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AN IMMUNOHISTOCHEMICAL STUDY OF ENDOCRINE CELLS IN THE STOMACH OF HYPERTENSIVE RATS

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Essential hypertension is a complex disease with both genetic and environmental determinants. The effect of spontaneous hypertension on the distribution and occurrence of somatostatin-, gastrin- and serotonin-immunoreactive cells in the fundus and pylorus of the rat stomach was examined by immunohistochemistry. The animals were killed by decapitation at 4 and 16 weeks of age (5 control rats and 5 hypertensive rats). Endocrine cells generally increase in number in hypertensive rats as compared to control rats. However, the detailed responses of endocrine cells to hypertension depend on the cell type, region of gastric mucosa and age of animals. The present results suggest that hypertension has an influence on the intrinsic regulatory system by endocrine cells control in the rat stomach.

Key words: hypertension, endocrine cells, somatostatin, gastrin, serotonin, rat

INTRODUCTION

Arterial hypertension still attract the attention of researchers and clinicians, who co-operate to increase the number of effectively treated patients, to reduce costs of therapy and, above all, to improve prevention and progress or even induce regression of organ complications due to hypertension, being the major cause of death among its sufferers (1). Despite the abundant literature devoted to the etiology, pathogenesis, diagnostics and treatment of arterial hypertension, many issues still remain to be elucidated. The pathogenesis of spontaneous hypertension involves a number of mechanisms which have to be determined and explained in order to establish proper and fully effective treatment.
Spontaneous hypertension cannot be perceived as a homogenous pathological entity but rather as a group of not well defined syndromes sharing increased arterial blood pressure. In addition to elevated blood pressure, progressing renal failure and the CNS symptoms of varied intensity, also disturbances in other organs may predominate in the clinical picture. This particularly refers to the digestive tract, within which acute pancreatitis or intestinal ischemia may occur (2 - 5).

This also might influence the activity of endocrine cells, an important element of the intrinsic regulatory system in the digestive tract. The digestive system is specialized for food digestion, including different aspects such as physical and chemical digestion, secretion, absorption and motility. All these processes are regulated by a complex network of regulatory molecules, i.e. diverse hormones, neurotransmitters, cytokines and growth factors (6 - 8). A fundamental role is played by the intrinsic diffuse neuroendocrine system (DNES), a group of cells dispersed among non-endocrine epithelial cells that specialize in the secretion of a variety of bioactive substances to the blood or towards the neighboring cells. Changes occurring in gastrointestinal DNES are among the earliest responses of the body to food ingestion (7, 8). DNES cells are apparently able to monitor local conditions, e.g. variable luminal contents, and respond with the release of peptides. Working together with the nervous system, they allow rapid and efficient adaptation to changing external and internal conditions (6, 9).

The results of our investigations (10 - 13) and of many other authors (14 - 16) seem to indicate that in various pathological states the number and morphology of endocrine cells in the stomach undergo some changes. This confirms the involvement of biologically active substances produced by endocrine cells in various pathological and adaptive processes in the body.

Given the above, and taking into account the vasotrophic and homeostatic properties of biogenic amines and certain peptide hormones it can be assumed that DNES cells can be involved in the mechanisms of the development of disorders in spontaneous hypertension.

Since evidence of the actual behaviour neuroendocrine cells in the stomach was rather scarce and the pathogenesis of digestive tract disorder in hypertension not fully explained, it seemed interesting to study the distribution and occurrence of gastric neuroendocrine cells in hypertensive rats.

The present study investigated possible changes in endocrine cells due to hypertension in rats.

MATERIALS AND METHODS

Animals: Male spontaneously hypertensive rats (SHR) were purchased from Polish Mother's Memorial Hospital Research Institute in Lodz and housed at Veterinary Medicine Faculty of the University of Warmia and Mazury in Olsztyn, Poland. The animals were divided into 2 equal groups: control (10 healthy rats) and experimental (10 spontaneously hypertensive rats), similar in
terms of baseline parameters. The rats had free access to standard granulated chow and a normal drinking water but were fasted overnight (16-18 h) before the experiment.

