

GASTRIN (G) AND ENTEROCHROMAFFIN - LIKE (ECL) CELLS IN THE STOMACH OF PORTOCAVAL-SHUNTED RATS – RADIOIMMUNOLOGICAL, IMMUNOCYTOCHEMICAL AND ULTRASTRUCTURAL STUDY

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The aim of the study was to monitor the changes observed in the concentration of gastrin, as well as the morphological and ultrastructural properties of pyloric G and fundic ECL cells in rats with a portocaval shunt (PCS).

Eight weeks after surgery, plasma and pyloric tissue gastrin concentrations were determined by radioimmunoassay. Sections from pyloric mucosa were immunostained for the identification of G cells, while oxyntic mucosa specimens were processed for Sevier-Munger detection of ECL cells. In addition, ultrastructural properties of G and ECL cells were determined by standard transmission electron microscopy. The results showed that the plasma gastrin levels were unchanged, while the pyloric gastrin concentrations were significantly increased in rats with PCS compared to the control animals ($p < 0.01$). The morphometric analysis showed a significant increase in the cell density of antral G cells ($p < 0.05$) and fundic ECL cells ($p < 0.01$), as well as an increased number of these cells per mm^2 of mucosa ($p < 0.01$). In addition, the ECL cell profile area was increased in rats with PCS. The predominance of very large vesicles and the reduction of normal cytoplasmic granules and vesicles was a prominent subcellular feature of the ECL. G-cells in PCS rats showed the presence of secretory granules of all types (electron-dense, electron-lucent and electron-pale). The number of electron dense granules was elevated. These ultrastructural patterns are compatible with the functional activation of G and ECL cells.

The present study suggests that PCS results in hyperplasia of G cells, as well as in both hyperplasia and hypertrophy of ECL cells. It is highly probable that the PCS enhances the sensitivity of the ECL cells to gastrin, thereby enabling them to respond to gastrin in an exaggerated manner.

Key words: portacaval shunt, rat, gastrin, enterochromaffin-like cells.

INTRODUCTION

The gastrin (G) cell is a classic gut endocrine cell organized with microvilli on the luminal surface that allow the G cell to detect the presence of food within the stomach. Gastrin released from the G cells into the circulation has three major effects which are mediated by gastrin/CCKb receptors. First, gastrin can stimulate the release of acid directly from the parietal cell. Second, gastrin can also stimulate acid secretion by enhancing the release of histamine from enterochromaffin-like (ECL) cells. Third, gastrin stimulates the release of somatostatin from gastric D cells (Reubi *et al.*, 1992; Sachs *et al.*, 1997; Sawada and Dickinson, 1997; Lindstrom *et al.*, 2001). As an endocrine hormone, gastrin is released via exocytosis from vesicles into the blood, and while previously it was believed to interact with receptors on the parietal cells thus resulting in the release of hydrochloric acid, it has now been found to function by stimulating the synthesis and release of histamine. Gastrin affects this regulation by binding to CCK2 receptors on ECL cells in the oxyntic mucosa, which causes the release of histamine (Lindstrom *et al.*, 2001). Gastrin not only acts on the stimulation, but also on the growth and maintenance of the cells of the gastric mucosa and on the proliferation of gastric stem and ECL cells (Koh and Chen, 2000).

The histamine-producing enterochromaffin-like (ECL) cells are the predominant enteroendocrine cell type in the acid-producing part of the rat stomach (Hakanson *et al.*, 1986; Prinz *et al.*, 2003). The antral hormone gastrin and a new pituitary messenger, adenylyl cyclase-activating peptide (PACAP), potently stimulate histamine synthesis, storage, and secretion by ECL cells by activation of the histamine synthesizing enzyme, histidine decarboxylase (HDC) and by cellular proliferation (Sachs *et al.*, 1997; Prinz *et al.*, 2003). Gastrin stimulates the transcriptional activity of histidine decarboxylase (Prinz *et al.*, 2003; Zhao *et al.*, 2003). This hormone binds to G-coupled gastrin/cholecystokinin B receptors resulting in a production of inositol phosphates (IP) in ECL cells. These findings suggest that in the neuroendocrine cell type IP₃ and elevated concentrations of Ca²⁺ play a key role in histamine secretion (Zanner *et al.*, 2002). Other stimulatory receptors such as muscarinic, adrenergic, peptidergic, and cytokine receptors have been found, as well as inhibitory receptors such as somatostatin and galanin (Sachs *et al.*, 1997; Ericsson *et al.*, 2003; Prinz *et al.*, 2003; Zhao *et al.*, 2003).

Long term stimulation of ECL cells by gastrin induced a significant proliferation of ECL cells. Such hypertrophy of ECL cells is particularly evident in patients with gastrin-secreting tumors (Reubi *et al.*, 1992).

