IMMUNOHISTOCHEMISTRY OF GRELIN-POSITIVE CELLS IN THE PANCREAS ISLETS OF THE RAT

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ABSTRACT

After the discovery of the hormone ghrelin research on it is intensified in recent years. They are mainly clinical researches or studying of its physiological action. Fewer morphological experiments therefore histochemical expression of the cells that produce it, are relatively rare. The purpose of this study was by immunohistochemical methods to localize ghrelin-positive cells in the pancreas islets. We use Ghrelin (H-40) antibody (Santa Cruz-USA), and for the visualization of the results - detection system EnVision™ FLEX Mini Kit, High Ph (DAKO - k8024 HRP). Histomorphometric study performed by Michael Vinther Image Analyzer, Version 1.33 (Mee Soft, Germany).

Key words: ghrelin, antibody, bilious epithelium, biliary ducts

INTRODUCTION

Ghrelin's discovery in 1999 by Kojima et al. (1) opened a new frontier in the understanding of energy homeostasis regulation (2) since ghrelin is the only circulating peptide hormone that stimulates food intake and adiposity in humans (3)

Ghrelin is mainly produced in the stomach from a distinct group of endocrine cells located within the gastric oxyntic mucosa (4). The same authors describe that ghrelin-positive cells are observed along the gastrointestinal tract and pancreas (4).

In the rat ghrelin homologs have been identified in human and other mammals (1). The ghrelin action is not equal in different animal species. Ghrelin stimulates feeding in rats; however, intracerebroventricular injection of ghrelin strongly suppressed feeding in neonatal chicks (5).

Ghrelin-immunoreactive cells are also found in the duodenum, jejunum, ileum, and colon (6, 7). In the intestine, ghrelin concentration gradually decreases from the duodenum to the colon.

The pancreas is a ghrelin-producing organ. Analyses combining HPLC and ghrelin-RIA

revealed that ghrelin and des-acyl ghrelin both exist in the rat pancreas (8). However, the cell type that produces ghrelin in the pancreatic islets remains controversial, whether it be the α cells, β cells or the newly identified islet ϵ cells. (8, 9). Werup et al. investigate the ghrelin regulation of development of novel islet cells in human pancreas (10) and expression of ghrelin in the novel endocrine cells type in rat pancreas islets (11).

The protein Nkx2.2 is essential for the differentiation of islet α cells and β cells, and lack of Nkx2.2 in mice results in replacement of pancreatic endocrine cells by cells that produce ghrelin (9). The role of ghrelin in insulin secretion is under debate. Ghrelin has been shown to inhibit insulin secretion in some experiments and stimulate insulin release in others (12). Some investigators contend that ghrelin of the stomach induces hyperglycemia and reduces insulin secretion in humans (13) but other authors maintain contention that ghrelin presented in pancreatic alpha-cells of humans and rats and stimulates insulin secretion (8). This idea is supported by Lee et al. (14). According to them ghrelin, as a new gastrointestinal endocrine peptide stimulates insulin secretion: enteric distribution,

ontogeny, influence of endocrine, and dietary manipulations.

According Kojima and Kangawa plasma ghrelin and insulin levels are affected by blood glucose level; high glucose suppresses ghrelin secretion and stimulates insulin secretion (15) and therefore the glucose level in experiments may be important. Date et al. (8) report that ghrelin stimulates insulin release in the presence of high levels of glucose (8.3 mM) but ghrelin had no effect on insulin release in the context of a basal level of glucose (2.8 mM).

AIM AND METHODS

The aim of this investigation is to localize the ghrelin in the pancreas islets of the rat.

The material was fixed in 4% aqueous formaldehyde for 48 hours, then embedded in paraffin in accordance with the requirements of 56°C standard paraffin inclusion. Using ultramicrotome (Ultracut, Germany) did slice thickness 4 µm.

Figure 1. Ghrelin immunoreactive cell in a pancreas islet.

Other authors confirm these results (6, 16). Some investigators have reports for expression of ghrelin-positive cells in the rat (17, 18) and other authors described a characterization of gastric ghrelin cells in adult and fetal tissues of human and other mammals (19).

CONCLUSION

Ghrelin-immunoreactive cells are found in all parts of gastrointestinal tract but their quantity is in varying degrees in the different digestive organs. A little some single ghrelin-positive cells are found in pancreas islets.

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For realization of this aim we used an immunohistochemical method. Ghrelin immunoreactivity was determined on sections using Ghrelin (H- 40) SC- 50 297 Company Santa Cruz, USA. Results visualized with detection system Daco - En Vision FLEX - Mini Kit.

RESULTS AND DISCUSSION

Ghrelin-positive cells are found in some pancreas islets. They are single cells but they are clearly visible (**Figure 1**). There are many islets without immunoreactive ghrelin cells in a field of vision.

An association between stomach and pancreas obviously manifests that ghrelin immunopositive cell density is lower in the pancreas in comparison with gastric mucosa. This is according to Kojima and Kangawa (15) that was identified ghrelin in the oxyntic gastric mucosa. They described it in highest concentrations in this region.

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