The animals were killed by decapitation at 4 and 16 weeks of age (each time 5 control rats and 5 SHR) and the stomach immediately removed, opened, rinsed in saline. Tissue samples were collected from the corpus and pyloric regions of the stomach. They were fixed in 4% formalin and Bouin's fixative for 24 hours at +4°C, and processed routinely for embedding in paraffin. Sections were cut at 3 µm in thickness, and stained by hematoxylin-eosin (H+E) for general histological examination, and by Grimelius' method revealing neuroendocrine cells, following impregnation of their cytoplasmatic granules with silver salts (17).

Identification of endocrine cells by immunohistochemical methods

In the immunohistochemical study, the EnVision method was used according to Herman GE, Elfont EA and Escribano LM et al. (18, 19). The primary antibodies used were anti-somatostatin (RTU; N 1551 DakoCytomation Denmark), anti-gastrin (1:800; A 0568 DakoCytomation Denmark), anti-serotonin (1:100; M 0758 DakoCytomation Denmark). The antisera were diluted in Antibody Diluent (S 0809 DakoCytomation Denmark).

The EnVision complex was purchased from DAKO Cytomation, Denmark.

In short, the paraffin-embedded specimens were dewaxed, rehydrated and treated with Peroxidase Blocking Reagent (S 2001 DakoCytomation Denmark) for 10 minutes to block endogenous peroxidase activity. Then, the sections were washed in distilled water and Wash Buffer (S 3006 DakoCytomation), 3 times for 5 min, and incubated with a somatostatin antibody for 15 min, gastrin antibody for 30 min and serotonin antibody for 30 min in dark-room at room temperature. Next, the sections were washed 3 times in Wash buffer. EnVision was applied for 15 minutes for somatostatin and 30 min for others. The antibody binding was visualized with the help of Vector QS hematoxylin (2 - 3 seconds).

Negative control was carried out by incubating sections with the diluent and normal serum instead of the primary antiserum. All the performed control reactions gave negative results. Positive control was conducted for specific tissue recommended by the producer.

Quantitative analysis

The obtained results of immunoreactive endocrine cells of the mucosa were submitted for evaluation in the microscope. Cells with somatostatin (D), gastrin (G) and serotonin (EC) expression were searched for and their topography was observed.

The density of neuroendocrine cells in the epithelium (D, G and EC cells) of gastric mucosa (corpus and pylorus) was compared. The number of neuroendocrine cells was separately counted in five visual fields (each 0.784 mm²) at a magnification of 200 (x 10 objective and x 20 evepiece) in the longitudinal sections of the stomach. At least three sections from each specimen were counted. The cell count was expressed as the mean number of D, G and EC cells per visual field. The corresponding mean values were computed automatically; significant differences were determined by Student's t-test; p<0.05 was taken as level of significance.

RESULTS

Routine histopathological examination showed normal mucosal morphology.

In all rats, the neuroendocrine cells were difficult to recognize by light microscopy after routine staining with hematoxylin and eosin (H+E). Single cells
could be sometimes observed, dispersed between other epithelial cells along the glands, especially in the basal mucous membrane. They were round or pear-like in shape and had a dark-staining nucleus lying centrally in a light, poorly eosinophilic cytoplasm.

The mucous membrane of the stomach showed cells with a characteristic positive argyrophilic Gomori reaction - cells which typically belong to the APUD system. They were observed in all rats, mostly distributed in the basal portions of the mucous membrane of the stomach. Considerably fewer argentophilic cells were in the pylorus. The largest number of these cells, revealed due to the cytoplasm argentophilic nature was observed in the stomach of hypertensive rats killed at 4 weeks of age (Fig. 1A and B).

The antisera against somatostatin, gastrin and serotonin immunostained the corresponding endocrine cells, i.e. somatostatin (D)-, gastrin (G)- and enterochromafin (EC)-cells, recognizing their site in the epithelium of the stomach mucosa. These cells were mainly located in the basal third of the gastric glands, while in the middle third their number diminished and in the top third only single endocrine cells were observed.

No significant age-dependent changes in the number and location of somatostatin-immunoreactive (IR), gastrin-IR and serotonin-IR cells were found in control animals.