Previous studies have shown that both PCS and long term hypergastrinaemia (induced by gastrin infusion or treatment with antisecretagogues) give rise to ECL cell hyperplasia and changes in the ultrastructure of the ECL cells that are compatible with functional activation (Larson *et al.*, 1988; Böttcher *et al.*, 1989). Moreover, in rats PCS results in an increased number of the ECL cells, despite the fact that serum gastrin concentration is not elevated by PCS (Chen *et al.*, 1993).

The present study describes morphological and ultrastructural properties of pyloric G and fundic ECL cells of Wistar rats with PCS, eight weeks after surgery. This study was undertaken in order to shed further light on how the ECL and G cells respond to metabolic and endocrine abnormalities in PCS.

MATERIAL AND METHODS

Two-month-old male Wistar rats, weighing approximately 230 g were kept in separate cages at room temperature in a 12-h dark, 12-h light regimen. They had free access to a conventional chow diet and tap water. The study was approved by the Institutional Ethics Committee for Animal Experiments. The animals were divided into two groups: control group (C rats; n=11) and rats with surgical portacaval shunt (PCS rats; n=27). On day one of the experiment the rats underwent end-to-side portacaval anastomosis according to Lee and Fisher (1961) as modified by Bismuth *et al.* (1963). The surgery was performed under ether anesthesia. All the animals were sacrificed by cervical dislocation eight weeks after surgery. Twenty-four hours before sacrifice the feed was withdrawn, but free access to tap water was not denied.

Plasma preparation. Prior to cervical dislocation, blood samples were collected by intracardial puncture. Full blood samples for estimation of fasting serum gastrin levels were placed in ice/chilled tubes (5 ml) containing EDTA (2mg) and proteinase inhibitor (Trasyol, 2,500 KIU). Plasma was extracted using standard procedure and stored below -70°C for subsequent analysis.

Tissue extract preparation. Each antral specimen was washed with saline solution, measured and placed in to the tube with 1 ml of distilled water. Gastrin extraction was performed in a water bath on 95 °C for 10 minutes, the supernatant was collected and after cooling stored below -70 °C for further analysis.

Radioimmunoassay (RIA). Plasma and tissue gastrin were determined under basal conditions using RIA protocol provided by *Affiniti* (UK) with rabbit anti-human gastrin 17 antiserum. Final dilutions were for serum 1:100,000 and for the tissue 1:500,000. Intra-assay and inter-assay coefficients were 9,0 and 8,4 respectively. Sensitivity of the method was 1 pmol/l.

Immunohistochemical / histochemical study. Sections from pyloric mucosa were stained for identification of G cells, using a conventional streptavidin-biotin immunohistochemical technique (LSAB+/ HRP kit, DAKO, Denmark) (Miller, 2002). For the detection the histamine-producing enterochromaffin-like (ECL) cells, the oxyntic mucosa sections were processed for Sevier-Munger histochemical method (Bancroft, 2002).

Routine electron microscopy. Small specimens of antrum and fundus were immediately placed in a mixture of 2% glutaraldehyde in 0.2M sodium cacodylate buffer, pH 7.4, and fixed in the same fixative for 20h at 4°C. After postfixation for 1h in 1% osmium tetroxide in cacodylate buffer, the specimens were dehydrated in graded ethanol and embedded in Epon 812^R. The blocks were sectioned with an LKB ultratome II. Ultrathin sections were double-stained with uranyl acetate and lead citrate before examination in an Opton 109 electron microscope. G and ECL

cells were identified as described previously (Varagić *et al.*, 1996; Sach *et al.*, 1997; Sawada and Dickinson 1997; Koko *et al.*, 1998).

Morphometric analysis of G and ECL cells by light microscope. Three sections at intervals of 50 μm were analyzed for both G and ECL cell numbers and volume density. Weibel multipurpose test system containing 42 points and 21 lines was used to evaluate these stereological parameters (Varagić *et al.*, 1996; Koko *et al.*, 1998).

Morphometric analysis of G and ECL cells detected by electron microscope. Morphometric analysis was performed using the methods described previously (Varagić *et al.*, 1996; Koko *et al.*, 1998). The cell and nuclear profile areas were estimated by drawing the appropriate region of G-cells with the help of Camera Lucida attached to a Reichert microscope and analyzed with an image analyzing system (MOP 3 Videoplan; Carl Zeiss).

Statistical analysis. All values are expressed as mean \pm SD. Statistical analysis of data was made using the Mann-Whitney nonparametric test and $p < 0.05$ was accepted as significant.

RESULTS AND DISCUSSION

The gastrin plasma levels were unchanged (Figure 1), while the concentration of pyloric gastrin was significantly increased in rats with PCS compared to the control group ($p < 0.01$) (Figure 2).

