Kragujevac J. Sci. 45 (2023)195-206. UDC 576.382:612.323:611.018.73:577.175.73
doi: 10.5937/KgJSci2345195G *Original scientific paper*

GASTRIN-PRODUCING G CELLS OF GASTRIC MUCOSA IN DEXAMETHASONE-TREATED RATS

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(Received March 29, 2023; Accepted May 08, 2023)

ABSTRACT. The systemic administration of glucocorticoids has not only strong beneficial anti-inflammatory and immunosuppressive effects but also numerous adverse health effects. Therefore, this study aimed to investigate the stereological and ultrastructural characteristics and distribution of gastrin-producing G cells in rat antral mucosa after 12 days of intraperitoneal administration of 2 mg/kg dexamethasone (DEX). In both groups of rats (DEX and control), the G cells were located at the base of the antral mucosal crypts, but in the DEX-treated rats, they were also found near the gastric lumen and showed an altered morphology compared to the basal cells. There were significant differences in the number of G cells per unit area and unit volume of antral mucosa between the groups studied. In the DEX-treated rats, the modified morphology and position of the antral G cells within the gastric units indicate their modified activity and possible impaired regulation of gastric acid production.

Keywords: dexamethasone, gastrin, antrum, rats.

INTRODUCTION

Enteroendocrine cells (EECs) belong to the diffuse endocrine system found in the epithelium of the gastrointestinal mucosa. EECs synthesize and secrete signalling molecules and hormones involved in the endocrine and paracrine regulation of digestive functions and metabolism of nutrients ingested with food. The different types of these EECs, either diffusely distributed or more or less restricted to a specific part of the digestive tract, are traditionally named after their main secretory product. EECs are classified as "open" and "closed" cells, the former extending their projections toward the gastrointestinal lumen to detect changes in its content, whereas the latter do not physically communicate with the lumen. Therefore, the secretory activity of "open" EECs is primarily regulated by changes in the lumen content, whereas the activity of "closed" EECs depends on neural and humoral control pathways (LATORRE *et al*., 2016).

The stomach (gaster) is a dilated part of the alimentary canal located between the oesophagus and duodenum. It serves to convert food into chyme which passes into the small intestine for further digestion and absorption. In rats, the stomach is anatomically composed of the forestomach, which is lined by non-glandular mucosa and serves for the temporary storage of partially digested food, and the cardia, fundus and antrum, which contain glandular mucosa (GÄRTNER, 2002; VDOVIAKOVÁ *et al*., 2016). In different anatomical parts of the stomach, mucosal glands differ slightly in their cellular composition, but EECs are always present, albeit in small numbers. Gastric EECs include enterochromaffin cells (EC), enterochromaffin-like cells (ECL), D, G, P and A cells, and a small number of L cells (WORTHINGTON *et al*., 2018; HUNNE *et al*., 2019).

The cells of the gastric mucosa, both the surface mucous cells and the glandular cells, including EECs, are derived from gastric stem cells. Although currently debated, it appears that two distinct populations of gastric stem cells exist in the gastric glands, one in the isthmic region and the other at the base of the gland (BARTFELD and KOO, 2017). The lifespan of EECs is 45-60 days, which is significantly longer than that of most other cells in the gastric mucosa, suggesting that the life cycle of EECs is independent of the life cycle of neighbouring epithelial cells (THOMPSON *et al*., 1990; FRICK *et al*., 2017).

Gastrin-producing G cells are predominantly located in the antrum of the stomach and, in much smaller numbers, in the duodenum and pancreas (THOMAS *et al*., 2003). In rats, the G cells are usually "open", flask-shaped cells with narrow apical extension-bearing microvilli that project into the lumen of the gland (OOMORI *et al*., 1993). After food ingestion, the altered chemical composition of the lumen content, together with gastrin-releasing peptide and vasoactive peptide released after vagal stimulation from the enteric neurons innervating G cells, triggers the secretion of gastrin (FELDMAN *et al*., 1978; BERTHOUD, 1996). Gastrin then stimulates gastric acid secretion both directly by stimulating the parietal cells and indirectly by positively regulating the histamine secreted by the ECL cells, which moreover itself acts as an acid secretagogue (OHNING *et al*., 1996; FRIIS-HANSEN *et al*., 1998). On the other hand, somatostatin released from D cells acts as a paracrine inhibitor of gastrin secretion (SCHUBERT and REHFELD, 2020). In addition, gastrin has recently been shown to control the normal division of gastric stem cells in a paracrine manner (CHANG *et al*., 2020).

