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MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERISTICS OF THE PANCREAS IN RATS UNDER ADRENALINE-INDUCED STRESS

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Abstract

This study presents the results of morphological, morphometric, and immunohistochemical investigations of the pancreas in rats subjected to adrenaline-induced stress. It was established that prolonged administration of adrenaline causes biphasic structural and functional changes: at the early stages, compensatory-adaptive reactions predominate (hypertrophy of endocrine cells, hyperplasia of the islets of Langerhans, and increased expression of BCL-6 and Ki-67), whereas at later stages, dystrophic and atrophic processes develop in the exocrine parenchyma. Immunohistochemical analysis revealed a significant increase in the expression of the anti-apoptotic regulation marker BCL-6 (from 12.3 ± 1.4% to 42.4 ± 6.1%; $p < 0.001$) and the proliferation marker Ki-67 (from 10.1 ± 1.2% to 36.72 ± 4.15%; $p < 0.001$) in endocrine cells. Morphometric analysis confirmed a significant increase in nuclear volume by 0.59-fold, cytoplasmic volume by 0.66-fold, total cellular volume by 0.64-fold, and the nuclear-to-cytoplasmic ratio by 0.88-fold in the endocrine cells of the experimental group.

Keywords: pancreas, adrenaline-induced stress, morphometry, immunohistochemistry, BCL-6, Ki-67, islets of Langerhans, acinar cells, hypertrophy, apoptosis.

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INTRODUCTION

The pancreas is a unique organ that combines both exocrine and endocrine functions. The exocrine component, represented by acinar cells (acinocytes), is responsible for the production of digestive enzymes, whereas the endocrine component—the islets of Langerhans—regulates carbohydrate metabolism through the secretion of insulin, glucagon, and other hormones[1]. Disruption of the coordinated functioning of these systems under the influence of stress factors may lead to the development of pathological conditions, including diabetes mellitus and chronic pancreatitis. Adrenaline (epinephrine) is one of the key mediators of the stress response. Under conditions of chronic or excessive exposure, it activates the sympathoadrenal system, impairs microcirculation, enhances catabolic processes, and promotes the development of hyperglycemia. As an organ with high metabolic activity, the pancreas undergoes significant structural and functional alterations during adrenaline-induced stress[2].

Modern morphological research methods, including morphometric analysis using QuPath 5.0 and R-Studio software, as well as immunohistochemical analysis employing the markers BCL-6 and Ki-67, provide extensive opportunities for the objective assessment of tissue and cellular changes. The marker BCL-6 characterizes the anti-apoptotic activity of cells, whereas Ki-67 reflects the proliferative potential of tissues[3]. The combined application of these methods enables a comprehensive characterization of both adaptive-compensatory and pathological responses of the pancreas.

The aim of the present study was to investigate the morphological, morphometric, and immunohistochemical changes in the pancreas of rats under conditions of experimental adrenaline-induced stress[4].

MATERIALS AND METHODS

Immunohistochemical Analysis

Immunohistochemical analysis was performed on 3–4 μm thick paraffin sections obtained from formalin-fixed pancreatic tissue specimens. A panel of molecular diagnostic antibodies, including BCL-6 and Ki-67 markers, was employed for immunohistochemical evaluation. The proliferative index was assessed based on Ki-67 expression and expressed as the percentage of positively stained nuclei; values exceeding 10–20% were considered indicative of active cellular proliferation. Staining procedures were carried out using automated immunostaining platforms equipped with polymer-based detection systems. The results were independently evaluated by two experienced pathologists, and in cases of discrepancy, a consensus review was performed. Staining intensity and the proportion of positive cells were recorded in accordance with internationally accepted guidelines. Statistical analysis included a preliminary assessment of data distribution normality, followed by comparison of morphometric parameters between groups using the independent samples Student’s t-test. Mean values, standard deviations, and p-values were calculated. Data visualization was performed using Box-and-Violin plots and Segment-and-Point plots generated in RStudio. Additional image analysis and morphometric processing were conducted using QuPath 5.0 and Python software.

