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Original Research Article

Alterations of rat stomach endocrine cells under renovascular hypertension

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ABSTRACT

Purpose: The aim of the present study was to perform immunohistochemical and ultrastructural analysis of gastrin-, synaptophysin (SY)- and atrial natriuretic peptide (ANP)-positive cells in the pylorus of "two kidney, one clip" (2K1C) renovascular hypertension model in rats.
Material/methods: In order to identify neuroendocrine (NE) cells, immunohistochemical reactions were performed with the use of specific antibodies against gastrin, SY and ANP. Gastric NE cells were also examined using an electron microscope.
Results: The present study revealed a twofold increase in the number of gastrin- and SY-positive cells and a significant decrease in the number of ANP-immunoreactive (IR) cells in the pyloric mucosa of 2K1C rats. Test results obtained with an electron microscope confirmed a change in the activity of the stomach endocrine cells of hypertensive rats.
Conclusions: Immunohistochemical and ultrastructural investigations demonstrated the impact of renovascular hypertension on the neuroendocrine system in the rat stomach. The changes in the total number and ultrastructure of DNES cells proved their undeniable role in the modulation of gastric dysfunction, as a consequence of deregulation of homeostasis-maintaining systems.

1. Introduction

Development of arterial hypertension is a complex process involving multiple interrelated mechanisms. In hypertension, besides stimulation of the main axis responsible for the regulation of the cardiovascular system, disorders also occur in the activity of diffuse neuroendocrine system (DNES) cells. The main role of DNES cells is to regulate the intercellular and intersystem interrelations for the stability of internal environmental of the body. Several experimental studies have demonstrated that hypertension causes disturbances in the secretion of neuropeptides and biogenic amines in the majority of organs, including the digestive system [1,2]. However, the influence of renovascular hypertension on neuroendocrine (NE) cells distributed in the gastrointestinal tract is still not fully examined.

The gastric NE cells, a highly specialized mucosal cell subpopulation, produce a wide range of hormones with a specific regional distribution. These hormones, beyond playing an essential role in the internal regulation of gut motility, absorption and gastric secretory function, might also affect the systemic vascular tone and blood pressure [3]. Gastrin, the main stimulator of the activity of parietal cells and intestinal peristalsis, shows arterial pressure reducing properties [3]. Moreover, the latest clinical reports have described a greater increase in postprandial serum gastrin level in patients with hypertension as compared to normotensive adults [4]. Likewise, a rise has been experimentally demonstrated in the number of gastrin-containing cells in the stomach of spontaneously hypertensive rats [1]. The above-mentioned findings suggest that gastrin might participate in the development of hypertension.

To visualize the cells belonging to DNES, researchers frequently use synaptophysin (SY) as a marker of NE cells. Synaptophysin can be found in gastrointestinal tract mucosa, where it co-localizes mainly with gastrin, somatostatin and serotonin [5]. Thus, the possible changes in SY distribution might illustrate the disturbances in gastrointestinal DNES in the state of hypertension.

Gastric NE cells also synthesize atrial natriuretic peptide (ANP), which is actively involved in the regulation of the cardiovascular system and sodium tubular reuptake [6,7]. It has been found that the peptide modulates gastric and ileum motility and indirectly regulates gastric acid release *via* influencing somatostatin and gastrin secretion [8–10]. The available literature data suggest the involvement of ANP in the pathogenesis of secondary hypertension

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[11]. It has been demonstrated that ANP serum level and expression of peptide mRNA in heart tissue is higher in rats with renovascular hypertension. However, up to date there have been no reports considering the distribution of ANP-containing cells in the gastrointestinal tract in hypertension state. In addition to the release of hormones, gastric NE cells form a complex network of interdependence in the regulation of local processes. The knowledge about functioning of the digestive system in various pathological states might be essential for understanding the systemic changes that occur during disease development.

Many investigations have indicated that in various pathological states the number and morphology of NE cells in the stomach undergo some changes [1,2,12]. This seems to confirm the involvement of biologically active substances produced by gastroendocrine cells in various pathological and adaptive processes in the body [1,2,12].

The aim of the current study was to perform immunohistochemical and ultrastructural analysis of gastrin-, SY- and ANP-IR cells in the pylorus of "two kidney, one clip" (2K1C) renovascular hypertension model in rats.

2. Material and methods

2.1. Experimental animals

Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international law and with guidelines for the use of animals in biomedical research [13]. The study was performed on twenty (20) young male Wistar rats, with body weight at the beginning of the experiment within 160–180 g. The rats were housed in polypropylene cages in groups of two or three rats per cage and received laboratory chow and tap water *ad libitum*. After a one-week period of acclimatization, the systolic blood pressure (BP) of each rat was measured, and the surgical procedure for induction of renovascular hypertension and sham operation was performed.