Corticosteroids are synthetic derivatives of natural steroid hormones produced by the adrenal cortex (WILLIAMS, 2018). Because of their potent anti-inflammatory and immunosuppressive effects, they are commonly used in the treatment of allergies, autoimmune and inflammatory diseases (CZOCK *et al*., 2005). Like most other medications, corticosteroids have some adverse effects, including those on the digestive system (LIU *et al*., 2013). For example, the association between gastric ulcers and therapy with the potent synthetic glucocorticoid, dexamethasone is particularly well-established (BANDYOPADHYAY *et al*., 1999).

Considering the deleterious effect of dexamethasone on gastric mucosa regarding the role of gastrin in regulating gastric acidity, we hypothesize that the acidification of gastric content might be due to the direct stimulation of G cells by dexamethasone to produce gastrin. Therefore, this study aimed to investigate the effects of prolonged treatment with dexamethasone on the stereological and ultrastructural characteristics and distribution of G cells in rat antral mucosa.

MATERIALS AND METHODS

Animals and tissue preparation for light microscopy

Twenty male Wistar rats (from the vivarium of the Vinča Institute of Nuclear Sciences, Belgrade, Serbia) aged 30 days, and weighing approximately 128 g, were individually caged under standard laboratory conditions (21±1°C, 12 h/12 h light/dark cycle), fed a stan-

dard diet and water, both *ad libitum*. After a five-day acclimation period, they were randomly allocated into two groups of 10 animals each. The rats received 2 mg/kg dexamethasone (DEX group) or saline (C group) intraperitoneally daily for 12 days. At the end of the experiment, the animals were fasted overnight, weighed and sacrificed under ether anesthesia. The experiment was performed according to the rules for animal care proposed by the Serbian Association for Laboratory Animal Science. Tissue samples of the antrum were fixed in Bouin's solution (8 hours), dehydrated and embedded in paraffin.

Immunohistochemistry

For detection of G cells, sections were immunostained with the labelled streptavidin-biotin technique (LSAB+/HRP kit, Dako Carpinteria, CA), using monoclonal antiserum against gastrin 17/CCK 8 (diluted 1:500, incubated overnight at 4°C; by courtesy of Dr. J. Rehfield, Univ. Aarhus, Denmark). Immunoreactivity was visualized with DAB/H_2O_2 in the dark, and the result of the immunohistochemical reaction was noticeable in the form of a brown deposit. The cell nuclei were counterstained with Mayer's hematoxylin.

Adipose tissue of the mesentery completely devoid of examined antigen was processed in the same way as gastric tissue and used as a negative control. The additional slide with gastric tissue treated with PBS instead of primary serum served as reagent control.

Electron microscopy

Tissue specimens were fixed in 3% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in ethanol and propylene oxide, and embedded in Epon 812. LKB ultramicrotome (Leica, Wetzlar, Germany) was employed for preparing the ultrathin sections, which were double contrasted with uranyl acetate and lead citrate, and studied under a Philips CM12 electron microscope (Eindhoven, Holland).

Stereological analysis

Stereological analysis was performed on the sections of antral tissue stained with hematoxylin and eosin, which were analyzed using an Olympus light microscope (Olympus DP70, Hamburg, Germany). The Weibel multipurpose test system (42 points/21 lines, WEIBEL, 1979) was used to collect all quantitative data via a point-counting method at the original magnification 20x. The volume density of antral G cells, and their number per mm^2 and per mm^3 of mucosa (numerical density) were determined. All parameters were calculated using standard stereological equations, as previously described (UKROPINA *et al.*, 2012).

Statistics

The statistical analysis of the data was carried out using the SPSS program (IBM SPSS Statistics, Version 20, Inc. 1989-2011, USA). All obtained numerical values were subjected to a paired t-test with a level of significance set at $p \leq 0.05$. Only cells with nuclei seen in the mucosal epithelium were counted.

RESULTS

Histological analysis

G cells were present in the basal part of the glands in both groups of rats, but in the DEX group, these cells were also found in the upper part of the glands, even near the gastric

lumen (Fig. 1). The roundish cells were mainly located basally (Fig. 1b, lower frame), whereas the spindle-shaped cells were mainly found in the upper part and near the gastric lumen (Fig. 1b, upper frame).

