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Immunohistochemical Expression of Ghrelin Receptor GHS-R1 in the Gastrointestinal Tract

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Ghrelin is a gastrointestinal hormone which performs the function of a ligand for secretory receptors of growth hormone in the adenohypophysis. In recent years, studies reveal the presence of ghrelin receptors outside the CNS. The aim of the study is an immunohistochemical proof of the presence and localization of ghrelin receptor GHS-R1 in the human gastrointestinal tract. Biopsy specimens from stomach – body and antrum pylorus and duodenum are subjected for the expression of ghrelin receptor GHS-R1. Immunohistochemical study of GHS-R1 found its expression in biopsy material from three locations. The presence of receptors for ghrelin in the gastrointestinal tract indicates its paracrine action. There are ghrelin receptors in the epithelial cells of gastric glands and Lieberkuhn crypts of the duodenum that can directly affect their secretion. By binding to receptors in smooth muscle cells ghrelin also influences motility of the gastrointestinal tract.

Key words: ghrelin, ghrelin receptor, GHS-R1, gastrointestinal tract.

Introduction

Ghrelin is a gastrointestinal hormone which performs the function of a ligand for secretory receptors of growth hormone in the adenohypophysis (growth hormone secretagogue receptor, GHSR). As a releasing factor for growth hormone ghrelin intervenes in the regulation of many metabolic processes. In recent years, studies reveal the presence of ghrelin receptors outside the CNS, in peripheral tissues. Ghrelin receptor GHS-R1 is established in placenta, lung, uterine myometrium, in vegetative afferent nerve fibers in the gastrointestinal tract.

The aim of the study is an immunohistochemical proof of the presence and localization of ghrelin receptor GHS-R1 in the human gastrointestinal tract.

Materials and Methods

The materials about the morphologic research of the EC cells were obtained by fibrogastroscopy performed 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 pylorus of the stomach and superior portion of the duodenum. There were no endoscopic pathological changes in the gastric mucosa of these patients.

The material is studied by immunohistochemistry using the ABC method with primary antibody for ghrelin receptor GHS-R1 (rabbit polyclonal antibody Ghrelin – Santa Cruz Biotechnology USA) at a dilution of 1:100. Positive response to ghrelin is presented by fine brown granulation. The specificity of the immunohistochemical reaction was confirmed by negative controls, the specific antibody was replaced with a buffer (PBS) or normal non-immune serum.

Results

Immunohistochemical study of ghrelin receptor GHS-R1 found its expression in biopsy material from three locations – in the stomach body and antrum pylorus and in the duodenum. In the stomach body mucosa GHS-R1 receptors were visualized by brown fine granulation in some cells of the fundic glands. These cells were few in number.

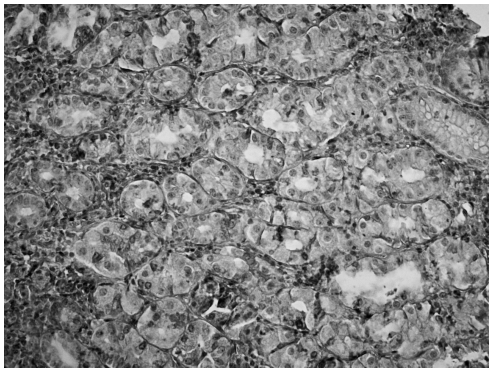

Fig. 1. Biopsy specimens from the body of the stomach. Immunohistochemical expression of GHS-R1 in the fundic glands. Paraffin preparation. Magnification × 20

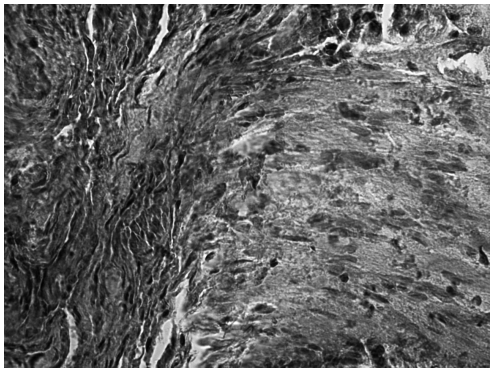

Fig. 2. Biopsy specimens from the antrum of the pylorus. Immunohistochemical expression of GHS-R1 in the smooth muscle cells of the gastric wall. Paraffin preparation. Magnification × 40