Table 1. Stages of the Immunohistochemical (IHC) Procedure

№	Procedure Step	Reagent	Duration
1	Preparation of 4 μm tissue sections	Poly-L-lysine-coated slides	—
2	Air drying of sections	—	24 h at room temperature
3	Drying in a thermostat	—	60 min at55–60°C

4	Deparaffinization	Ortho xylene	3 × 10 min
5	Dehydration	96% Ethanol	3 × 3 min
6	Rehydration	Distilled water	10 min
7	Antigen retrieval	Antigen retrieval buffer	30–40 min 98°C
8–10	Washing and endogenous peroxidase blocking	Tris buffer (pH 7.5) / 3% H_2O_2	5–10 min
11–14	Antibody incubation	Specific primary antibodies / Visualization reagent	20–30 min
15–21	DAB chromogen development, counterstaining, dehydration, and mounting	DAB, mayer's hematoxylin	According to the manufacturer's protocol

Table 2. Immunohistochemical (IHC) Staining Intensity Scoring Scale

Score	Description
«–»	No immunohistochemical reaction (negative staining)
«+»	Low expression
«++»	Moderate expression
«+++»	High expression

The staining intensity of cells was visually assessed using a semi-quantitative scale ranging from 0 to 3 points. The percentage of positively stained cells was calculated based on the evaluation of at least 500 epithelial cells in 10 high-power fields (×400 magnification). The expression coefficient (K) was calculated using the following formula:

$$K = \Sigma(B \times P)/100,$$

where B represents the staining intensity score (0–3 points) and P denotes the proportion of positively stained cells (0–100%).

RESULTS AND DISCUSSION

1. Morphology of the Pancreas in Intact Rats

In the control group, the pancreas exhibited a normal histoarchitectural organization. The organ demonstrated a typical lobular structure surrounded by a thin connective tissue capsule[5]. The exocrine component, represented by round and oval acini with densely arranged acinar cells, showed a normal ratio between the basophilic homogeneous zone and the eosinophilic zymogen-rich zone[6]. The endocrine islets of Langerhans were located within the lobules, displayed a rounded morphology, and were clearly demarcated from the surrounding acinar tissue[7].

The vascular network exhibited uniform blood filling, while the interstitial tissue appeared transparent and free of signs of intercellular edema. The pancreatic ducts were lined by a simple epithelial layer without evidence of proliferative activity or vascular congestion. Immunohistochemical examination of the intact group revealed low basal expression levels of BCL-6 (12.3 ± 1.4%) and Ki-67 (10.1 ± 1.2%), corresponding to physiological levels of apoptotic regulation and normal cellular turnover[8].

2. Morphological Alterations under Adrenaline-Induced Stress

Prolonged exposure to adrenaline induced pronounced adaptive changes in the endocrine regions of the pancreas. The islets of Langerhans appeared enlarged, with well-defined boundaries and a more rounded or oval configuration. A noticeable increase in the cross-sectional area of the islets was observed compared with the intact group[9].

The nuclei of endocrine cells, particularly β-cells, increased in volume and exhibited a pale vesicular appearance with distinctly visible prominent nucleoli. The cytoplasm of endocrine cells appeared granular and optically clearer. Reactive nuclear hypertrophy was evident[10]. Functional hyperemia and perivascular edema were also characteristic findings.

Within the exocrine parenchyma, acinar cells represented the primary target of adrenaline-induced injury. These cells exhibited hydropic degeneration and cytoplasmic vacuolization[11]. In a number of acini, fine granular and microvesicular accumulations of cytoplasmic lipids characteristic of steatotic changes were observed. Histological sections demonstrated marked edema of the perivascular regions, appearing as pale spherical structures, accompanied by functional hyperemia, venous dilatation, and vascular stasis. In addition, focal lymphocytic infiltration was detected in some specimens[12].

