



CHARACTERISTICS OF GHRELIN POSITIVE CELLS OF THE STOMACH IN THE RAT

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ABSTRACT

Ghrelin is a novel hormone which is mainly produced by distinct endocrine cells in the fundus of stomach. The aim of this investigation is to discover ghrelin positive endocrine cells in the stomach mucosa. We applied immunohistochemical methods and antibodies Ghrelin (H- 40) SC- 50 297, Company Santa Cruz, USA. We visualized results with detection system Dako - En Vision FLEX - Mini Kit. Ghrelin positive cells were visualized in the depth of the mucosa of the stomach fundus, close to the border with ventricular lamina propria.

Key words: ghrelin, ghrelin positive cells, stomach, stomach, fundus

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 fundus of stomach. Although many previous studies grelin producing denied any cells in the rest of the gastrointestinal tract, in other experiments it was detected not only in the stomach but also in the intestine, testes, pancreas 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.

According to Wren et al. (2001) Ghrelin enhances appetite and increases food intake in humans, and the same year Cummings et al. (2001) study the role of plasma ghrelin level and its relation to the beginning of human nutrition. Date (2000) discloses that the newly hormone ghrelin was synthesized in (distinct) endocrine cells of the gastrointestinal tract of rats and humans. Sun et al (2007) determine the role of ghrelin on glucose homeostasis in humans.

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Ariyasu (2001), after several years of research points to the important role of the stomach as the main source of ghrelin circulating in the

bloodstream and grelin-like immunoreactive plasma levels in humans and Romero et al (2010) published that peptide mainly secreted by gastric mucosa and has been implicated in the regulation of eating behavior and weight balance.

Van der Lely et al (2004), after many years of research made biological, physiological, pathophysiological and pharmacological profile of ghrelin and Purnell et al (2003) studied the physiological interactions. According to them, this hormone is correlated with insulin levels and cholesterol levels in the blood.

PURPOSE AND OBJECTIVES

The purpose of this study is to establish the existence of grelin positive cells in stomach and duodenum mucosa in the rat.

To accomplish this goal, we set the following tasks:

Collection and preparation of biological material for immunohistochemical study.

Impact on the taken material with ghrelin antibody (Ghrelin (H- 40) SC- 50 297, Company Santa Cruz, USA).

Display immunohistochemical reaction with Dako - En Vision FLEX - Mini Kit system. Monitoring and documentation of results.

MATERIAL AND METHODS

The biological material taken in vivo from the lining of the stomach lining from the bottom of the stomach in the rat race. Riyal is taken in compliance with all requirements for animal welfare according to the respective European directive and the permission of the Ethics Committee of the Medical Faculty, University of Thrace, Stara Zagora, Bulgaria.

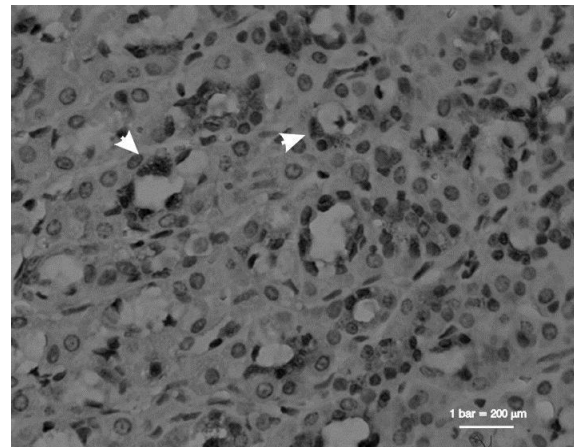
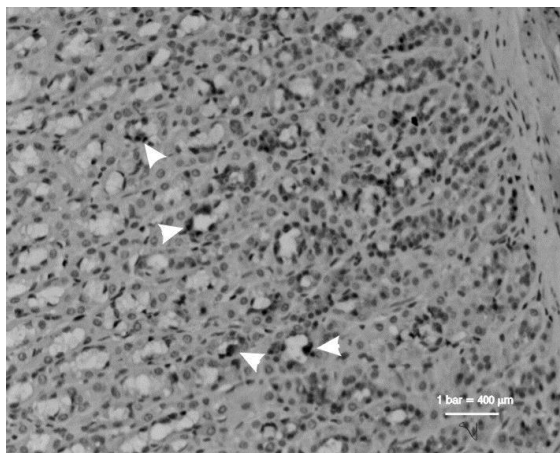
The material fixed in 10% aqueous formaldehyde for 48 hours, then embedded in paraffin in accordance with the requirements of 56°C standard paraffin inclusion. Using

ultramicrotome (Ultracut, Germany) did slice thickness 4 µm.