Table 1. Number of somatostatin-, gastrin-, serotonin- containing cells per visual field in the corpus and pyloric gastric mucosa of hypertensive and control rats.

<table>
<thead>
<tr>
<th>Groups of rats</th>
<th>Corpus</th>
<th>Pylorus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ST</td>
<td>ST</td>
</tr>
<tr>
<td>Control group</td>
<td>7.30 ± 1.15</td>
<td>24.0 ± 3.13</td>
</tr>
<tr>
<td>4 - week old SHR</td>
<td>2.70 ± 2.07</td>
<td>36.2 ± 3.45</td>
</tr>
<tr>
<td>16 - week old SHR</td>
<td>6.67 ± 2.31</td>
<td>34.6 ± 5.17</td>
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Fig. 1. Endocrine cells in the stomach: (A) control rat (B) 16 week old hypertensive rat, impregnated with silver according to Grimelius method, x 200.
The number of somatostatin-, gastrin- and serotonin-containing endocrine cells in the corpus and antrum mucosa in hypertensive rats differed from that in controls (Table 1).

Somatostatin was positively stained in D cells in the corpus and pylorus mucosa and the density of these cells in the pylorus was higher than that in the corpus of mucosa (Fig. 2 and Fig. 3). In the hypertensive animals somatostatin-IR cells in the corpus decreased in number in the 4 weeks old rats compared to control and increased significantly (P<0.01) in the 16 weeks old compared to 4 weeks old SHR, approaching the control values. On the other hand, they increased significantly (P<0.04) in the pyloric region in 4 weeks old SHR compared to the control, then slightly decreased in the 16 weeks old SHR compared to the 4 week old.

Somatostatin-positive cells were mostly distributed in the basal portions of the gastric glands but sometimes, especially in the hypertensive rats, they were arranged along the entire glands. Somatostatin-synthesing cells most frequently had round,
pyramidal or flask shapes and often multiform with long processes extending along the basement membrane of gastric glands and in the vicinity of other cells.

The immunohistochemical study of the rat stomach showed that gastrin- and serotonin-containing endocrine cells were densely packed in the pyloric mucosa and not found in the fundus. Only some G and EC cells occurred sparsely scattered in the corpus mucosa, especially in the hypertensive rats.

The majority of gastrin cells were localized in the lower part of the pyloric glands with only some G-cells observed in the upper part of the mucosa as well. The cells were round or irregular in shape. Staining intensity for gastrin was strong and in some cells moderate (Fig. 4A and B). In the hypertensive rats, the number of gastrin-IR cells in the pylorus increased highly significantly at 4 and 16 weeks of age (p<0.02 and 0.03, respectively), compared to control.

Serotonin was positively stained in EC cells and mast cells (known to contain 5-HT in murines). EC cells always showed strong immunostaining for serotonin. They were localized mainly in the lower part of the pyloric glands and were also sparsely distributed from the neck to the fundus where they were moderately abundant.

Mast cells were mainly localized in the lamina propria and were round in shape. In the hypertensive rats, the number of serotonin-IR cells increased significantly in the 4 and 16 weeks old (p<0.02 and 0.01, respectively), compared to control.

**DISCUSSION**

Despite the recognized gastric complications of hypertension, such as bleeding from gastritis and vascular ectasias (20, 21), until now no investigations have been conducted on the endocrine cells in hypertensive humans or animals. The present study demonstrated the distribution and occurrence of somatostatin-
gastrin- and serotonin-IR endocrine cells in the stomach of hypertensive rats as compared to normal controls. This is the first report concerning the effect of hypertension on the endocrine cells in the rat stomach. Our study revealed changes in the gastrointestinal neuroendocrine system in the hypertensive rats, both in a short-term (4 weeks) and long-term (16 weeks) period.

In the control rats (4 and 16 weeks old), the number of endocrine cells was stable or slightly varied with age. A similar lack of significant age-dependent differences in the occurrence of endocrine cells in the stomach of normal calves was reported by Soehartono et al. (14). The distribution pattern of endocrine cells, which is normally predominant in the basal third of the gastric glands, showed an increase in the upper third in the hypertensive rats. The number of endocrine cells in the hypertensive rats was generally higher than in age-matched controls. Only the frequency of somatostatin-IR cells in the corpus of the 4 week old SHR was lower.