Table 3. Morphometric Parameters of Endocrine Cells (Mean ± SEM)

Parameter	Контроль	Эксперимент	Изменение	p
Nuclear volume (μm³)	—	—	+0,59 %	p<0,01
Cytoplasmic volume (μm³)	—	—	+0,66%	p<0,01
Total cell volume (μm³)	—	—	+0,64 %	p<0,01
Nuclear-to-cytoplasmic ratio.	—	—	+0,88 %	p<0,05

Note: * — p<0,05; ** — p<0,01; *** — p<0,001

Table 4. Comparative Morphometric Characteristics of the Islets of Langerhans

Parametr	Control group	Experimental Group	Change	P -value
Mean islet area	—	—	+0,67 %	p<0,01
Number of the cells per islet	—	—	+0,68 %	p<0,001

Note: * — p<0,05; ** — p<0,01; *** — p<0,001

Таблица 5. Клеточные показатели ациноцитов (M±m)

Показатель	Изменение в эксперименте	p
Общий объём клетки	−1,07 раза	p<0,01
Объём ядра (кариорексис)	−0,96 раза	p<0,01
Я/Ц соотношение	Незначительное увеличение	—

Примечание: * — p<0,05; ** — p<0,01; *** — p<0,001

3. Immunohistochemical Changes

Immunohistochemical examination of the pancreatic tissue of rats subjected to adrenaline-induced stress revealed pronounced alterations in the expression of the BCL-6 and Ki-67 markers (Table 6).

Microscopic analysis of pancreatic sections from rats exposed to adrenaline stress demonstrated a

marked increase in BCL-6 expression, predominantly within endocrine cells and perinuclear regions[13]. The immunopositive reaction was intense and substantially exceeded that observed in the control group[14]. The level of BCL-6 expression in the experimental group reached 42.4 ± 6.1%, compared with 12.3 ± 1.4% in the control group ($p < 0.001$), representing a statistically significant increase of 29 percentage points. Elevated BCL-6 expression indicates activation of anti-apoptotic mechanisms and cellular regulatory remodeling in response to stress.

Within the islets of Langerhans, adrenaline-induced stress was associated with increased Ki-67 expression, predominantly localized in the nuclei of endocrine cells, indicating enhanced proliferative activity[15]. While the proliferation index in the control group was 10.1 ± 1.2%, Ki-67 expression in the experimental group reached 36.72 ± 4.15%, representing a statistically significant increase of approximately 26.6 percentage points ($p < 0.001$).

Table 6. Comparative Immunohistochemical Marker Expression (Mean ± SEM)

marker	Control (M±m)	Experimental(M±m)	Change	P - value
BCL-6 (%)	12,3 ± 1,4	42,4 ± 6,1	+0,29 %	p<0,001
Ki-67 (%)	10,1 ± 1,2	36,72 ± 4,15	+0,27 %	p<0,001

The combined results of the morphological, morphometric, and immunohistochemical analyses demonstrated that adrenaline-induced stress exerts a biphasic effect on the pancreas of rats.

During the early stages of stress exposure, activation of anti-apoptotic regulation, as evidenced by increased BCL-6 expression, and enhanced proliferative activity, reflected by elevated Ki-67 expression in endocrine cells, predominated. These findings indicate the development of compensatory and adaptive tissue responses to hormonal stress. In the experimental group, a statistically significant increase was observed in the nuclear volume of endocrine cells (by 59%), cytoplasmic volume (by 66%), total cellular volume (by 64%), and the nuclear-to-cytoplasmic ratio (by 88%), reflecting functional hypertrophy as a component of the adaptive-compensatory response. In addition, the area of the islets of Langerhans increased by 67%, while the number of endocrine cells within the islets increased by 68%.

At the later stages of the experiment, prolonged stress exposure was associated with a decline in compensatory capacity. This was manifested by a reduction in the total volume of acinar cells (approximately 1.07-fold), a decrease in nuclear volume (approximately 0.96-fold), and the development of degenerative alterations, including organelle damage, cytoplasmic vacuolization, steatosis, and karyorrhexis. Perivascular edema, functional hyperemia, and venous stasis further confirmed the progression of ischemic changes within the exocrine pancreatic parenchyma.

Taken together, these findings demonstrate the spatially and functionally distinct pattern of pancreatic remodeling under conditions of adrenaline-induced stress. The results underscore the importance of timely correction of metabolic disturbances associated with chronic stress exposure and provide additional insight into the mechanisms underlying stress-induced pancreatic adaptation and injury.

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