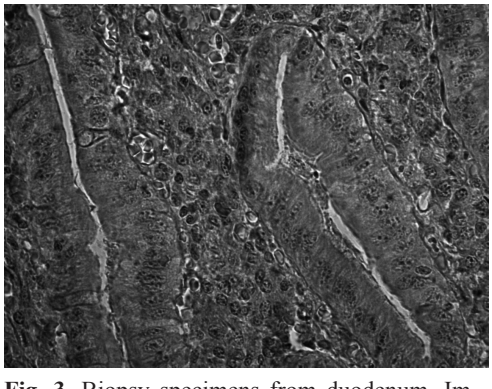

Fig. 3. Biopsy specimens from duodenum. Immunohistochemical expression of GHS-R1 in the epithelial cells of the duodenal villus. Paraffin preparation. Magnification × 40

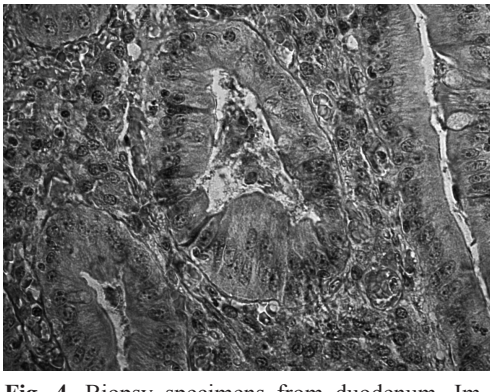

Fig. 4. Biopsy specimens from duodenum. Immunohistochemical expression of GHS-R1 in the glandular cells of the crypts of Lieberkuhn. Paraffin preparation. Magnification × 40

They were located mainly in the central parts of the glands unevenly among other epithelial cells (**Fig. 1**). In the antrum biopsy immunohistochemical reaction is positive for smooth muscle cells. Bundles of parallel leyomyocytes showed brown granulations that can cover whole cells (**Fig. 2**). In the duodenum the receptor is expressed in both the epithelial cells coating the intestinal villi, and in the glandular cells of the crypts of Lieberkuhn (**Figs. 3, 4**).

Discussion

Ghrelin receptor GHS-R1 is a G-coupled peptide. It was first cloned in 1996 from adenohypophysis and hypothalamus [1]. Binding of ligands to this receptor leads to the secretion of growth hormone. In 1999 ghrelin was identified as an endogenous ligand for this receptor [5]. The receptor has two isoforms – GHS-R1a and GHS-R1b. GHS-R1a is a mature polypeptide of 366 amino acid sequences and 7 transmembrane domains. GHS-R1b is an immature polypeptide with 289 amino acid residues and 5 transmembrane domains. Recently some authors found the presence of ghrelin receptors outside of the CNS. O'Brien et al. [4] reported the expression of ghrelin and GHS-R1 in the smooth muscle cells of myometrium of the uterus during pregnancy, childbirth and in the uterine wall outside of pregnancy. Ghrelin has autocrine and paracrine effects that decrease contractility of the leyomyocytes and provide protection of pregnancy [4]. Ghrelin has a number of effects on the cardiovascular system. In vitro and in vivo it has a powerful vasodilatating effect. Ghrelin decreases the tonus of smooth muscle cells of the vascular wall. Endothelial cells of the microcirculatory bed express ghrelin and GHS-R1. *In vitro* ghrelin stimulates cell proliferation, migration and angiogenesis [3]. There is evidence of GHS-R1 receptor in the gastrointestinal tract of rats and guinea pigs that affect gastrointestinal motility [2].

Conclusion

The presence of receptors for ghrelin in the gastrointestinal tract indicates its paracrine action. There are ghrelin receptors in the epithelial cells of gastric glands and Lieberkuhn crypts of the duodenum that can directly affect their secretion. By binding to receptors in smooth muscle cells ghrelin also influences motility of the gastrointestinal tract. Evidence of the existence and distribution of ghrelin receptors in the human gastrointestinal tract is important for the clinical practice – possible therapeutic use of ghrelin analogues; agonists and antagonists of ghrelin receptors and knowledge of their side effects and complications.

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