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CAN DOXAZOSIN INHIBIT THE HYPERTENSION-INDUCED CHANGES OF ENDOCRINE CELLS IN THE STOMACH OF SPONTANEOUSLY HYPERTENSIVE RATS?

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We have previously shown that the endocrine cells in the stomach increase in number in spontaneously hypertensive rats (SHR) that suggests that the hypertension has an influence on the intrinsic regulatory system by endocrine cells control in the stomach of rats. The aim of the present study is to find differences in the density of neuroendocrine (NE) cells of stomach rats and composition in doxazosin treated SHR compared to untreated animals. Fragments of the pyloric region were collected at 12 weeks of age. Paraffin-embedded sections were stained with H+E and by silver impregnation. To identify NE cells, immunohistochemical reaction (IR) was performed with the use of a specific antibody against somatostatin, gastrin, serotonin and chromogranin. It was revealed that the distribution density of IR-endocrine cells all searched types was considerable lower in the pyloric mucosa of hypertension animals treated with doxazosin compared to SHR untreated and was on level healthy rats. The present study demonstrated that doxazosin inhibit the hypertension-induced changes of endocrine cells in the stomach of SHR.

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

Abbreviations: SHR – spontaneously hypertensive rats; NE – neuroendocrine; DNES – diffuse neuroendocrine system; IRE – immunoreactive endocrine;

INTRODUCTION

Doxazosin is used to treat the symptoms of an enlarged prostate (benign prostatic hyperplasia or BPH), which include difficulty urinating, painful
urination, and urinary frequency and urgency. It is also used alone or in combination with other medications to treat high blood pressure. Doxazosin is in a class of medications called alpha-blockers. It lowers blood pressure by relaxing the blood vessels so that blood can flow more easily through the body (1).

Hypertension is the most common cardiovascular disease in humans and causes morphological and functional changes in myocardium, vascular smooth muscle or endothelium, and abnormalities in physiological regulatory system for blood pressure, including neurotransmitters and humoral factors (2, 3). At the same time the participation and the role of biogenic amines and peptide hormones (which are produced by the diffuse neuroendocrine system (DNES) cells located in different organs) in endogenous mechanisms in of organs diseases are still unknown (4).

Recent data on identification of the same and similar physiologically active substances, acting within the nervous system as neurotransmitters and neurohormones, and locally or remotely as hormones within the endocrine system, enables both system to be incorporated into the universal DNES (5, 6). It is well-known that the nervous and endocrine systems have well-established and very closed related interrelations to regulate systemic homeostasis that involves the production and secretion of a variety of cellular mediators known as regulatory peptides (peptide hormones, cytokines, chemocines, integrins and others). Peptide hormones, cytokines and other related molecules regulate homeostasis in the tissue of external systems that facilitate restoration of local homeostasis (7).

The endocrine cells of the stomach are a highly specialized mucosal cell subpopulation. Within the gastrointestinal tract a large number of hormones have been identified, so that the gastroenteropancreatic tract is now recognized as the largest endocrine organ of the whole human body (8). The DNES of stomach is remarkably heterogeneous and is composed at least 14 different cell types produce a wide range of hormones with a specific regional distribution. Besides releasing hormones into the bloodstream to act on distant tissues, the DNES cells of the stomach constitute a complex regulatory network whose function includes the local fine tuning of secretion, absorption, motility, mucosal cell proliferation, and possibly immune-barrier control. Considering their multiple systemic and local roles, DNES cells may need to be considered an active cell subpopulation that may adopt and respond to different physiological and pathological stimuli (5).

The aim of our study is to find differences in the number of neuroendocrine cells of stomach rats and composition in doxazosin treated SHR compared to untreated animals.

MATERIALS AND METHODS

Experimental model

Male spontaneously hypertensive rats were purchased from Polish Mother’s Memorial Hospital Research Institute in Lodz, Poland.
All experimental procedures involving animals and their care were approved by local authorities and conducted in conformity with the national and international laws and Guidelines for the Use of Animals in Biochemical Research.