2.2. 2K1C renovascular hypertension procedure

After the rats were anesthetized by exposure to pentobarbital (40 mg/kg, i.p.), a 3-cm retroperitoneal flank incision was performed under sterile conditions. The left kidney was exposed and the renal artery was carefully dissected free of the renal vein. The renal artery was then partially occluded by placing a silver clip with an internal diameter of 0.20 mm on the vessel. The wound was closed with a running 3-0 silk suture ($n = 10$). Sham operated rats ($n = 5$) underwent identical surgical procedures, except that a clip was not applied to the renal artery. Five ($n = 5$) rats were not been exposed to any surgical procedures.

After a six-week period of the renal artery clipping procedure, the systolic arterial pressure was measured. Conventional noninvasive blood pressure in conscious rats was measured using the tail-cuff method [14]. The BP measurements were considered valid only when three consecutive readings did not differ by more than 5 mmHg. The average of the three measured values was then recorded. After this time, eight 2K1C rats developed stable hypertension (mean blood pressure values 163 ± 3.2 mmHg).

2.3. Method of experimental material collection and fixation

Six weeks after the renal artery clipping procedure, the animals were killed and the stomach was immediately removed, opened (along the long curvature) and rinsed in saline. Tissue samples were collected from the pyloric region of the stomach. They were fixed in 4% buffered formalin for 24 hours at + 4 °C, and processed routinely for embedding in paraffin. Sections were cut at 4 μm in thickness, and stained by hematoxylin–eosin (H+E) for general histological examination.

2.4. Identification of endocrine cells by immunohistochemical method

In the immunohistochemical study, the EnVision method was used according to Herman GE, Elfont EA [15]. Immunohistochemical reactions to find gastrin, ANP and SY in gastroendocrine cells were performed on paraffin stomach sections of the animals studied. In these analyses, specific anti-gastrin (1:800; A 0568 Dako Denmark A/S Produktionsvej 42, DK-2600 Glostrup, Denmark), anti-synaptophysin (1:20; Clone SY38, Nr kat. M0776 Dako Denmark A/S Produktionsvej 42, DK-2600 Glostrup, Denmark) and anti-ANP antibodies (1:2000; Nr kat. H-005-24, Phoenix Pharmaceuticals Inc, 530 Harbor Boulevard, Belmont, CA 94002, USA) were applied. The antiserum was diluted in Antibody Diluent (S 0809 Dako Denmark A/S Produktionsvej 42, DK-2600 Glostrup, Denmark).

2.5. Immunohistochemical reaction procedure

In short, the paraffin-embedded specimens were dewaxed, rehydrated and treated with Peroxidase Blocking Reagent for 10 min to block endogenous peroxidase activity. Then, the sections were washed in distilled water and Wash Buffer (S 3006 Dako Denmark A/S Produktionsvej 42, DK-2600 Glostrup, Denmark), 3 times for 5 min, and incubated with gastrin and synaptophysin antibodies for 30 min and ANP for 1 hour, in a humidified chamber at room temperature. Next, the sections were washed 3 times in Wash buffer. The antibody binding was visualized with the help of EnVision (+) HRP Kit (K4007 for mouse and K4011 for rabbit antibodies; Dako Denmark A/S Produktionsvej 42, DK-2600 Glostrup, Denmark) containing Labelled Polymer-HRP. Vector QS hematoxylin was used (2–3 s) for cellular nuclei staining. Negative control was carried out by incubating sections with normal rabbit and mouse serum (Confirm Negative Control Rabbit Ig Nr 760-1029, Mouse 760-2014, Ventana) instead of primary antiserum. All the performed control reactions gave negative results (no staining). Positive control was conducted for specific tissue recommended by the producer, which is human pylorus for rat gastrin, rat brain for SY and rat atrium for rat ANP.

2.6. Electron microscopy

The samples were fixed in 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2) at 4 °C overnight, and next post-fixed for 1 hour in 2% osmium tetroxide, and then were dehydrated in graded ethanol solutions and propylene oxide and embedded in epon. Ultrathin sections (60 nm) were cut on Leica Reycher Ultracut S, contrasted with uranyl acetate and lead citrate, and examined in OPTON 900 PC electron microscope. Gastroendocrine cells were identified and evaluated on the basis of secretory granules.

2.7. Quantitative analysis

The results of immunohistochemical staining were submitted for evaluation in an Olympus Bx50 microscope. Gastrin-, SY- and ANP-IR cells were searched for and their topography was observed. Immunopositive cells were counted in five (5) randomly selected microscopic fields, each field of 0.785 mm^2, in magnification of 200× (20× the lens and 10× the eyepiece) in the longitudinal sections of the stomach. Three (3) sections of each rat were analyzed. The numbers of positively stained cells were presented