



CHARACTERISTICS OF GHRELIN POSITIVE CELLS IN EXTRAHEPATIC BILE DUCTS OF THE RAT

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ABSTRACT

Ghrelin is a newly discovered hormone that associated with the metabolism and produced mostly by endocrine cells in the stomach fundus. There are data that there is a ghrelin production at other places: testis, pancreas, thyroid gland. The aim of this investigation is to discover ghrelin positive endocrine cells in the extrahepatic bile ducts of the rat. We applied immunohistochemical methods and antibodies Ghrelin (H- 40) SC- 50 297, Company Santa Cruz, USA to the bile mucosa. We visualized results with detection system Daco - En Vision FLEX - Mini Kit. Rare ghrelin positive cells were visualized in the mucosa of the extrahepatic bile ducts.

Key words: ghrelin, ghrelin positive cells, extrahepatic bile duct, mucosa

INTRODUCTION

Ghrelin is a newly discovered hormone that most researchers associated with the metabolism and which is mainly produced by distinct endocrine cells in the digestive system – mostly in the fundus of stomach.

In many studies was described that ghrelin producing cells are localised in the gastrointestinal tract, but they was detected not only in the stomach but also in the intestine, in the intra- and extrahepatic tract and in a very other places in- and outside the digestive system. From the time of its discovery ghrelin is subject to diverse study by scientists worldwide.

28 amino acid peptide ghrelin was discovered by Kojima et al. (1999) as a growth-hormone-releasing acylated peptide from stomach. According Maksud et al. (2011) this hormone appears to play a role in appetite regulation and in the control of energy homeostasis. There is a relevance of ghrelin in human energy balance and its circulating levels are negatively associated with obesity (Ariyasu et al., 2001; Tschop et al., 2001).

Date et al. (2000) discloses that the newly hormone ghrelin was synthesized in (distinct) endocrine cells of the gastrointestinal tract of rats and humans. According to the same author (Date et al., 2000) ghrelin induces increased secretion of hydrochloric acid in the stomach by the action on the central nervous system .

Ariyasu et al. (2001) points to the important role of the stomach as the main source of ghrelin circulating in the bloodstream and ghrelin-like immunoreactive plasma levels in humans and Tschop et al. (2001) found that ghrelin level in the blood decrease in people with obesity.

Broglio et al.(2001) determines that ghrelin produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans and Toshinaj et al. (2001) investigate upregulation of ghrelin expression in the stomach upon fasting, insulin-induced hypoglycemia, and leptin administration. Some authors (Lu et al, 2002) have a publication about Immunocytochemical observation of ghrelin-containing neurons in the rat arcuate nucleus and Rindi et al. (2002) publishes a characterization of gastric ghrelin cells in man and other mammals. They study many adult and fetal tissues.

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Van der Lely et al. (2004), after many years of research made biological, physiological, pathophysiological and pharmacological profile of ghrelin and Kojima&Kangava. (2005) studied the physiological interactions. According to them, this hormone is the basis of the regulation of all functions of gastrointestinal tract. The same year they determine the structure and function of ghrelin.

Some authors search other organs – normal and pathological. Raghay et al. (2006) report for ghrelin localization in rat and human thyroid and parathyroid glands and tumours.

According Wierup et al. (2007) ghrelin and motilin are cosecreted from a prominent endocrine cell population in the small intestine. They recognize the role of these hormones in digestive tract.

More of experiments are in stomach, small intestine or pancreas. There are too many reports about ghrelin positive cells in extrahepatic biliary ducts.

PURPOSE AND OBJECTIVES

The purpose of this study is to establish the existence and characteristics of ghrelin positive cells in extrahepatic biliary tract in the rat.

To accomplish this goal, we set the following tasks:

1. Collection and preparation of biological material for immunohistochemical study.
2. Impact on the taken material with ghrelin antibody.
3. Display immunohistochemical reaction with a detection system.
4. Monitoring and documentation of results.

MATERIAL AND METHODS

The biological material taken in vivo from the lining of the ductus choledochus and vesica felea in the rat race (Wistar) (Figure 1). Rival is taken in compliance with all requirements for animal welfare according to the European directive and the permission of the Ethics Committee of the Medical Faculty, University of Thrace, Stara Zagora, Bulgaria.

We fixed the material in 10 % aqueous formaldehyde for 48 hours, then embedded in paraffin in accordance with the requirements of 56°C standard paraffin inclusion. Using ultramicrotome (Ultracut, Germany) we did slice thickness 4 µm..



Figure 1. Experimental rat of species Wistar.

We applied immunohistochemical methods Ghrelin (H-40) SC-50 297, Company Santa Cruz, USA. Results visualized with detection system Daco - En Vision FLEX - Mini Kit. The place of our investigation was the bile bladder mucosa (Figure 2).

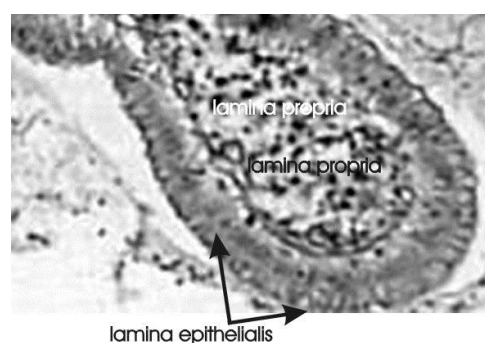


Figure 2. Part of the bile bladder wall in rat.

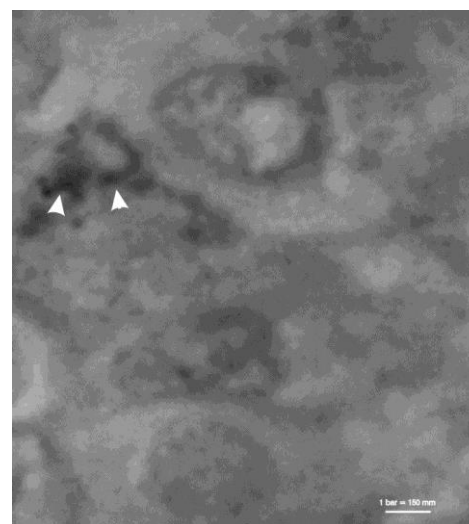


Figure 3. Ghrelin positive cell in the bile bladder wall.

RESULTS

Ghrelin positive cells were visualized in the depth of the mucosa of the biliary bladder, close to the lamina propria. Rare cells containing granules with ghrelin was found. Ghrelin positive cells are not found in all investigated cases (Figure 3).

DISCUSSION

Ghrelin is produced in distinct endocrine cells located within the extrahepatic ducts mucosa. There are not many reports about ghrelin positive cells in the extrahepatic bile ducts (Date, 2000).

There are two reasons for ghrelinpositive cells rarity:

1. Ghrelinproducing cells are rare in bile ducts.
2. Anatomical objects are too small and investigation is difficult.

CONCLUSIONS

In the digestive system is the availability of ghrelinpositive cells.

The greatest amount of cells containing the ghrelin is detected in the fundus of stomach. Ghrelinpositive cells are relatively underrepresented in the bile ducts at least found there in small quantities.

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