Study assumptions, aim, schedule and model of animal treatment were approved by the Senate Committee for Supervision of Experiments on Humans and Animals, Medical University of Białystok Nr 2001/16.

The study was performed on 15 young male Wistar rats, their body weight at the beginning of the experiment within 180 - 200 g (the mean body weight: 190 ± 10 g). The animals were kept in lighted and ventilated conditions with room temperature and maintained day and night rhythm. The rats had a free access to standard granulated chow and drinking water was available but were fasted overnight (16 - 18 h) before the experiment.

The animals were divided into 3 equal groups: two control groups - (5 healthy rats and 5 spontaneously hypertensive rats) and experimental group - 5 spontaneously hypertensive rats treated with doxazosin (1mg/100 g in drinking water per day), similar in terms of baseline parameters. The rats were anesthetized by pentobarbital, administered interperitoneally (i.p.) at a dose of 50 mg/kg then, the animals were killed by cutting of heard at 12 weeks of age and the stomach immediately removed, opened, rinsed in saline. The tissue sample was collected from the pyloric regions of stomach. They were fixed in Bouin’s fluid for 24 h 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, and by Grimelius’ method revealing neuroendocrine cells, following the impregnation of their cytoplasmatic granules with silver salts (9).

**Identification of endocrine cells by immunohistochemical methods**

In the immunohistochemical study, the EnVision method was used according Herman GE, Elfont EA and Escribano LM et al. (10, 11). The primary antibody used are anti-somatostatin (RTU; N 1551 DakoCytomation Denmark), anti-gastrin (1:800; A 0568 DakoCytomation Denmark), anti-serotonin (1:100; M 0758 DakoCytomation Denmark) and anti-chromogranin A (RTU; N 1535 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 min 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, serotonin antibody for 30 min and chromogranin A antibody for 20 min in dark-room at room temperature. Then sections were washed 3 times in Wash buffer. EnVision was applied for 15 min for somatostatin, and 30 min for others. The antibody binding was visualized with the help of Vector QS haematoxylin (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 and positive control was conducted for specific tissue recommended by producer.

**Observation, photomicrograph and count**

Five rats used for each studied groups, 5 specimens of each antibody of each animals were observed and photomicrographed under the Olympus Bx50 light microscope, with video circuit and a Pentium 120 PC computer with Lucia G (Nikon) software for microscope image analysis. The results of immunoreactive endocrine (IRE) cells with somatostatin (D), gastrin (G), serotonin...
(EC) and chromogranin A (ECL) expression were searched for and their topography was observed. The dark brown positive cells on section were separately counted in five visual fields (each 0.784 mm$^2$) at a magnification of 200 (x 10 objective and x 20 evepiece) in the longitudinal sections of the stomach. The average number of IR cells from 5 fields selected randomly in each specimen was the IRE cell number in each animal. The average number of 5 rats of each group was quantified IRE cell’s distribution density. The number of endocrine cells was counted and expressed as the mean ± SEM per visual field. 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

DNE cell types, distribution and density in the pyloric region of the stomach of three studied groups are listed in Table 1. It can be seen from Table 1 that the all types of searched endocrine cells were the highest distribution density in the pyloric epithelium of SHR untreated on doxazosin. The distribution density of IRE cells was considerable lower in the pyloric mucous of hypertension animals treated with doxazosin compared to SHR untreated with doxazosin and was on level healthy rats.

Routine histopathological examination showed normal mucosal morphology. In all studied rats, endocrine cells recognition was very difficult at the level of light microscope, following H+E staining. It was sometimes possible to see single cells, distinguished among other cells of epithelium, especially in the basal mucous membrane, by dark staining of the nucleus, centrally located in light, poorly eosinophylic cytoplasm. Routine studies by means of H+E staining cannot, by any means, be used for the identification of DNES cells.

The mucous membrane of the stomachs showed cells with a characteristic positive argentophilic Gomori reaction – cells which typically belong to the APUD system. They were observed in all rats, mostly distributed in the basal portions of mucosal membrane of the pyloric. Considerable less argentophilic cells were in the pylorus of healthy rats and in doxazosin treated SHR. The largest number of the endocrine cells, revealed due to the cytoplasm argentophilic nature observed in the stomach of SHR.

