

## Immunohistochemical expression of ghrelin and leptin in newborn rats

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Ghrelin is produced in the stomach, stimulates the release of GH from the anterior pituitary through the GH receptors, thus activating hunger. Using different methods the hormone is identified in the endocrine cells of the stomach and the Langerhans islands, but the phenotype and the morphological characteristics of the ghrelin-producing cells have not yet been discovered. Leptin is a hormone produced by the adipocytes. It plays a role in body weight regulation by signaling to the brain to reduce hunger and burn more calories. The complex interactions of ghrelin and leptin in appetite regulation are not yet elucidated. The aim of the present work was to study the morpho-functional link of ghrelin and leptin by finding out immunohistochemical expression presence and localization of both hormones in rats. Immunohistochemical reactions for leptin and ghrelin by the ABC method with primary polyclonal antibodies (Santa Cruz, USA) were fulfilled on paraffin sections of fragments of subcutaneous adipose tissue and stomach of newborn Wistar rats. Our study demonstrated positive expression of leptin in the adipose cells of the subcutis but not in the stomach mucosa cells. Immunohistochemical reaction for ghrelin was detected in some of the cells of the stomach glands in the newborn rats. Adipocytes did not express ghrelin. Our results, revealing the localization and activity of leptin and ghrelin in the investigated tissue, suggest their close relations and interaction in the regulation appetite and metabolism from birth.

*Key words:* ghrelin, leptin, gastrointestinal tract, rat

### Introduction

The morphological structure and functions of the gastrointestinal tract are yet to be completely studied. This is demonstrated by the discovery of a new hormone, produced by the gastrointestinal cells, called ghrelin. Ghrelin is an oligopeptide, composed of 28 aminoacids, isolated for the first time from a rat stomach [1]. Its secretion increases when the stomach is empty. Diet rich in lipids decreases ghrelin's plasma level while a low protein diet increases it [2]. Through specific receptors ghrelin binds with diencephalon nuclei and stimulates the feeling of hunger [3,4]. It is known that ghrelin is a releasing factor for growth hormone, acts as a ligand for the secretory receptors of growth hormone (GH-R) in adenohypophysis. As a releasing factor for the growth hormone it takes part in the regulation of metabolism. Growth hormone itself stimulates the growth of bones and soft tissues, acts on protein, lipid and carbohydrates metabolism. Ghrelin was identified by the methods of immunochemistry and RT-PCR in the endocrine cells of ventricular mu-

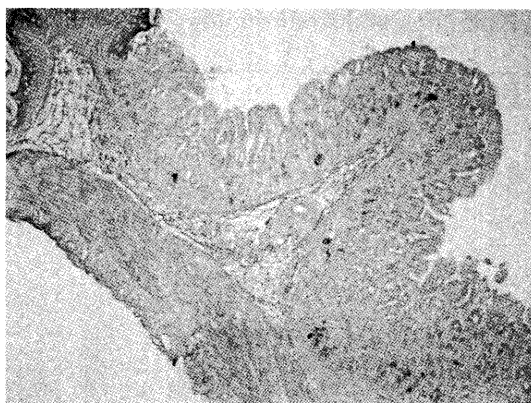


Fig. 1. Stomach of an one-day-old newborn rat. The mucosa of the esophagus passes in the mucosa of the stomach. We can observe pars cardiaca and part of the fundus of the stomach. All tissue layers of the gastric wall are formed. (x 10).

the stomach and the subcutaneous tissue were investigated by the ABC method for 12 hours using primary antibodies for: 1. ghrelin (rabbit polyclonal antibody Ghrelin – Santa Cruz Biotechnology, USA) dilution 1:100 at 4°C and 2. primary polyclonal rabbit antibody for leptin (Santa Cruz Biotechnology, USA) dilution 1:200 at 4°C. DAB was used as a chromogen. Leptin was expressed by brown granular substance and ghrelin was expressed by black granular substance in the cytoplasm of the cells. Negative controls, with buffer (PBS) or normal non-immune serum used instead of the specific antibodies, were used to verify the specificity of the immunohistochemical reactions to any examined antigen. In those samples no product from the corresponding reaction was present.



Fig. 2. Stomach of an one-day newborn rat – pars cardiaca. Single ghrelin-positive cell in the bottom of the cardia's gland. We observe an immunohistochemical reaction of moderate intensity. The cytoplasm in the enlarged basal part of the cell is filled with fine black granules Paraffin preparation (x 20).

cosae and Langerhans islets in human [5]. But the type of the endocrine cells that secrete it has not yet been identified. Leptin is a hormone produced by the adipocytes. It plays a role in body weight regulation by signaling to the brain to reduce hunger and burn more calories. The aim of the present work was to study the morpho-functional link of ghrelin and leptin by finding out immunohistochemical expression presence and localization of both hormones in rats.

