BIOGERONTOLOGY

Effect of Tetrapeptide Pancragene on Functional Morphology of the Pancreas in Rats with Experimental Diabetes Mellitus

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The regulatory effect of tetrapeptide pancragene on the morphology and function of pancreatic tissues was shown in rats with diabetes mellitus, which suggests good prospects of this drug.

Key Words: *pancreas; diabetes mellitus; morphometric study; cell marker expression; tetrapeptide pancragene*

Diabetes mellitus (DM) is a highly prevalent disease and an important social and medical problem. According to the WHO data, more than 4% of the population suffer from this pathology [2,3]. The main trend in DM treatment is replacement hormone therapy and symptomatic treatment [1]. At the same time, pathogenetic therapy is required for inhibition of the progress of DM, prevention of complications, and improvement of the quality of life. The effects of pathogenetic treatment are related to modulation of functional activity of cells realizing the para- and autocrine regulation in tissues [5,6]. Tetrapeptide pancragene (Lys-Glu-Asp-Trp) regulating metabolic processes in the pancreatic tissue was synthesized at St. Petersburg Institute of Bioregulation and Gerontology [4].

We studied the effect of pancragene on functional morphology of the pancreas in rats with experimental DM.

MATERIALS AND METHODS

The study was carried out on 28 rats divided into 4 groups, 7 per group: 1) intact rats; 2) rats with DM induced by injection of 50 mg/kg streptozotocin; groups 3 and 4 animals received pancragene orally and intramuscularly, respectively, after the development of experimental streptozotocin-induced DM. The material was collected 20 days after streptozotocin injection and 10 days after pancragene treatment.

The fragments of the pancreas were fixed in 10% neutral formalin. The material was dehydrated in a Leica TP1020 automated station and embedded in paraffin. Paraffin sections (5 μ) were mounted on poly-L-lysine-coated slides (Sigma).

The sections were stained with hematoxylin and eosin for scanning microscopy. Immunohistochemical studies of A and B cells of pancreatic islets were carried out using monoclonal antibodies to insulin (Dako; 1:50) and glucagon (Dako; 1:75). The balance between proliferative activity and apoptosis in the acinar tissue was studied using monoclonal antibodies to the proliferating cell nuclear antigen (PCNA), clone PC10 (Dako; 1:300) and p53 proapoptoric protein (Dako; 1:50). Immune staining was identified by the avidine-biotin complex method with Dako LSAB 2 visualization kit.

Morphometry was carried out using a computer image analysis system consisting of a Nikon Eclipse

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E400 microscope, Nikon DXM1200 digital camera, PC, and Videotest-Morphologiya 4.0 software.

Ten visual fields were analyzed at ×400 in each case. Optical density of marker expression was expressed in arbitrary units. The area of expression was determined (the ratio of area occupied by immunopositive cells to total area of cells in visual field in percent). These parameters reflect the intensity of synthesis and accumulation of the studied hormones and signal molecules.

The differences between the groups (comparison of the means) were evaluated using Student's *t* test.

RESULTS

In group 1 (control), the connective tissue septae divide the glandular tissue in pancreatic preparations into lobules formed by cell accumulation (acinuses). The acinuses lay compactly and have no clear-cut orientation. The cells forming the acinuses are mainly pyramidal; round nuclei with large nucleoli are shifted to the basal part. Acidophilic granules are seen in the cytoplasm of acinar cells. Langerhans islets are formed by 4 types of endocrine cells: A cells (producing glucagon), B cells (producing insulin), D-cells (producing somatostatin), and PP-cells (producing pancreatic polypeptide). The bulk of cells (80%) are B cells occupying the central parts of the islets. A cells, producing glucagon, are located at the periphery of the islets.

Computer analysis of microscopic images showed that the mean area of insulin-producing B cell expression is significantly larger than the area of expression of glucagon-producing A cells (Figs. 1, 2). The mean optical density of immunohistochemical reaction for B cells was higher than for A cells.