<table>
<thead>
<tr>
<th>Groups of rats</th>
<th>Pyloric</th>
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<tr>
<td></td>
<td>ST</td>
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<tr>
<td>Control group</td>
<td>18.8 ± 2.51</td>
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<tr>
<td>SHR untreated doxazosin</td>
<td>30.2 ± 4.35</td>
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<tr>
<td>SHR with doxazosin</td>
<td>20.1 ± 3.25</td>
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Table 1. DNES cells types and number per visual field in the pyloric region of control rats, untreated doxazosin SHR and doxazosin treated SHR.
The antisera against somatostatin, gastrin, serotonin and chromogranin A immunostained the corresponding endocrine cells, i.e. somatostatin (D)-, gastrin (G)-, enterochromafin (EC)- and chromogranin (ECL)- cells, recognizing their site in the epithelium of the pyloric mucosa. The endocrine cells were distributed throughout the glands and the superficial epithelium. They 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, especially in SHR. According to distribution density ECL-cells were found to be the most frequent endocrine cells, followed by G-, D- and EC-cells. DNE cells distributed between columnar epithelium were scattered or piled in distribution, and diverse in shape, sometimes had cytoplasmic process.

All IRE cell types and the distribution density was lower in doxazosin treated SHR compared to SHR approaching near the control values.

Somatostatin-positive cells were mostly distributed in the basal portions of the glands of the stomach, but sometimes especially in SHR were arranged along the entire glands and have round, pyramidal or flask shapes, and often multiform with long processes extending along the basement membrane of gastric glands and in nearby others cells. The majority of D-cells showed a strong immunostaining for somatostatin (Fig. 1A, B, C).

The most of G cells were round or irregular in shape. Staining intensity for gastrin was strong and in some cells moderate (Fig. 2A, B, C).

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 but were also sparsely distributed along the entire glands. Mast cells were mainly localized in the lamina propria and were round in shape. In doxazosin treated SHR the number of serotonin-IR cells was on control level and distinct lower compared to the SHR (Fig. 3A, B, C).

ECL cells displayed lower density CgA immunoreactivity in doxazosin treated SHR than in SHR. The majority of CgA- positive cells simultaneously showed a moderate or weak immunostaining for CgA in doxazosin treated SHR whereas staining intensity of CgA in the same cells in pyloric mucosa of SHR untreated with doxazosin was strong (Fig. 4A, B, C).

DISCUSSION

The distribution and density of occurrence of somatostatin-, gastrin-, serotonin, and chromogranin-IR endocrine cells were clarified in the pyloric of the stomach of normal, SHR untreated with doxazosin and hypertension animals treated with doxazosin. These endocrine cells types are typical of the pyloric mucosa region, the rat (12).
Fig. 1. Somatostatin-positive cells mainly in the basal of the pyloric mucosa (A) control rat, (B) hypertensive rat, (C) hypertensive rat with doxazosin treated, x 200.
Fig. 2. Gastrin-immunoreactive cells in the pyloric region (A) control rat, (B) hypertensive rat (significant increase in the number of G cells), (C) hypertensive rat with doxazosin treated, x 200.
Fig. 3. Serotonin-immuno-reactive cells in the basal of the pyloric mucosa (A) control rat, (B) hypertensive rat, (C) hypertensive rat with doxazosin treated, x 200.
Fig. 4. Chromogranin A - positive cells mainly in the basal of the antral mucosa (A) control rat, (B) hypertensive rat (significant increase in the number of ECL cells), (C) hypertensive rat with doxazosin treated, x 200.
We have previously shown that the endocrine cells in the stomach increase in number in SHR that suggests that the hypertension has an influence on the intrinsic regulatory system by endocrine cells control in the stomach of rats (13).

The most significant finding of this study was that doxazosin inhibit the hypertension-induced changes of endocrine cells in the stomach of spontaneously hypertensive rats.

The present study demonstrated that the distribution density of all types of searched endocrine cells in the pyloric mucosa of hypertension rats treated with doxazosin was considerable lower as compared with SHR and was on level of control animals.