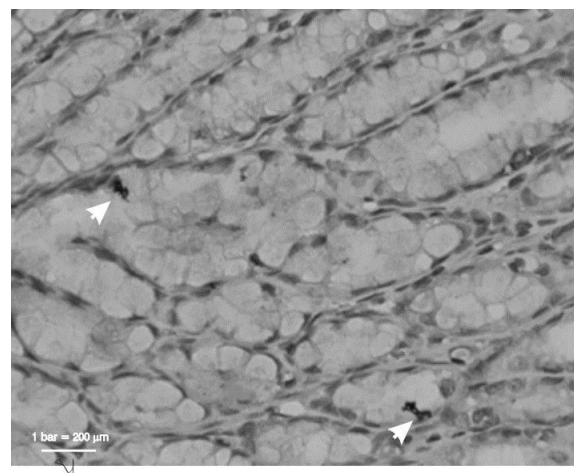
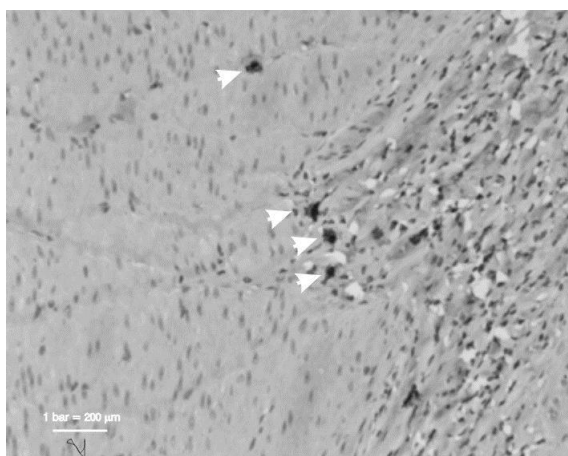
We applied immunohistochemical methods Ghrelin (H- 40) SC- 50 297 Company Santa Cruz, USA. Results visualized detection system Dako - En Vision FLEX - Mini Kit.

RESULTS

Grelipositive cells were visualized in the depth of the mucosa of the fundus of the stomach, fundus ventriculi, close to the border with lamina propria of the mucosa. Individual cells containing granules with ghrelin are found in proximity to the muscle layer. The intensity of the color is different in different cells. Grelipositive cells in the stomach mucosa are found in all investigated cases (**Figures 1-3**, magnification x200; **Figures 2-4**, magnification x400).



Figures 1-2. Grelipositive cells in the depth of the stomach mucosa.



Figures 3-4. Grelipositive cells in the depth of the stomach mucosa.

DISCUSSION

Ghrelin is a peptide mainly secreted by gastric mucosa and has been implicated in the regulation of eating behavior and weight balance (Maksud et al, 2011). Ghrelin is produced basic in distinct endocrine cells located within the gastric oxyntic mucosa. Our results are in accord of many publications (Broglia et al, 2001; Toshinai et al, 2001). Ghrelinpositive cells was found in deep layers of stomach mucosa. This is in accord with investigations of de la Cour et al (2001), Ariyasu et al (2001) and others. The density of ghrelin-immunoreactive cells in the stomach oxyntic mucosa was manifested.

CONCLUSION

In the digestive system is the availability of grelinpositive cells. The greatest amount of cells containing the ghrelin is detected in the mucosa of the stomach fundus. There are some ghrelin-immunoreactive cells near the muscular layer and rare – between muscle fibres.

REFERENCES

1. Ariyasu H, Takaya K, Tagami T, Ogawa Y, Hosoda K, Akamizu T, Suda M, Koh T, Natsui K, Toyooka S, Shirakami G, Usui T, Shimatsu A, Doi K, Hosoda H, Kojima M, Kangawa K & Nakao K. Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. *Journal of Clinical Endocrinology and Metabolism* 2001, 86: 4753–4758. (doi:10.1210/jc.86.10.4753)
2. Broglia F, Arvat E, Benso A, Gottero C, Muccioli G, Papotti M, van der Lely AJ, Deghenghi R & Ghigo E. Ghrelin, a natural GH secretagogue produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans. *Journal of Clinical Endocrinology and Metabolism* 2001 86 5083–5086.
3. Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE & Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 2001 50 1714–1719.
4. Date Y, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Saganuma T, Matsukura S, Kangawa K & Nakazato M. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology* 2000 141-142.
5. Dornonville de la Cour C, Bjorkqvist M, Sandvik AK, Bakke I, Zhao CM, Chen D & Hakanson R. A-like cells in the rat stomach contain ghrelin and do not operate under gastrin control. *Regulatory Peptides* 2001 99 141–150.
6. Maksud, Fabiana A N, Jairo S Alves, Marco T C Diniz and Alfredo J A Barbosa, Density of ghrelin-producing cells is higher in the gastric mucosa of morbidly obese patients. *European Journal of Endocrinology*, 2011, 165: 57-62.
7. Purnell JQ, Weigle DS, Breen P & Cummings DE. Ghrelin levels correlate with insulin levels, insulin resistance, and high-density lipoprotein cholesterol, but not with gender, menopausal status, or cortisol levels in humans. *Journal of Clinical Endocrinology and Metabolism* 2003 88 5747–5752.
8. Romero, Amparo, Henriette Kirchner, Kristy Heppner, Paul T Pfluger, Matthias H Tscho and Ruben Nogueiras. GOAT: the master switch for the ghrelin system? *European Journal of Endocrinology* (2010) 163: 1–8.
9. Sun Y, Asnicar M & Smith RG. Central and peripheral roles of ghrelin on glucose homeostasis. *Neuroendocrinology*, 2007, 86: 215–228.
10. Toshinai K, Mondal MS, Nakazato M, Date Y, Murakami N, Kojima M, Kangawa K & Matsukura S. Upregulation of ghrelin expression in the stomach upon fasting, insulin-induced hypoglycemia, and leptin administration. *Biochemical and Biophysical Research Communications* 2001 281 1220–1225.
11. van der Lely AJ, Tschop M, Heiman ML & Ghigo E. Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin. *Endocrine Reviews* 2004 25 426–457.
12. Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillon WS, Ghatei MA & Bloom SR. Ghrelin enhances appetite and increases food intake in humans. *Journal of Clinical Endocrinology and Metabolism* 2001 86 5992.