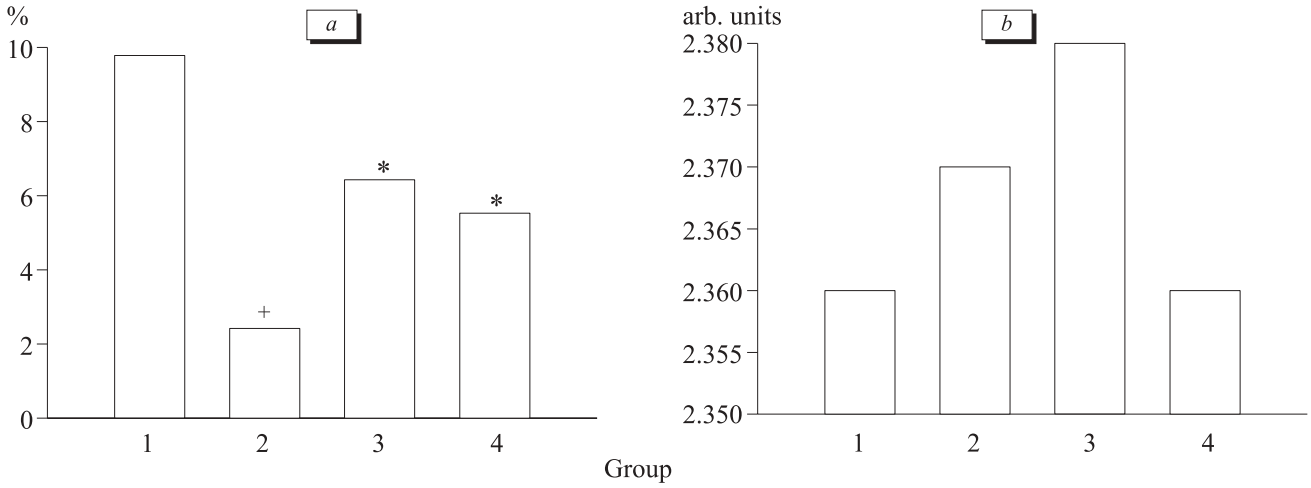

Fig. 1. Effect of pancragene on insulin expression by pancreatic B cells. *a*) mean area of insulin expression; *b*) mean optical density of insulin. Here and in Fig. 2: $p<0.05$ compared to +group 1; *group 2.

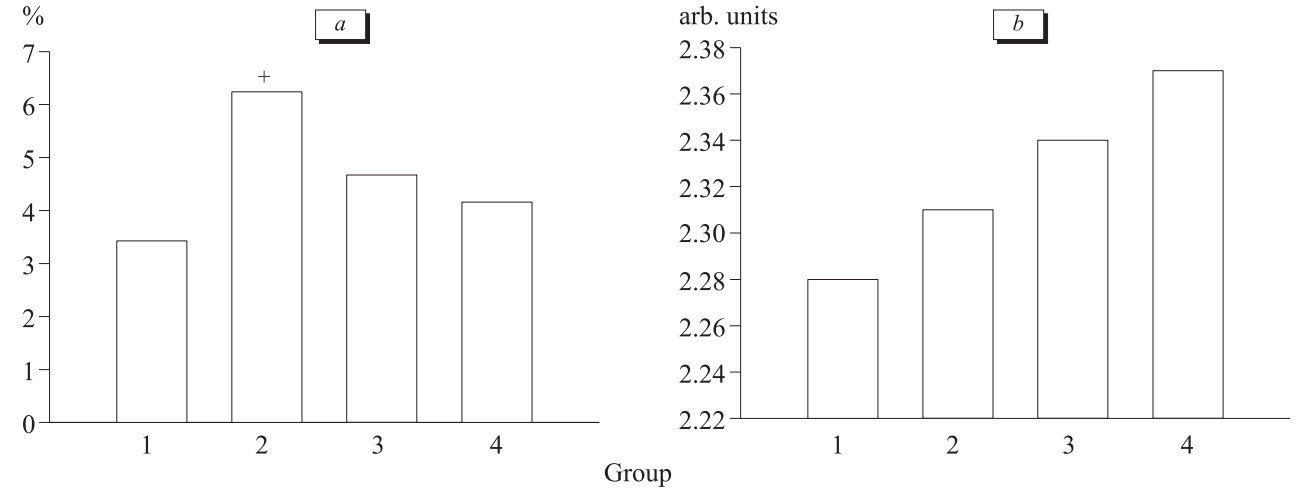

Fig. 2. Effect of pancragene on glucagon expression by pancreatic A cells. *a*) mean area of glucagon expression; *b*) mean optical density of glucagon.

