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Original scientific paper

HISTOLOGICAL, ULTRASTRUCTURAL AND STEREOLOGICAL ANALYSES OF PANCREATIC ISLETS IN GLUCOCORTICOID-TREATED RATS

Radmila M. Glišić[1]*, Maja M. Čakić-Milošević[2], Mirela M. Ukropina[2], Ana S. Mitrovski Bogdanović[1], Vesna D. Stanković[3]

[1]University of Kragujevac, Faculty of Science, Department of Biology and Ecology, Radoja Domanovića 12, PO Box 60, 34 000 Kragujevac, Republic of Serbia
[2] University of Belgrade, Faculty of Biology, Institute of Zoology, Studentski trg 16, 11 000 Belgrade, Republic of Serbia
[3]University of Kragujevac, Faculty of Medical Sciences, Department of Pathology, Svetozara Markovića 69, 34000 Kragujevac, Republic of Serbia

*Corresponding author; E-mail: radmila.glisic@pmf.kg.ac.rs

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ABSTRACT. Dexamethasone is a synthetic glucocorticoid commonly used in both human and veterinary medicine. Prolonged treatment with dexamethasone can lead to serious metabolic side effects such as insulin resistance and diabetes. In this study, the morphological, immunohistochemical, and ultrastructural changes in the rat pancreatic islets were investigated after dexamethasone treatment (2 mg/kg, intraperitoneally for 12 days). The results showed that the volume density, mean diameter and profile area of islets were significantly increased in dexamethasone-treated animals. The volume density, profile area and mass of B-cells increased, whereas the volume density of A- and D-cells decreased, as did the number of A-cells per μm^2 of islet. Mild insulin immunopositivity of the centrally located B-cells together with altered ultrastructural features indicated their loss of function. The results of the present study suggest adverse effects of dexamethasone on pancreatic endocrine function that may ultimately seriously affect glucose homeostasis.

Keywords: pancreas, islet, dexamethasone, rat, histology, stereology.

INTRODUCTION

Glucocorticoids are steroid hormones of the adrenal cortex whose synthesis is controlled by the hypothalamic-pituitary-adrenal axis. They exert their effects by binding to specific intracellular receptors that act as nuclear transcription factors and regulate the transcription of target genes. The physiological actions of glucocorticoids include regulation of carbohydrate, lipid and protein metabolism, particularly in the context of stress response and maintenance of homeostasis. In addition, both naturally occurring glucocorticoids and their synthetic analogues are known for their potent anti-inflammatory and immuno-

suppressive effects, so they are commonly prescribed as medications for various chronic inflammatory diseases, allergies, autoimmune disorders, and even some tumors (LIU and GOODWIN *et al*., 2020). However, a growing body of data from the literature indicates that prolonged systemic administration of supraphysiological doses of corticosteroids is associated with adverse effects, some of which may result in serious long-lasting or permanent health problems (BUCHMAN, 2001; ORAY *et al*., 2016).

The endocrine pancreas is a diffuse organ consisting of numerous islets (islets of Langerhans) distributed throughout the organ. Each islet contains several types of endocrine cells whose secretory products are involved in the regulation of blood glucose levels and the proper functioning of the digestive system. The most important pancreatic cells are the most abundant insulin-producing B-cells, as well as glucagon-producing A-cells, and somatostatin-producing D-cells. Far less numerous and not always detectable in islets are E-cells and PP-cells, which are responsible for the synthesis and secretion of ghrelin and pancreatic polypeptide, respectively (BONNER-WEIR, 1991). The peptide hormone insulin is directly responsible for controlling peripheral glucose uptake. The effects of insulin are closely linked and coordinated with the effects of other pancreatic hormones, which together regulate blood glucose homeostasis (JAIN and LAMMERT, 2009). To maintain euglycemia, functional B-cell mass is dynamically regulated and determined by the size and number of B-cells. The number of these cells depends on the balance between the formation and death of B-cells. The main mechanism for the increase in B-cell mass in adulthood is mitotic proliferation of preexisting islet B-cells (DOR *et al*., 2004), but they can also be derived from other islet endocrine cell types (GUZ *et al*., 2001), from non-islet cells (LIPSETT and FINEGOOD, 2002), or from pancreatic or bone marrow stem cells (ZULEWSKI *et al*. 2001; IANUS *et al*., 2003).