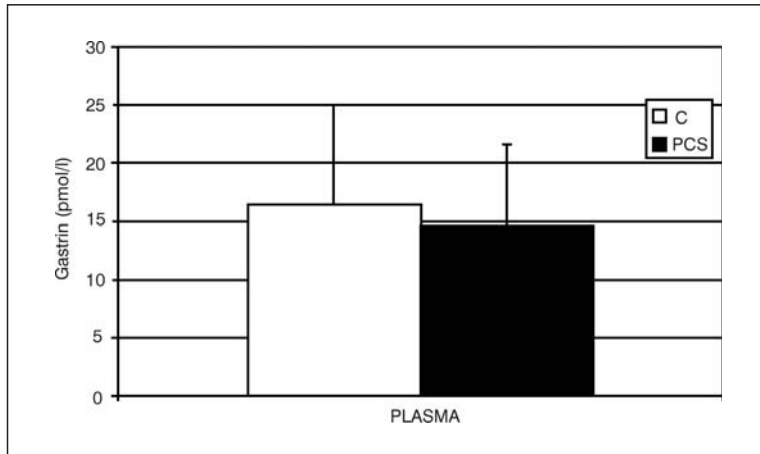


Figure 1. Basal plasma levels of gastrin in C and PCS rats

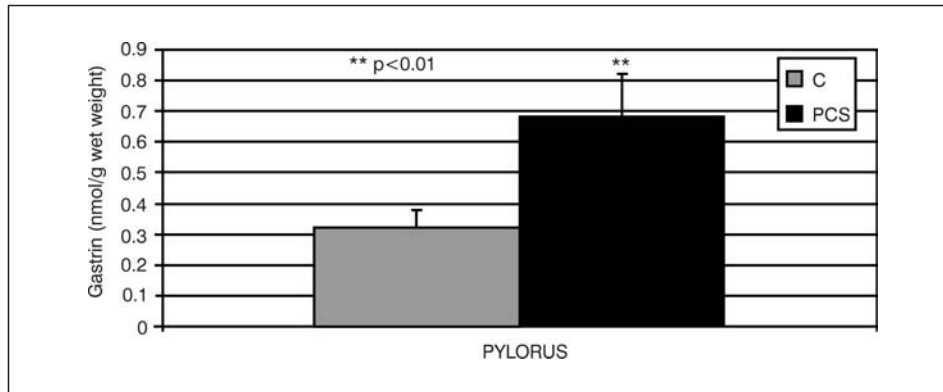


Figure 2. The concentrations of gastrin in tissue extracts of pylorus in C and PCS rats

The results of the morphometric analysis of G-immunoreactive cells in the pylorus and ECL-immunoreactive cells in the fundus of the stomach in control and PCS rats are presented in Table 1. The morphometric analysis carried out on stained pyloric and fundic stomach mucosa of PCS rats, showed a significant increase in cell density of G ($p < 0.05$) and ECL ($p < 0.01$) cells, as well as an increase in the number of these cells on mm^2 of mucosa ($p < 0.01$). In addition, the ECL cell profile area was increased in rats with PCS. Also, PCS induced changes of both ECL and G cells at the ultrastructural level. The predominance of very large vesicles (vacuoles) and a reduction of normal cytoplasmic granules and vesicles was a prominent subcellular feature of ECL. G cells in PCS rats showed the presence of secretory granules of all types (electron-dense, electron-lucent and electron-pale), but the number of electron dense granules was elevated (Figure 3a,b). Taken together, these ultrastructural patterns are consistent with the functional activation of G and ECL cells.

Table 1. Morphometric analysis of pyloric G-cells, and of fundic ECL-cells in C and PCS rats

G cells	C (n=10)	PCS (n=25)	P
Number / mm^2 mucosa	77.9+26.1	121.8+44.9	<0.05
Volume density (%)	2.2+0.07	4.0+0.03	<0.01
Cell profile area (mm^2)	67.2+9.6	63.9+7.0	NS
ECL-cells	C (n=10)	PCS (n=25)	P
Number / mm^2 mucosa	637.9+67	931.8+101	<0.01
Volume density (%)	19.2+1.6	24.0+0.9	<0.05
Cell profile area (mm^2)	73.2+6.0	123.9+10	<0.01