The mechanisms involved in the genesis of the hypertension appear to involve several different components including augmented sympathetic nerve activity (14), altered function of arterial chemoreceptors (3,15), elevated levels of circulating norepinephrine (16), decreased vascular responses to nitric oxide (2), increased plasma concentrations of endothelin (17) and others (18).

The endocrine cells of gastrointestinal epithelium sense the luminal contents and through secretions at their basolateral side signal both to other epithelial cells and to subepithelial cells, including smooth muscle, neurons, and inflammatory cells. Some of the features of these cells are clearly neurone-like (5). It is well-known that the nervous and endocrine systems have well-established and very closed related interrelations to regulate systemic homeostasis that involves the production and secretion of a variety of cellular mediators known as regulatory peptides (peptide hormones, cytokines, chemocines, integrins and others). Peptide hormones, cytokines and other related molecules regulate homeostasis in the tissue of origin, either via local actions or by recruitment of external systems that facilitate restoration of local homeostasis (6, 19).

Functionally, DNES cells are receptor-secretory cells, with their receptors located on the surface of the cellular membrane, the cells reacting by secretion to certain stimuli. The receptors of endocrine cells are capable of receiving chemical stimuli from blood or tissues (20). The primary target of hormonal action (paracrine effect) is the direct environment of neuroendocrine cells, first of all, the endothelium and the muscular coat of blood vessels, nerve fibres, and cells of the connective tissue (21). Following the classical endocrine theory, once the hormones permeate into blood vessels, they head towards distant target organs, i.e. the more distant object of hormonal effects (5, 22).

NE cells are dispersed throughout the epithelium of the stomach and have granules containing 5-HT, and various peptide hormones are concentrated around the basolateral surface of the cell. Many different factors as stroking the stomach mucosa, increase in intracellular cAMP concentration evoke release peptide hormones (6). The levels of cAMP are regulated by G proteins and associated adenylyl cyclase signaling has been shown to be implicated in a variety of cellular functions, including vascular permeability (23, 24) and catecholamine release (25) all of which play a key role in the regulation of blood pressure (26). Yuan et
al. (26) and others have shown an increased expression of G proteins in heart and aortas from SHR. That enhanced expression of this G proteins and resultant decreased levels of cAMP in response to various hormones, may be one of the contributing factors in the pathogenesis of hypertension.

Furthermore, release of peptides is controlled by the autonomic nervous system. The parasympathetic innervation by the vagus nerve constitutes an important link between the central nervous system and the gastrointestinal tract (27), and it has a significant influence on the secretory function of the stomach. Contradictory results have been reported about the changes in the number of endocrine cells such as chromogranin, serotonin, gastrin and somatostatin cells, after vagotomy (28, 29, 30).

For the last years, a link between NE cells and hypertension has been postulated. Central nervous system controls the sensitivity of endocrine cells of the gastrointestinal epithelium via receptors known to be present on endocrine cells. Sympathoadrenal activity mediated by catecholamine acts on cardiovascular target cells to increase blood pressure (31).

α₁-adrenoceptors play a significant role in many essential physiological processes, such as control of vascular tone, cardiac contraction, and the regulation of smooth muscle activity (32 - 34). Several recent studies (35, 36) have demonstrated that α₁-adrenergic antagonists decreased 24-h BP in patients with essential hypertension by inhibiting sympathetic nervous activity (37).

The idea and assumptions of the performed own studies are morphological and anatomical in character, rather than physiological, thus the explanation of the pathophysiological mechanism of the NE cell number changes and doxazosin inhibit the hypertension-induced changes in the stomach of SHR may be just a merely hypothetical consideration.

In conclusion, we have provided the first evidence showing that inhibition of the sympathetic nervous activity by doxazosin, an α-blocking agent given through 12 weeks (1 mg/100g in drinking water per day), prevented the development of hypertension-induced changes of endocrine cells in the stomach of spontaneously hypertensive rats.

The interplay between the hormonal products of gastric endocrine cells and nerve regulation or feed-back functional modulation still deserves careful study in various conditions before their full impact on clinical practice can be clarified.

REFERENCES


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