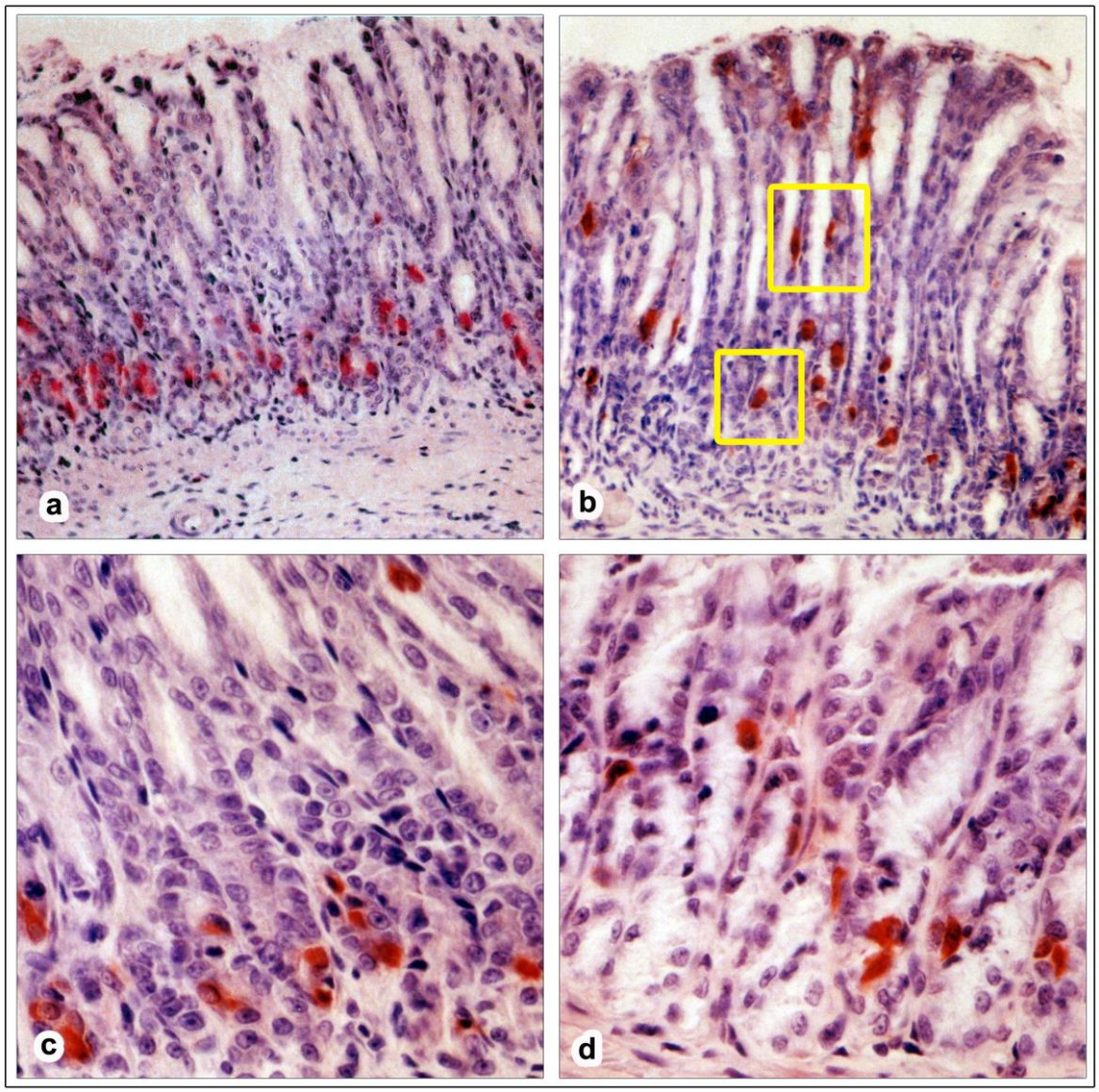

Figure 1. Gastrin-immunopositivity in the antral mucosa of C (a, c) and DEX groups of rats (b, d). In both groups, the immunopositive cells were observed in the basal part of the glands. In the DEX group, they were also found in the upper part of the glands as well as in the base of the gastric pits, very close to the gastric lumen (b); lower frame - roundish cells, upper frame - spindle-shaped cells; original magnification: a, b - 20x; c, d - 40x.

