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EXPRESSION OF CHROMOGRANIN (CGA) IN RAT PANCREAS DURING ALLOXAN DIABETES

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ქრომოგრანინ CgA-ს ექსპრესია პანკრეასში ალოქსანური დიაბეტის დროს

ივ.ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი, ალ.ნათიშვილის მორფოლოგიის ინსტიტუტი, კლინიკური და ექსპერიმენტული პათოლოგიის დეპარტამენტი

რეზიუმე

კვლევის მორფოლოგიური მეთოდების გამოყენებით შესწავლილია ვირთაგვების პანკრეასი ალოქსანური დიაბეტის დროს. კვლევაში განსაკუთრებული ყურადღება დაეთმო გლიკოპროტეინ ქრომოგრანინ A-ს ექსპრესიის შესწავლას. ცნობილია, რომ CgA მიეკუთვნება გრანინების ჯგუფს, ის დიდი რაოდენობითაა პანკრეასის ნეირონებში, ნეიროენდოკრინულ და ენდოკრინულ უჯრედებში და მნიშვნელოვან როლს თამაშობს სეკრეტორული გრანულების გენერაციაში. უჯრედების იდენტიფიცირებისა და მათი ექსპრესიის ხარისხის დასადგენად გამოყენებული იყო ამ უჯრედებისათვის სპეციალურად შექმნილი მარკერი, ხოლო NE უჯრედების უკეთესი ვიზუალიზაციის მიზნით ასევე ვაწარმოებდით ვერცხლით შეღებვას. კვლევით დადგენილია, რომ საშუალო მიმდინარეობის ალოქსანური დიაბეტის დროს CgA-ს ძლიერი ექსპრესია აღინიშნებოდა პანკრეასის NE ნაწილობრივ ენდოკრინულ უჯრედებში და ინტრალობულური სადინრების უჯრედებში. მიღებულ შედეგებზე დაყრდნობით შეიძლება ვივარაუდოთ, რომ საშუალო სიმძიმის ალოქსანური დიაბეტის დროს აღინიშნება NE უჯრედების უჯრედშიდა ფუნქციის ზრდა, რაც თავისმხრივ გულისხმობს გრანულების მომწიფების, სეკრეციის და გრანულების უჯრედშიდა შენახვის პროცესების გაძლიერებას. სადინარების ინტრალობულურ ეპითელურ უჯრედებში CgA დადებითი ექსპრესია მიუთითებს რომ ის შეიძლება იყოს მნიშვნელოვანი მარკერი პანკრეასის ღეროვანი უჯრედების იდენტიფიცირებისთვის.

Introduction. Chromogranin A (CgA) lipoprotein is one of the important representatives of the group of granins. CgA is found in neuroendocrine, endocrine, and nerve cells [2,21]. CgA is a granulogenic factor that regulates secretory functions in tissues. CgA participates in the generation of secretory granules, and its breakdown products are biologically active peptides [4,8,21]. CgA helps regulate secretion in granules and produces several degradation products after secretion. Some of the products obtained after CgA degradation alter hormonal functions in autocrine and paracrine ways [9,18].

Recent studies [13,15,16,17,19,20,23] have shown that CgA and some of its breakdown products, namely (pancreastatin, catestatin, vasostatin 1, WE-14) play an important role in the pathogenesis of various forms of diabetes [4,6,14,18,24], but the exact mechanisms of their action are still unclear. Several studies [12,17,20] have reported higher levels of pancreastatin and WE-14 in patients with type 1 and type 2 diabetes [12].

According to a number of authors [1,11,13,15,18], pancreastatin, catestatin, and vasostatin play an important role in metabolic syndromes, namely obesity, diabetes, and others. Pancreastatin affects insulin [4,17,23], weakening insulin sensitivity and enhances inflammatory processes, while pancreastatin inhibitory peptide (PSTi8) improves insulin sensitivity and regulates glucose homeostasis [6,17,24]. In contrast, catestatin CST increases insulin sensitivity and reduces inflammatory reactions. According to a number of studies [12,17,20,23,24] pancreastatin and catestatin regulate pancreatic β-cell insulin secretion and glucose metabolism. The metabolic effect of the peptide fragments obtained after CgA cleavage allows us to speculate on their possible participation in the pathophysiology of diabetes and to use it as a

biomarker for the study of pancreatic neuroendocrine and endocrine cells in diabetes [3,13,14,18.22]. Based on the above, the aim of the paper is to determine the expression of CgA and its role in the pancreas of rats during Alloxan diabetes.