NS – non significant

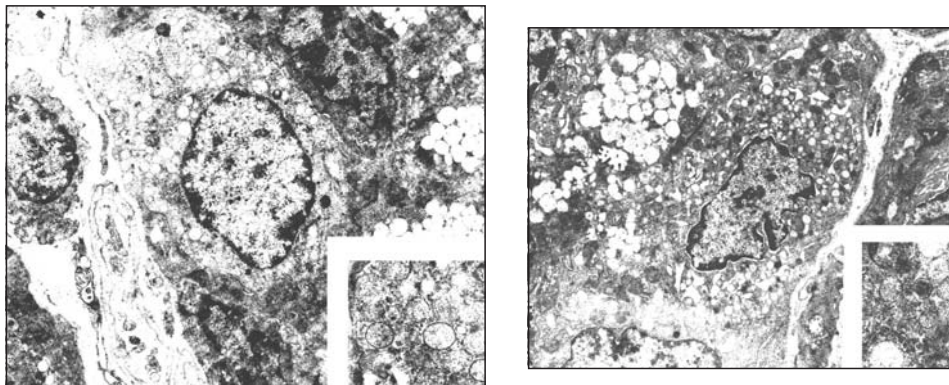


Figure 3a,b. Electron micrographs showing G cells of control (a) and portacaval-shunted (b) rats. Note that G cells of control rat containing predominantly electron-pale granules (insert), whereas G cells of rat with PCS showed the presence of increased number of electron-dense granules (insert)

Earlier studies due to the trophic effects of PCS on the rat stomach the ECL cells in the oxyntic mucosa increase in number (Axelson *et al.*, 1990; Chen *et al.*, 1993). In addition, Axelson and collaborators have shown that in rats, PCS results in an increased number and volume density of ECL cells despite the fact the plasma gastrin concentration was not elevated (Axelson *et al.*, 1990). PCS in the rat is known to enhance the gastrin-induced trophic stimulation of the ECL cells of the rat stomach (Ekelund *et al.*, 1985; Axelson *et al.*, 1990). It has been suggested that PCS increases the circulating levels of intestinal agents which enhance the trophic effect of gastrin on the ECL cells. Many factors originating from the gastroentero-pancreatic region accumulate in the circulation following portacaval shunt (Ekelund *et al.*, 1985, Hakanson *et al.*, 1986). The PCS evoked enhancement of the trophic effects of cholecystokinin (CCK)-A receptor activation on the pancreas and of CCK-B receptor activation in the ECL cells in the oxyntic mucosa. This is associated with enhanced expression of CCK-A receptor mRNA in the pancreas and of CCK-B receptor mRNA in the oxyntic mucosa (Nylander *et al.*, 1997).

In our study, the number of G and ECL cells was increased after shunting. The combination of PCS and elevated gastrin mucosal content in the pyloric mucosa produced a greatly enhanced ECL hypertrophy, with a marked elevation of cytoplasmic vacuoles, as described previously (Radosavljević *et al.*, 2001). The increase of ECL cells in the oxyntic mucosa of PCS rats can be explained by impaired degradation of intestinal substances in the liver exerting a highly specific trophic effect on the ECL cells or causing expression of gastrin receptors on these cells. We have suggested that PCS enhances the sensitivity of the ECL cells to gastrin, thereby enabling them to respond in an exaggerated manner.

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GASTRIN I ENTEROHROMAFIN - SLIČNE ĆELIJE U ŽELUCU PACOVA SA PORTO-KAVNIM ŠANTOM – RADIOIMUNOLOŠKA, IMUNOCITOHEMIJSKA I ULTRASTRUKTURNA STUDIJA

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SADRŽAJ

Kod pacova sa porto-kavnim šantom (PCS) kao modelom hronične insuficijencije jetre, ispitivane su promene u brojnosti i ultrastrukturnim karakteristikama antralnih G- i fundusnih ECL ćelija. Određivana je i bazalna koncentracija gastrina u plazmi i tkivnom ekstraktu sluzokože antruma RIA metodom. Za detekciju G ćelija antruma želuca korišćena je imunohistohemijska metoda, dok je za identifikaciju ECL ćelija fundusa, korišćena histohemijska metoda po Sevier-Munger-u.

Koncentracija gastrina u plazmi bila je nepromenjena, a u tkivnom ekstraktu pilorusa povećana ($p < 0,01$) u pacova s PKŠ. Takođe, kod ovih životinja došlo je do značajne proliferacije gastrin-produkujućih ćelija u antralnom delu ($p < 0,05$) i ECL ćelija u fundusnom delu želuca ($p < 0,01$), kao i do hipertrofije ECL ćelija ($p < 0,01$). Ultrastrukturne promene ECL ćelija, u smislu evidentnog povećanja broja vakuola na račun klasičnih granula i vezikula, kao i značajno povećanje broja elektron-gustih granula u G ćelijama, govore u prilog njihove funkcionalne aktivacije.

Objašnjenje za povećanje broja G ćelija u pacova sa PCS ostaje otvoreno, ali se hiperplazija i hipertrofija ECL ćelija kod ovih životinja može objasniti smanjenom degradacijom intestinalnih supstanci u jetri koje ispoljavaju trofični efekat na ECL ćelije ili povećanjem osetljivosti receptora za gastrin na ovim ćelijama.