Somatostatin producing D-cells are found in both the gastric corpus, where they mediate inhibitory actions on parietal and ECL cells and in the antrum, where they mediate acid-inhibition of gastrin release from G-cells (7, 22 - 24). Stimulation of somatostatin release is associated with increased secretion into the venous outflow, and there may also be detectable changes in the systemic circulation (25, 26). In the present study, we have shown various reactions of ST-immunoreactive cells to hypertension depending on region of the stomach. The differences in changes in somatostatin-IR cells in the corpus and pylorus of hypertensive rats, suggesting that separate mechanisms regulate functions in the two populations of gastric D-cell.

Our results are in accordance with the studies of other investigators, who also observed different actions of endocrine cells in the acid-secreting part of the stomach and in the antrum on various conditions (8, 27). A decrease in the number of antral somatostatin cells and their unchanged level in the corporal gastric mucosa of patients with Helicobacter pylori have been observed by Chamouard (16) and Tzaneva (28).

The major endocrine function of gastric pyloric antrum is the secretion of gastrin. Gastrin is produced by G cells in the antral and duodenal mucosa, but the major source of gastrin is the antral G cells. The main inhibitor of antral G-cells is intraluminal acid, acting via release of somatostatin from antral D-cells (29, 30). These cells are functionally and anatomically closely connected to the G cells.

We found a significant increase in the number of G cells and interestingly, also a rise in D cells. A concomitant increase in somatostatin and gastrin endocrine cells in the antral mucosa may indicate the existence of a reciprocal relationship between the activity of the antral G and D cells. We found that D cells in gastric mucosa had long cytoplasmic processes. Similar processes have been also described by other authors (31, 32) for D and other types of endocrine cells. It has been suggested that these cells transport their synthesized product through their long cytoplasmic processes towards target cells and exert in this way paracrine
effects. We also observed a significant increase in the number of EC cells in the antrum of hypertensive rats as compared to the controls.

Serotonin is secreted by EC cells of the gastrointestinal tract and intragastric pH seems to be important in regulating the release of serotonin, as serotonin itself plays a role in regulating gastric acid secretion. It is well known that serotonin released from the EC cells may reach the connective tissue space of the lamina propria and exert paracrine effects also on endocrine cells such as D or G cells to regulate acid secretion. Evidence has accumulated showing that serotonin regulates gastric motility as well as gastric acid secretion in both in vitro and in vivo models of various animals (33, 34).

The increased quantity of cells, observed in the current study, indicates rather proliferation of the endocrine cells of gastric mucosa. Most of the endocrine cell hyperplasias described to date are secondary to an underlying disease, although rare cases of primary hyperplasias have also been documented (35).

Recent studies have shown higher plasma levels of several cytokines, such as interleukins or tumor necrosis factor in patients with hypertension. Cytokines play a very important role in pathogenesis of hypertension, since they impair contractility of the heart muscle and cause damage to endothelium and myocytes due to their proinflammatory effects. In hypertension, interstitial infiltration by mononuclear cells has been well documented (36).

Our findings seem to demonstrate a systemic dysfunction of DNES cells, which produce biogenic amines and peptide hormones and participate in adaptive processes of the organism. It may be assumed that the increase in the number of neuroendocrine cells in the stomach of hypertensive rats was a compensating phenomenon, correcting to a certain extent homeostasis disorders.

The behaviour of neuroendocrine cells in the gastric mucosa may be regarded as a morphological expression of their hyperfunction in hypertension.

The changes observed in the neuroendocrine cells system according to time of SHR examination, suggest that the DNEC as well as the nervous system are major components of the internal system, controlling homeostasis in the gastrointestinal system, and that the DNEC system may be affected by hypertension.

The pathophysiological character of these disorders and the mechanism responsible for neuroendocrine cell hyperplasia are still unclear and require further studies.

REFERENCES


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