Animal care and maintenance. WMA DECLARATION OF HELSINKI-ETHNICAL PRINCIPLES FOR MEDICAL RESEARCH INVOLVING HUMAN SUBJECTS 2016

1. Directive 2010-63-EU on the Protection of Animals Used for Scientific Purposes 2010.
2. გზამკვლევი ლაბორატორიული ცხოველების მოვლისა და გამოყენებისათვის, 8th.ed., 2011.
3. All study procedures were implemented in accordance with the Institutional Guidelines for Animal Experiments at Bioethical Board of Al. Natishvili Institute of Morphology.

Material and Methods. The pancreas of rats has been studied in an experiment, namely in Alloxan diabetes. The experiment was conducted on 20 Wistar rats. Rats were divided into 2 groups of 10 control and 10 targets. We induced diabetes in the target animals by intraperitoneal injection of 120 mg of 10% Alloxan solution. Control and target animals were kept under standard vivarium conditions. Twenty male Wistar laboratory rats (aged eight weeks, ≈ 200g each) were housed 2 per cage in a room with a 12 h/12 h light/dark cycle and an ambient temperature of 22 to 25ºC. Free access to food and water was provided throughout the duration of the study. The body weight of each animal was measured at the beginning and at the end of the experiment. Diabetes was diagnosed by the blood glucose level. Blood glucose levels were measured (Glucometer IME-DC, FIA Biomed GmbH, Germany) in order to assess the development of diabetes. Blood samples were collected from the tail vein prior to the Alloxan injection as well as 2 and 24h. and 7, 14, 21, 27, 30 days after Alloxan administration. In addition, glucose level was measured two hours. An injection of a lethal dose of a sodium pentobarbital mixture (1%) was used for cessation of the experiment.

1 month after the beginning of the experiment, the experimental animals were slaughtered. Animals were removed from the experiment by intraperitoneal injection of 1% ethaminal-sodium.

Alloxan. Alloxan (2, 4, 5, 6-pyrimidinetetrone) is a toxic glucose analogue, which selectively destroys insulin producing cells in animals pancreas. It causes an insulin-dependent diabetes, so called Alloxan-induced diabetes.

Histological study. For histological examination, the pancreas samples were fixed in Carnuas's fixative mixture and Buen solution, then embedded in paraffin. 5 μm. Serial thickness slides were stained with hematoxylin-eosin.

Immunohistochemical research. Immunohistochemical study was performed according to the general scheme. 5 μm was used. Thick paraffin candles. To determine CgA expression and prevalence we used the following markers - polyclonal antichromogranin serum (SP-1). All procedures were carried out by the manufacturer with the recommendation of the company (BIO GENEX, USA). Results were visualized in 0.05% 3,3 diaminobenzidine hydrogen peroxide medium, nuclei were stained with hematoxylin. HardshipProvision-DPX. Silver staining allowed us to visually distinguish and characterize neuroendocrine cells and ductal cells of the pancreas [9,10].

Electron-microscopic research. The pancreas samples were fixed in 2.5% glutaraldehyde followed by a fresh 1% solution of osmium tetra-oxide in a colloidal buffer (pH 7.2-7.4) for 2 h at +4 °C. The samples were dehydrated in increasing concentrations of alcohol, poured over an araldite mixture, and polymerized for 24 h at +58 °C. A Reichert-42 Ultramicrotome (Vienna, Austria) was used to prepare sections of the samples, and the Reynolds method was used for Uranyl Acetate contrasting. The sections were covered with a silver-containing emulsion and observed under a microscope (Tesla-BS 500, Praha, Czech Republic, magnification of 3000-22000). The negative group images were magnified 3-5 times when printed.

One month after the administration of Alloxan, the glucose blood levels in Alloxan-treated group were 230±20mg/dL, and the body weight had decreased by 50-70 g and was 150-170 g compared to control group. After 2 hours Blood glucose level in Alloxan treated group was higher than in control.

Results. One month after the administration of Alloxan, the glucose blood levels in Alloxan-treated group were 230±20 mg/dL, and the body weight had decreased by 50-70 g and was 150-170 g

compared to control group. Histologically in Alloxan-treated group pancreas islets were of various size, some of the islets were atrophied, other hypertrophied and hyperemic. The islets structure was disorganized. Necrobiotic changes of β-cells were observed in the central part of the islets (e.g., destruction, degranulation, and vacuolization) compared with the intact control group. There was reduction of β-cells in the islet. Necrotized cell regions were surrounded by connective tissue. Fibrous inclusions were detected in the islet. The ultrastructure of the pancreatic islets of the rats from Alloxan-treated group was found to be markedly changed in comparison to those from control group. After one month from the beginning of Alloxan diabetes number of apoptotic cells increased, compared to intact group. Along necrotic changes in the β-cells were observed cells with highly condensed or fragmented nuclei, and cells without nuclei (Fig. 1.) [7].