Stereological analysis

The volume density of gastrin-producing cells in the antrum of the DEX-treated rats was increased, but without statistical significance (Fig. 2, Table 1). However, DEX treatment significantly increased the number of gastrin-producing cells per unit area and unit volume of the mucosa (Figs. 3 and 4, Table 1).

Electron microscopy

Ultrastructural examination revealed that the G cells of both groups of rats contained numerous pleomorphic granules, which were arbitrarily divided into four groups: (i) homogeneous granules with moderate electron density, (ii) homogeneous granules with high

electron density, (iii) granules with an eccentrically placed core of high electron density surrounded by a wide electron-lucent halo, and (iv) electron-lucent ("empty") granules. The G cells of the C group contained predominantly electron-lucent granules (Fig. 5a, b). In contrast, in the DEX group, the cytoplasm of G cells was mostly filled with granules of types (i), (ii), and (iii) (Fig. 5c, d).

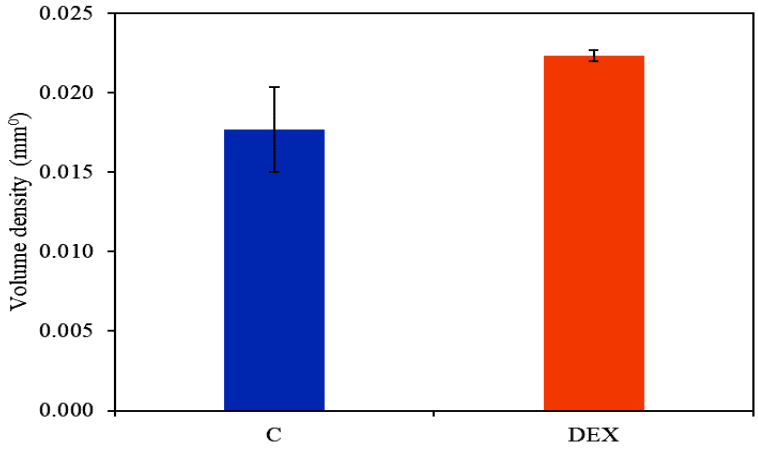

Figure 2. Volume density of G cells in C and DEX groups of rats, with error bars.

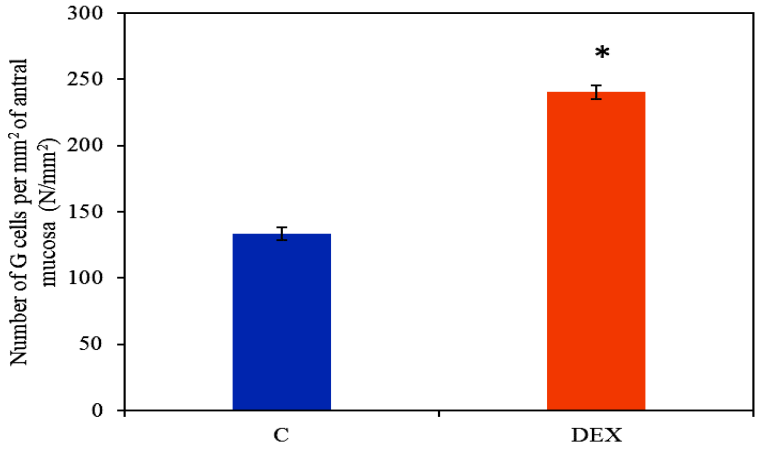

Figure 3. Number of G cells per mm^2 of antral mucosa in C and DEX groups of rats, with error bars.
* Indicate statistically significant increase.

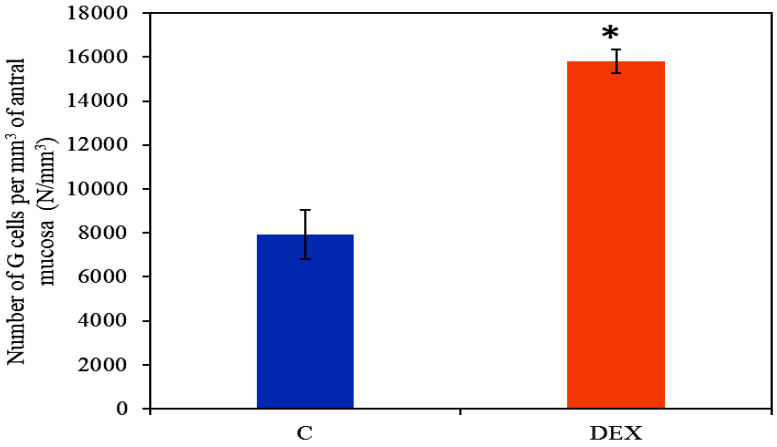

Figure 4. Number of G cells per mm^3 of antral mucosa in C and DEX groups of rats, with error bars.
* Indicate statistically significant increase.

