for 20–30% of the entire endocrine cell population [25].

According to the above mentioned data, the aim of our study was to investigate the early appearance of ghrelin endocrine cells and ghrelin receptors, their localization and number, as well as their relationship in the human duodenum during prenatal and early postnatal development. According to our previous results the highest number of closed-type ghrelin endocrine cells was found in the corpus stomach [17], while the number of opened-type ghrelin endocrine cells demonstrated gradual enhancement in direction from stomach to the duodenum [27]. To define the physiological role of ghrelin endocrine cells and their receptors during human ontogeny, it is important in the beginning to identify the time of appearance, morphological characteristics of the ghrelin endocrine cells and its receptors, their density and distributions. In addition, there have been studies about ghrelin cells and ghrelin receptors in the stomach [17,21], but no studies focused on their interaction in human fetal duodenum. Therefore, we investigated in detail the first appearance, density, distribution and morphological characteristics of ghrelin endocrine cells and ghrelin receptors in human fetal duodenum. The results of our study will improve understanding of ghrelin endocrine cells and ghrelin receptors role in development of human gastrointestinal tract.

Material and methods

Material

The tissue samples of complete wall of antrum ($n = 27$), corpus ($n = 43$) and duodenum ($n = 9$) from fetuses, 5 embryos and 5 infants, different ages and both sexes, were collected at legal abortions, premature deliveries, and immediate postnatal deaths, according to the principles of the Ethical Committee. The ages of embryos were determined by the measuring crown - rump length, the method by O'Rahilly [20], while measuring the length of the foot was used to determine fetal age, given that this measure correlates well with the gestation period. The examined samples of fetal origin were divided into three groups based on affiliation of particular trimester of pregnancy (9–15 weeks – the first trimester; 15–27 weeks – the second trimester; 27–42 weeks – the third trimester).

Tissue preparation

The examined tissue specimens were fixed in 10% buffered (PBS) formalin and paraffin embedded. Tissue blocks of human antrum, corpus and duodenum (five tissue sections of each paraffin blocks) were transversely cut in serial sections, 5 μm of thickness. Five consecutive sections from each level, taken at intervals of 20–50 μm, were stained with different antibodies. The Ethical Committee Faculty of Medicine, University of Niš, approved the study. On the tissue sections, stained with haematoxylin and eosin, normal morphology was observed in the antrum, corpus and duodenum wall.

Antibodies

For immunohistochemical analysis polyclonal and monoclonal antibodies against – ghrelin (polyclonal goat antibody, Santa Cruz Biotehnology, USA, sc-10368), – GHS-R1b (polyclonal rabbit antibody, Phoenix Pharmaceuticals, Catalog number: H-001-61), – GHS-R1a (polyclonal rabbit antibody, Phoenix Pharmaceuticals, Catalog number: H-001-62), – chromogranin A (monoclonal mouse antibody, DAKO A/S Denmark, M 0869) were used.

Immunohistochemical procedure

The antrum, corpus and duodenum tissue samples were fixed in 10% buffered formalin solution and embedded in paraffin. The tissue sections were cut at 5 μm, heated at 56 °C for 60 min, then deparaffinized and rehydrated through a series of xylenes and alcohols followed by an epitope retrieval step. Tissue sections were treated with 3% H_2O_2 solution in PBS to block endogenous peroxidase activity. After that, tissue sections of antrum and corpus of the stomach and duodenum were heated in microwave oven (at 680 W, in 10 mmol/L citrate buffer pH 6.0, for 21 min) for epitope retrieval. The next step was incubation of tissue sections with appropriate antibodies (against ghrelin, chromogranin-A, GHS-R1a and GHS-R1b) in the humid chamber for 60 min at room temperature. Immunostaining was performed using the streptavidin–biotin technique (LSAB+/HRP Kit, Peroxidase Labeling, K0690, DAKO Cytomation, Denmark). Immunoreactivity complex was visualized with DAKO Liquid DAB+ Substrate/Chromogen System (Code No. K3468), and counterstained with Mayer's hematoxylin (Merck). The antrum, corpus and duodenum tissue sections with omitted primary antibody were used as a negative control. The adult antrum, corpus and duodenum tissue sections, known to express chromogranin-A, ghrelin, GHS-R1a and GHS-R1b, were used as positive controls for immunohistochemical staining.

Morphometry

The number of immunoreactive ghrelin and chromogranin-A endocrine cells was determined in the glandular epithelium and epithelial cells of crypts and villi using a computer-supported imaging system connected to a light microscope (Olympus AX70) with the objective magnification of ×10. The area of the glandular epithelium was calculated using the following formula:

$$P = \frac{pd^2}{10^6}$$

where: P – surface; p – the number of grid points in the glandular epithelium; d – the size of the square network at a magnification of 200 (×10 objective and ×20 eyepieces). According to the above-mentioned formula, the area of glandular epithelium was