In Alloxan diabetes, neuroendocrine (NE) cells in the pancreas were scattered among other cells, and their identification by hematoxylin-eosin staining was often difficult. For this purpose, immunostaining with a specific marker for these cells and silver was used for the identification, visualization and characterization of NE cells [9,10].

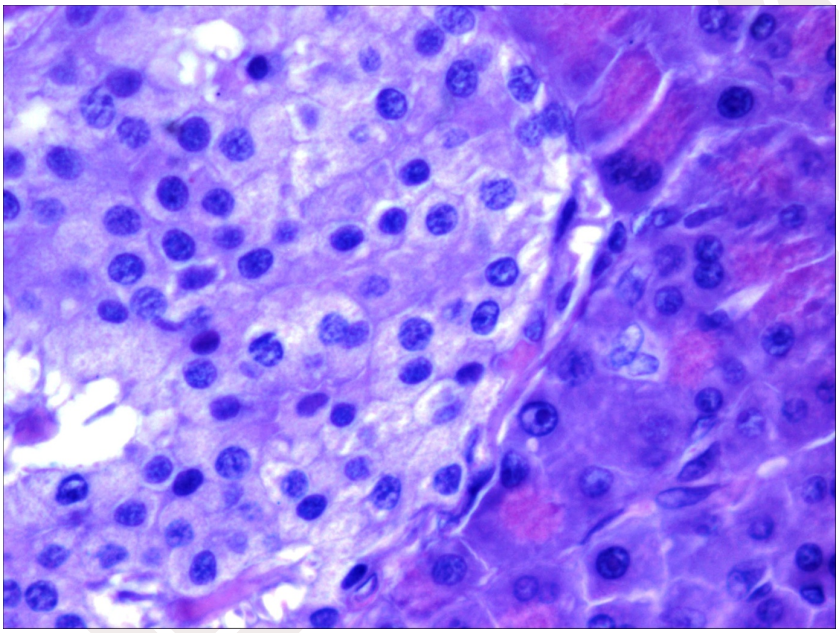

Fig. 1. Alloxan-treated group. Rats pancreas. The islets structure was disorganized. Necrobiotic changes of β-cells were observed of the islets (e.g., destruction, degranulation, and vacuolization). Shown magnification 10-40. (H@E)

Pancreas immunohistochemical and silver staining of the same tissue revealed that cells immunostained with CgA also showed an argyrophilic reaction using the Grimmelius method. As a result of immunohistochemical study, strong expression of CgA was noted of the neuroendocrine, endocrine and in the epithelial cells of intralobular ducts of the pancreas. Immunoreactive neuroendocrine cells were scattered throughout the pancreas (Fig. 2.) in contrast to the control groups, where CgA immunoreactivity was very weak or not observed at all. In studies by a number of authors [5], when examining the pancreas with light microscopy, CgA immunoreactivity was detected in the same cells that react with insulin, glucagon, and somatostatin antibodies. Intense expression of CgA was mainly observed in the vesicles of neuroendocrine cells, especially at their periphery, based on these data, it can be assumed that CgA is one of the main parts of secretory granules, which is also indicated by other authors in their works [5,6,8]. According to their research, at the ultrastructural level (protein A-gold technique), CGA immunoreactivity is limited only to the periphery of secretory vesicles, while hormones are localized mainly in the center of secretory vesicles, indicating that CGA is a component of the matrix of secretory vesicles of pancreatic cells. On electron-microscopic examination, NE cells had weakly stained cytoplasm, with large nuclei, vesicles, and abundant granules (Fig. 3.). According to some researchers [4], these vesicles contain several markers of endocrine differentiation, including CgA.

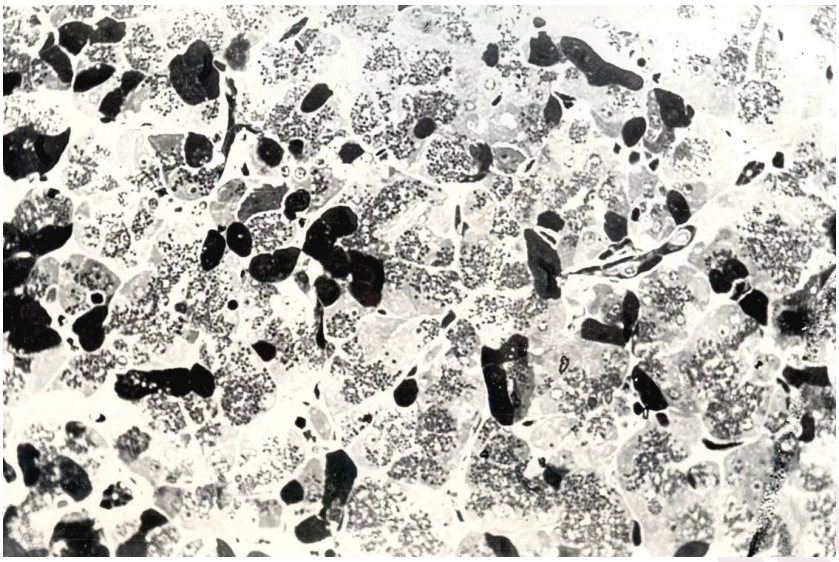

Fig. 2. Alloxan-treated group Rats pancreas. neuroendocrine cells are scattered throughout the pancreas. Shown magnification. 10-40. (Silver stain)