Table 1. Paired t-test of volume density, number of gastrin-producing cells per mm^2 of antral mucosa and number of gastrin-producing cells per mm^3 of antral mucosa, in C and DEX groups of rats.

		t	df	Sig. (2-tailed)
Pair 1	C V_V – DEX V_V	-2.0	2	.184
Pair 2	C N_A – DEX N_A	-23.18	2	.002
Pair 3	C N_V – DEX N_V	-8.12	2	.015

C – control rats; DEX – dexamethasone-treated rats; V_V – Volume density of gastrin-producing cells; N_A – Number of gastrin-producing cells per mm^2 of antral mucosa; N_V – Number of gastrin-producing cells per mm^3 of antral mucosa; significance level of $p \leq 0.05$; df – The degrees of freedom; t – The test statistic for the paired t-test.

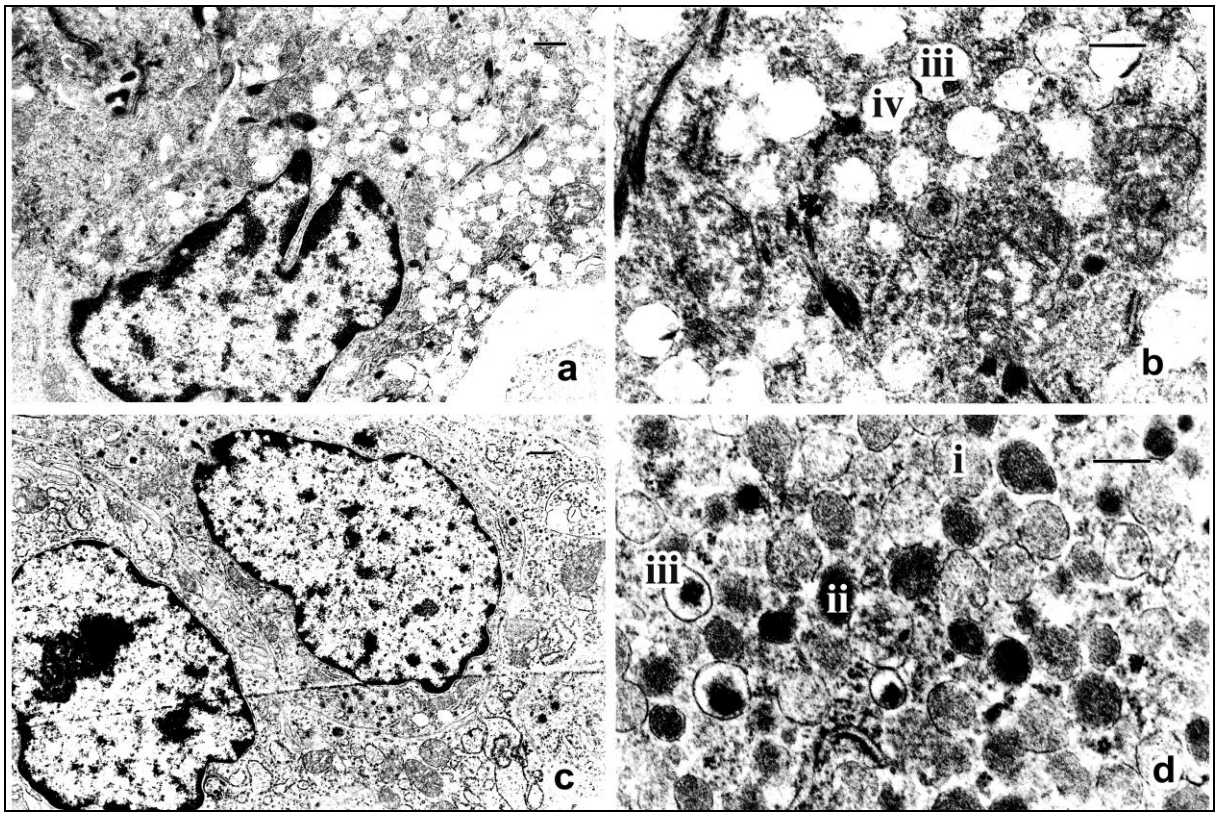

Figure 5. Electron micrographs of gastrin-producing cells and their granules in C (a – scale bar is 0.5 μm, b – scale bar is 1 μm) and DEX groups of rats (c – scale bar is 0.3 μm, d – scale bar is 1 μm); (i) homogeneous granules with moderate electron density, (ii) homogeneous granules with high electron density, (iii) granules with an eccentrically placed core of high electron density surrounded by wide electron-lucent halo, and (iv) electron-lucent ("empty") granules.