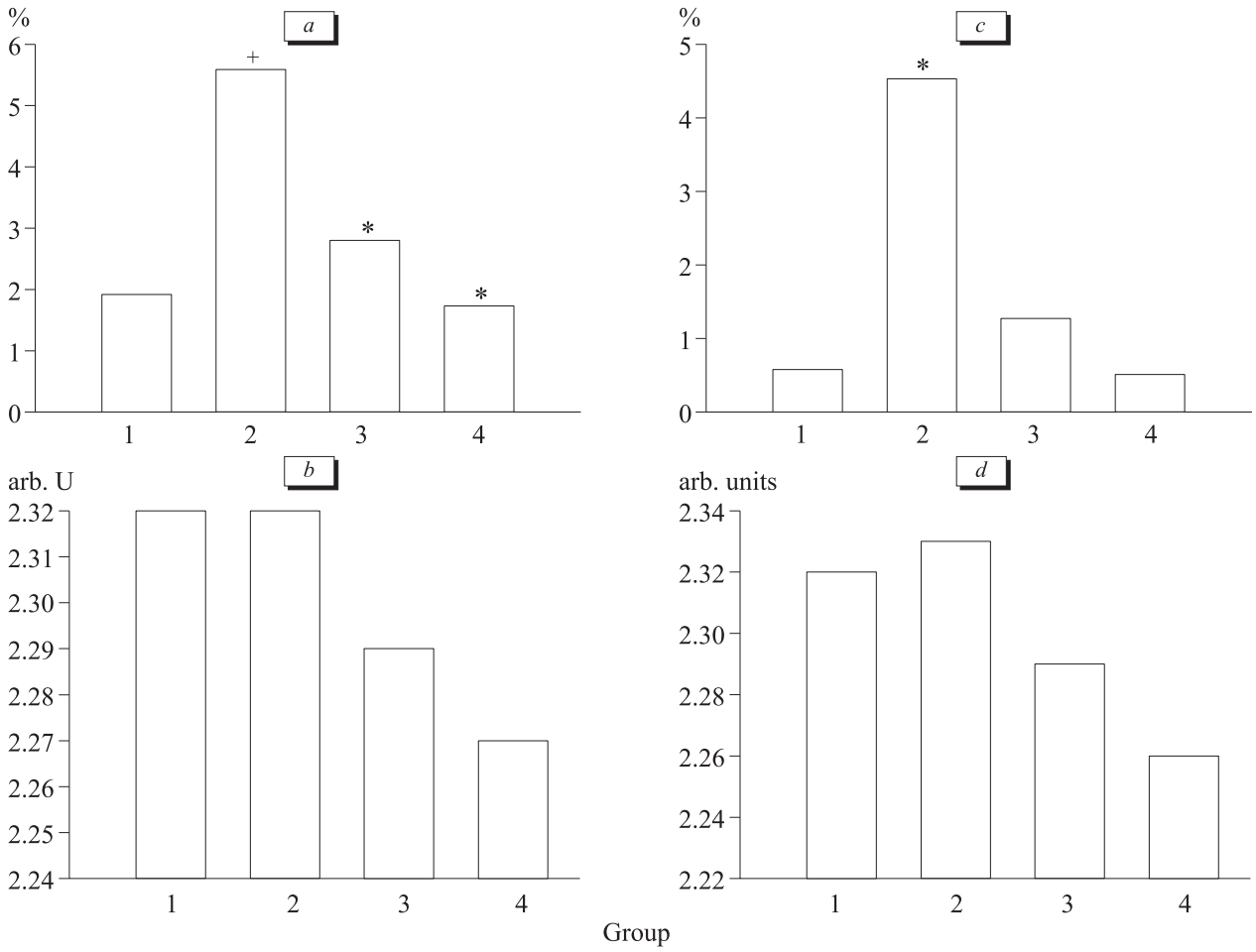

Fig. 3. Effect of pancragene on the expression of PCNA (proliferating cell marker; *a*, *b*) and p53 proapoptotic protein marker (*c*, *d*). *a*, *c*: mean area of expression: *b*, *d*: mean optical density.

The balance between proliferating exocrinocytes and apoptotic cells in the acinar tissue was as follows: the mean area of PCNA expression was significantly larger than of p53 expression (Fig. 3).

In group 2 with experimental DM, the mean area of insulin-producing B cells decreased under the effect of streptozotocin, while the mean area of glucagon-producing A cells increased 2-fold (Figs. 1, 2), the mean optical density of immunohistochemical reaction intensity remained virtually unchanged.

The mean area of PCNA expression in the acinar tissue increased, while its optical density remained unchanged (Fig. 3, *a*, *b*). The expression of p53 protein also increased (Fig. 3, *c*).

Hence, DM is associated with a decrease in insulin production by B cells and increase of glucagon synthesis by A cells. Exocrinocyte apoptosis and proliferation are activated.

Injection of pancragene was followed by pronounced compensatory changes in the pancreatic tissue; morphofunctional activity of the islet system and exocrine part of the gland (disordered under conditions of experimental DM) improved (Figs. 1-3).

Injection of streptozotocin led to the development of morphofunctional picture of DM in the pancreatic tissue. Streptozotocin stimulated proliferative activity and apoptosis induction in the acinar tissue. Pancragene injection led to the development of reparative and compensatory processes in the pancreas, which was seen from increased expression of insulin by B cells of the Langerhans islets and decreased production of glucagon by A cells. Proliferative activity of exocrinocytes and apoptosis approached the control levels.

The results indicate than pancragene is tropic to the pancreatic tissues and is characterized by a significant therapeutic effect restoring the functions of the endo- and exocrine compartments of the organ in oral and injection treatment.

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