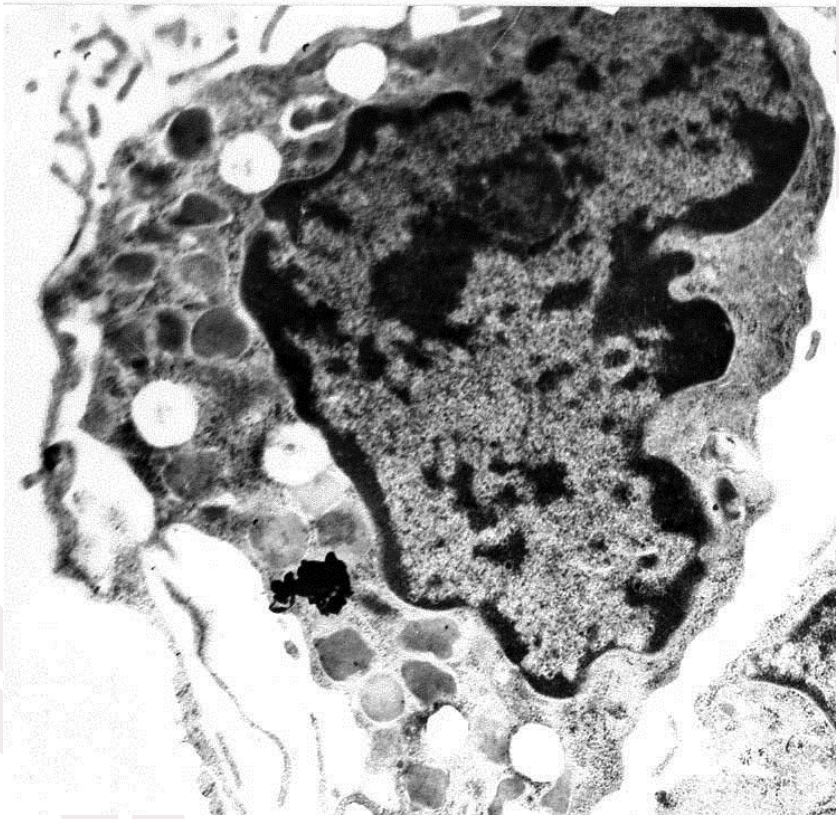

Fig. 3. Alloxan-treated group. Rats pancreas. Neuroendocrine cell. TEM-10000.

Strong expression of CgA was also detected in mainly alpha and partially remaining beta endocrine cells of pancreatic islets. Thus, during Alloxan diabetes of medium severity (glucose in the blood 230±20 mg%), in parallel with dystrophic, necrotic processes, CgA activation is observed in the cells of neuroendocrine, intralobular ducts, alpha and remaining beta cells of some islets. Based on the data obtained, it can be assumed that in Alloxan diabetes there is an increase in the intracellular function of NE cells, which affects the maturation of granules, secretion and intracellular storage of granules. The results of experimental study and available literature data [8,13,15,18], allow us to assume that CgA, in addition to their intracellular function, have another function, in particular, its breakdown products affect the development of diabetes.

Positive expression of CgA in intralobular epithelial cells of the pancreatic ducts suggests that it may also be an important marker for identifying pancreatic stem cells.

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SUMMARY

Using morphological research methods, the pancreas of rats with alloxan diabetes was studied. In the study, special attention was paid to studying the expression of chromogranin CgA. It is known that CgA-glycoprotein belongs to the group of granins. It is abundant in pancreatic neurons, neuroendocrine and endocrine cells and plays an important role in the generation of secretor granules. CgA-marker specially designed for these cells was used to determine the level of their expression, while silver staining was performed for better visualization of NE cells. Studies have shown that with moderate severity of Alloxan diabetes, a strong expression of CgA was observed in the NE of the remaining endocrine and also in the cells of the intralobular ducts of the pancreas. Based on the data obtained, it can be assumed that in Alloxan diabetes there is an increase in the intracellular function of NE cells, which affects the maturation of granules, secretion and intracellular storage of granules. Positive expression in intralobular epithelial cells of the pancreatic ducts suggests that CgA may also be an important marker for identifying pancreatic stem cells.

Keywords: Chromogranin, CgA, pancreas, Alloxan, Diabetes, Rat