## Material and methods

The study is carried out on fragments of subcutaneous adipose tissue and stomach of 10 one-day-old newborn Wistar rats. Paraffin sections of

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## Results

From the sectors under study of the digestive tract – esophagus, stomach and intestine, expression of ghrelin was received only in fragments from the stomach. The morphological characteristic of the gastric wall shows the typical picture of a tubular organ. All tissue layers of mucosa are well formed. The covering epithelium is a single-breasted cylindrical secretorius. In the home plate the glands are tightly arranged. Two or three rows of smooth muscle cells highlight the underlying loose connective tissue of the submucosa. In the thick muscular sheath are distinguished smooth muscle layers with circular, longitudinal

and oblique direction (Fig. 1). In the initial part of the stomach – pars cardiaca is established expression in single cells located on the bottom of the cardiacus glands. Immunohistochemical reaction was of moderate intensity. The black granules fill the entire cytoplasm of the endocrine cell (Fig. 2). In the fundus of the stomach is established a positive reaction in many cells from the bottom of the main glands. The expression of ghrelin in them is from weak to moderate (Fig. 3). Ghrelin positive cells are present in the body of the stomach. Here we observe a large number of endocrine cells with moderate intensity, as well as single cells with high intensity of immunohistochemical reaction. In these black granules tightly fill the entire cytoplasm of the cell (Fig. 4). Our immunohistochemical study demonstrated positive expression of leptin in the adipose cells of the subcutis. It is detected as a brown staining in the cytoplasm of the adipocytes which were typical mature unilocular cells – with one lipid drop occupying the whole cell and cytoplasm and nucleus pushed to the cell periphery. Subcutaneous adipocytes are not demonstrate positive immunohistochemical staining for ghrelin (Fig. 5.). Our study discovered positive expression of leptin in the subcutaneous adipose cells but none in the stomach mucosa. Immunohistochemical reaction of ghrelin was established in some cells of the stomach glands in newborn rats. Adipocytes did not express ghrelin.

## Discussion

Many authors do not find expression of ghrelin in stomach cells of newborn rat fetuses despite the presence of high plasma levels of ghrelin in the fetus. Nakahara et al. explain this fact with high permeability of the hemato-placental barrier for ghrelin. The hormone comes from the mother. It is established that the ghrelin which comes from the mother is one of the important factors that regulate the weight of the fetus during the late stages of pregnancy [6]. Hayashida T. et al. discover that the level of ghrelin in the postnatal period of newborn rats increases. Peak levels are reached during the second and third week after birth [7]. Our results also demon-

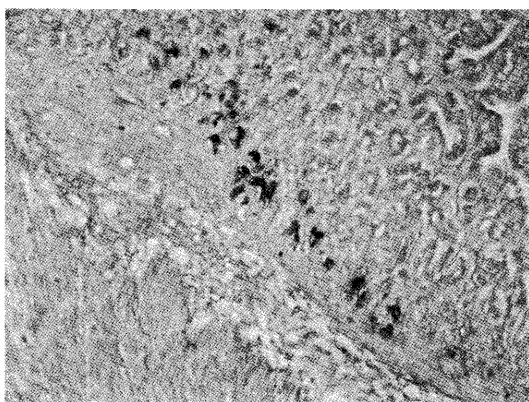


Fig. 3. Stomach of an one-day newborn rat – fundus. A large number of ghrelin-positive cells in the bottom of the main gland – gll. gastricae propriae. Immunohistochemical reaction with mild to moderate intensity. Paraffin preparation. (x 20).

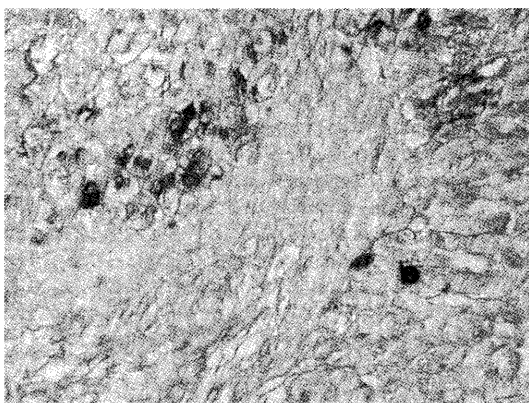


Fig. 4. Stomach of an one-day newborn rat – corpus. An area with a large number of endocrine cells with weak to moderate intensity of immunohistochemical reaction. Single ghrelin-positive cells with high intensity – the black granules tightly fulfil throughout the cytoplasm of the cell. Paraffin preparation. (x 40).

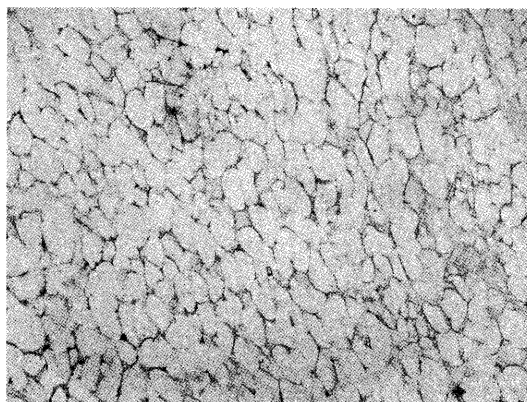


Fig. 5. Biopsy specimens from the subcutis. Leptin-positive adipose cells. Brown staining in the cytoplasm of the mature unilocular adipocytes. Paraffin preparation. (x20).

strate positive immunohistochemical expression of ghrelin in the stomach of one day old rats. K. Choi et al establish adipogenic effect of ghrelin on the white adipose tissue in vitro [8]. Other authors describe ghrelin as an inhibitor to some types of lipolysis [9]. W. Zhang et al. published opposite results on the activity of ghrelin on preadipocytic differentiation and proliferation. Study on cell lines with super expression of ghrelin revealed an inhibitory effect on adipogenesis [10].

## Conclusion

Differentiated ghrelin producing cells are present in the wall of gastrointestinal tract during the early neonatal period. Positive immunohistochemical activity of leptin is established in adipocytes from the subcutaneous fat tissue of experimental animals. Further studies on the receptors for both of those hormones and the cells that express them would shed some light on the debatable question about the lack or presence of direct effect of ghrelin on adipocytes and its direction – stimulating or inhibitory.

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