DISCUSSION

This study is part of a larger investigation of the morphological and functional properties of the enteroinsular axis in an experimental model of non-insulin dependent diabetes, in which dexamethasone was used as a diabetogenic agent. Despite the significant beneficial effects of dexamethasone in the treatment of numerous serious diseases and conditions, its adverse effects on multiple organs and organ systems, including the digestive tract, have been described (CHEN *et al.*, 2021). Dexamethasone is known to increase gastric acid secretion while inhibiting the inherent protective mechanisms of the gastric mucosa, making it more

susceptible to injury (BANDYOPADHYAY *et al*., 1999; LANG *et al.*, 2007). However, the way in which dexamethasone affects gastric acidity has not been fully elucidated.

The digestion of food that takes place in the stomach is a complex process that is under neuroendocrine control. One element of this control is the pool of G cells, whose secretory product, gastrin, is involved in maintaining the proper gastric acid level necessary for the breakdown of food components. By recognizing the products of protein digestion, G cells stimulate parietal cell activity directly through CCK-2 receptors, and indirectly through the mediation of histamine released by ECL cells (PRINZ *et al*., 1994; ZAVROS *et al*., 1998). Consistent with the negative feedback loop model, decreased pH in the gastric lumen stimulates D cells to secrete somatostatin, which in turn, inhibits the release of gastrin. Given the role of gastrin-producing G cells in controlling gastric acidity, this study focused on the effects of dexamethasone administration on gastric G cells.

In our experiment, although it is known that the administration of dexamethasone can lead to acute gastric erosion (FILEP *et al*., 1992) it was not found. The reason for this may be the different weight of the animals used in our experiment, and the longer period of treatment by the lower concentration of dexamethasone, twice lower than the one that caused gastric mucosal lesions in the previously mentioned study. The administration of corticosteroids disturbs the function of the stomach, changing the activity and mutual interactions of its cell populations. We found that treatment with dexamethasone resulted in a statistically significant increase in the number of G cells per unit area and unit volume of antral mucosa. These results are consistent with those obtained in other animal models after administration of corticosteroids (DELANEY *et al*., 1979; XYNOS *et al*., 1987). Moreover, these results suggest that our experimental model has similarities with the model of genetically diabetic (db/db) mice, a widely accepted animal model of non-insulin dependent diabetes (PINTO *et al*., 1995). Indeed, db/db mice have also been shown to have an increased number of G cells compared with non-diabetic animals.

When interpreting the meaning of increased abundance of a particular cell type, it usually means a concomitant increase in the secretory product of that cell type. Accordingly, we can assume that there was also an increased release of gastrin in our experiment. Although no direct measurement was performed, this assumption was supported by ultrastructural analysis of the granule content of the G cells in the control and experimental groups. It is believed that the electron-lucent granules in the cytoplasm of G cells from the control animals are granules that contained predominantly mature gastrin extracted during the preparatory procedures. In contrast, the pleiomorphic granules in the cytoplasm of the G cells from the DEX group are immature granules, the presence of which indicates continuous release and synthesis of gastrin (MORTENSEN and MORRIS 1977; VARNDELL *et al*., 1983).

To determine the topographic distribution of G cells in the gastric mucosa, we immunohistochemically labeled them with an anti-gastrin antibody. The results showed that G cells were present in the basal part of the gastric glands in both groups. In the dexamethasone-treated rats, G cells were also found in the upper part of the glands and even closer to the gastric lumen, at the base of the gastric pits. Considering that G cells are normally localised basally (CHEW, 2004), this result was unexpected. The same distribution pattern of G cells has been reported in the antral region of the mouse stomach (FRICK *et al*., 2016), but to the best of our knowledge, it has never been reported in the antrum of rats. More specifically, FRICK *et al*. (2016) described two morphologically distinct types of G cells with different distribution patterns: G cells with roundish bodies and short projections found mainly in the basal region of the glands, and G cells with elongated bodies and long projections found mainly in the upper region of the glands. On closer examination of the light microscopic images presented in this paper, we can see that this description is consistent with the morphology of G cells in the antral mucosa of rats treated with dexamethasone.