Islet amyloid polypeptide (IAPP or amylin) is another secretory product of B-cells, in addition to insulin. IAPP is stored in the "halo" of secretory granules along with proinsulin and C-peptide and is involved in the regulation of glycemia and energy homeostasis (SCHERBAUM, 1998; LUTZ, 2012). In type 2 diabetes (T2D), secreted IAPP converts from the soluble to the insoluble fibrillar form, thus creating amorphous deposits between islet cells (JAIKARAN and CLARK, 2001). IAPP deposits are a typical histopathological finding in T2D and exert cytotoxic effects on B-cells via mechanisms that are not fully understood (WESTERMARK *et al*., 2011; AKTER *et al*., 2016).

T2D is a metabolic disorder with multifactorial etiology characterized by impaired carbohydrate and fat metabolism. It results from a complex interaction of genetic background and environmental risk factors such as poor diet, physical inactivity, and even emotional stress (POUWER *et al*., 2010; MUREA *et al*., 2012). Overt T2D is usually preceded by a prediabetic phase characterized by peripheral insulin resistance, which may last for years or even decades in humans (TABÁK *et al*., 2012). The relative insulin deficiency in insulin resistance is initially compensated by increased insulin secretion, but it eventually ends with exhaustion, progressive functional failure and loss of B-cells (KAHN, 1994; SACKS and MCDONALD, 1996; CERNEA and DOBREANU, 2013).

To date, several studies have shown that there is an association between the administration of synthetic glucocorticoids (mainly dexamethasone) on the one hand and an increase in circulating insulin levels, blood glucose levels, and free fatty acid concentrations on the other (BARBERA *et al*., 2001). However, data on the mode of action of pharmacological doses of glucocorticoids on pancreatic B-cells are contradictory: induction of both proliferation and apoptosis has been reported (RAFACHO *et al*., 2009; SUKSRI *et al*., 2021). The effects of glucocorticoids on other pancreatic endocrine cells of the pancreas are even less clear.

Therefore, the main objective of this study was to investigate the morphological, immunohistochemical, and ultrastructural changes in pancreatic endocrine cells in male Wistar rats treated with high doses of dexamethasone for a prolonged period of time. The

results obtained in this study should contribute to a better understanding of the effects of externally imposed imbalance of glucocorticoid hormones on the structural and functional characteristics of pancreatic islets and their endocrine cells, and also demonstrate the importance of monitoring potential adverse events during systemic glucocorticoid therapy.

MATERIALS AND METHODS

Animals and tissue preparation for light microscopy

Twenty male Wistar rats, aged 30 days and weighing approximately 128 g, obtained from the vivarium of the Vinča Institute of Nuclear Sciences (Belgrade, Serbia) were used for the experiment. Animals were housed individually in metabolic cages and kept at 21±1°C, on a 12 h/12 h light/dark cycle. Standard laboratory rat food and tap water were freely available. After a five-day acclimation period, the rats were randomly divided into two equal groups (control group and dexamethasone-treated group) of 10 animals each. Animals in the dexamethasone-treated group were injected intraperitoneally with 2 mg/kg dexamethasone dissolved in saline, while the control animals received saline only. After 12 days of treatment, the animals were fasted overnight, weighed and sacrificed under ether anesthesia. The experiment was performed according to the rules for animal care proposed by the Serbian Association for Laboratory Animal Science. The pancreas was quickly removed, washed in cold saline, freed from fatty tissue on ice, and weighed. The total volume of the pancreas was determined by the immersion method. Tissue samples were taken from five previously determined pancreatic segments. After fixation in Bouin's solution (8 hours), tissue samples were routinely embedded in paraffin and stained with hematoxylin and eosin.

Immunohistochemistry

Immunohistochemistry was performed on 5 μm thick serial sections. To detect A-, B- and D-cells, sections were immunostained using the peroxidase-antiperoxidase (PAP) immunohistochemical staining technique of STERNBERGER (1986). Sections were incubated with antisera against glucagon (1:200; A565, Dako Agilent, Santa Clara, CA, USA), insulin (1:1000; A564, Dako Agilent, Santa Clara, CA, USA) and somatostatin (1:500; by courtesy of Dr J. Rehfeld, Univ. Aarhus, Denmark). Avidin biotin complex (ABC) technique was used to localize PP-cells (1:1000, AB939-I, Sigma-Aldrich). For IAPP detection, sections were immunostained with anti-amylin (1:100; B29-1; Euro Diagnostica, Holland) by the labeled streptavidin biotin (LSAB) technique. ABC and LSAB techniques were used in conjunction with Histostain® Bulk Kit (95-6143-B; Invitrogen, Waltham, MA, USA).