Given the increase in the number of G cells per unit area and unit volume of antral mucosa, this result raises the question of the origin of these unusually localised G cells – whether they are formed *de novo* or arose by transdifferentiation from surface and/or neck mucous cells. The latter seems less likely since the lifespan of mucous cells is very short, only three to five days (HATTORI and ARIZONO, 1988), so we assume that the G cells from the upper region are formed after stimulation of isthmic stem cells and their differentiation and migration.

The acidic environment of the stomach forces a constant and dynamic exchange of the resident epithelial cells. The renewal and repair of the gastric epithelium rely on two stem cell pools: the first at the base of the gastric glands consists of long-term, self-renewing multipotent stem cells and the second consists of highly proliferative cells in the isthmic part of the gastric glands. Cells from the base of the gland migrate to the isthmic region where they proliferate and form committed progenitor cells that can migrate toward the gastric pit or gland base and differentiate into all types of gastric epithelial cells (BARKER *et al.*, 2010; BJERKNES and CHENG, 2002). Moreover, even fully differentiated epithelial cells can undergo redifferentiation or transdifferentiation, thus maintaining the cellular homeostasis of the tissue (XIAO and ZHOU, 2020). The finding in this study that the volume density of G cells remained unchanged despite the increased number of G cells suggests a likely increase in the mass of other cell types, based on the high renewal potential of gastric stem cells. Our previous research showed that dexamethasone treatment caused an increase in the number of gastric serotonin (EC) cells, as well as a tendency to decrease somatostatin cells (D cells) (GLIŠIĆ *et al*., 2006, 2022). At present, we do not have sufficient data to interpret the possible mechanisms of stem cell activation, so we can only hypothesize about the pathways involved in this process. One mechanism could involve gastrin-mediated paracrine activation of isthmic stem cells, whereas the other could be based on changes in the microenvironment caused by the systemic effects of dexamethasone.

There are also some reports that G cells contain peptides other than gastrin, such as PYY, TRH-like peptides, VIP, proenkephalin gene-derived peptides, xenopsin, ACTH, hCG-α (CHEW, 2004), whose physiologic role in the stomach is unclear. It is known that the action of dexamethasone alters gene expression (TSURUFUJI *et al.*, 1979; MENKE *et al.*, 2012; MURANI *et al.*, 2019), so the unexpected position of G cells near the gastric lumen and their altered morphology may indicate a slight shift in their activity toward synthesis and release of the different secretory product and thus their altered function in a new gastric microenvironment.

The evidence obtained suggests that dexamethasone regulates gastric acid secretion indirectly through its stimulatory effect on gastrin-producing G cells. The unexpected but highly interesting finding of this study is the discovery of an unusual distribution pattern of G cells in the antral mucosa of rats treated with dexamethasone, which, together with the increase in the number of G cells and ultrastructural features corresponding to secretory active cells, indicates impaired negative regulation of G cell activity. However, this study is limited by the lack of information on the levels of gastrin and its negative regulator somatostatin in tissue and plasma and on gastric acid levels. Further work is needed to address these and other unanswered questions and to shed more light on the complex mechanisms regulating digestive function.

In conclusion, this study demonstrated that dexamethasone treatment affected the distribution pattern, stereological parameters, and ultrastructural characteristics of rat antral G cells, suggesting that G cells are potential targets of dexamethasone side effects. In this experimental setting, dexamethasone did not cause any noticeable histopathologic lesions of the gastric mucosa, so the changes described can be considered to be within the adaptability of the tissue.

Acknowledgments

The experimental part of this work was performed at the Institute of Medical Research in Belgrade. The authors would like to thank Prof dr Vesna Koko for her contribution to the design and conduct of the experiments and for critical comments during the preparation of the manuscript. A part of the results was first presented at the 3rd Serbian Congress for Microscopy (September 25-28, Belgrade, 2007, pp. 183–184). The authors would also like to thank Ms. Leposava Jovanović for her excellent technical assistance and Ms. Vesna Glišić, holder of the Diploma in Professional Writing (Professional Editing and Proofreading) from Cengage Education, formerly Australian College of Journalism, for her linguistic editing of the text. This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, Agreement No. 451-03-47/2023-01/ 200122.

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