Immunoreactive sites were visualized with DAB/H_2O_2 (0.7 mM diaminobenzidine-HCl and 0.002% H_2O_2 in 0.05 M Tris-HCl, pH 7.6), for 5 min in the dark. The result of immunohistochemical reaction was visible as brown deposits. The cell nuclei were counterstained with Mayer’s hematoxylin. Skin tissue completely clear of the antigens studied was processed in the same manner as pancreatic tissue and used as a negative control. Reagent control was performed on an additional slide with pancreatic tissue treated with non-immune serum instead of primary serum.

Electron microscopy

Small pieces of pancreatic tissue (approximately 1 mm^3) were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 3 hours, postfixed in 1% osmium tetroxide in the same buffer for 1 hour, dehydrated in a series of ethanol of increasing concentrations and propylene oxide, and embedded in Epon 812. Ultrathin sections were

prepared using an LKB ultramicrotome (Leica, Wetzlar, Germany), double contrasted with uranyl acetate and lead citrate, and examined under a Philips CM12 electron microscope (Eindhoven, Holland).

Stereological analysis

Stereological analysis was performed at the level of light microscopy. For this purpose, sections from five pancreatic segments of each animal were used. Each tissue block was serially sectioned, and three sections per block, taken at 50 μm apart, were stained with hematoxylin and eosin and analyzed with an Olympus light microscope (Olympus DP70, Hamburg, Germany). All quantitative data were obtained by a point-counting method using the Weibel multipurpose test system (42 points/21 lines, WEIBEL, 1979) at x200 magnification. These sections were used to determine islets volume and numerical density, absolute volume, total number, mean diameter and profile area. Based on the mean diameter, islets were classified as small (<200 μm), medium (200-400 μm) and large (>400 μm) (VON MACH *et al*., 2003). Immunohistochemically stained sections were used to determine volume density, mass, profile area, and number of each endocrine cell types per μm^2 of islet, as well as the total number of cells per islet. Standard stereological equations were used to calculate all of the above parameters, as previously described (UKROPINA *et al*., 2012).

Statistics

All data were subjected to a Student's t-test and results are presented as mean ± standard error of the mean (SEM). The normality of the distribution for the parameters studied was determined using the Kolmogorov-Smirnov test, before examining the differences. The analysis revealed the presence of a normal distribution. Statistical significance was set at $p<0.05$.

RESULTS

The results for body weight and pancreas weight and volume are shown in Table 1. As can be seen from this table, the final body weight of rats from the dexamethasone-treated group was significantly lower compared with the control group. Regular measurement of body weight showed that the control rats gained weight continuously during the experiment, whereas the animals from the dexamethasone-treated group began to lose weight on the fifth day of the study (data not shown), so that their average body weight at the end of the experiment was lower than at the beginning. The absolute weight of the pancreas and the volume of the pancreas were significantly reduced in the treated animals, whereas the relative weight of the pancreas was similar in the control group and the dexamethasone-treated group.

Table 1. Body weight and pancreatic weight and volume.
Results are presented as the mean ± SEM. * $p<0.05$.

	Control	**Dexamethasone**
Initial body weight (g)	127.0±1.53	128.6±1.43
Final body weight (g)	163.8±3.56	113.9±4.39*
Body weight change (g)	+36.7±3.69	-14.6±4.24*
Pancreatic weight (g)	1.0 ± 0.08	0.7 ± 0.09*
Relative pancreatic weight (%)	0.6±0.06	0.6±0.09
Pancreatic volume (cm^3)	0.9±0.08	0.7±0.09*

Histology and immunohistochemistry

In the control group, the endocrine islets appeared scattered randomly throughout the pancreas. They were mostly oval and of widely varying size. Immunohistochemical staining showed that islets in the control group contained a core of strongly insulin-immunopositive B-cells surrounded by a mantle of non-B cells (Fig. 1a). In the dexamethasone-treated group, B-cells were also present in the mantle zone (Fig. 1b). In this group, especially in the large islets, the centrally located B-cells showed weak immunoreactivity, which may indicate depletion of insulin-containing granules, whereas the immunoreactivity of the peripherally located B-cells was much stronger. Insulin-positive hypertrophied cells, single or in small clusters, were also detected in the exocrine pancreatic tissue of dexamethasone-treated rats (Fig. 1c).

Uniformly weak IAPP immunoreactivity was found in the islets of control rats (Fig 1d). However, a stronger IAPP reaction was detected in all islets of dexamethasone-treated rats (Fig. 1e).

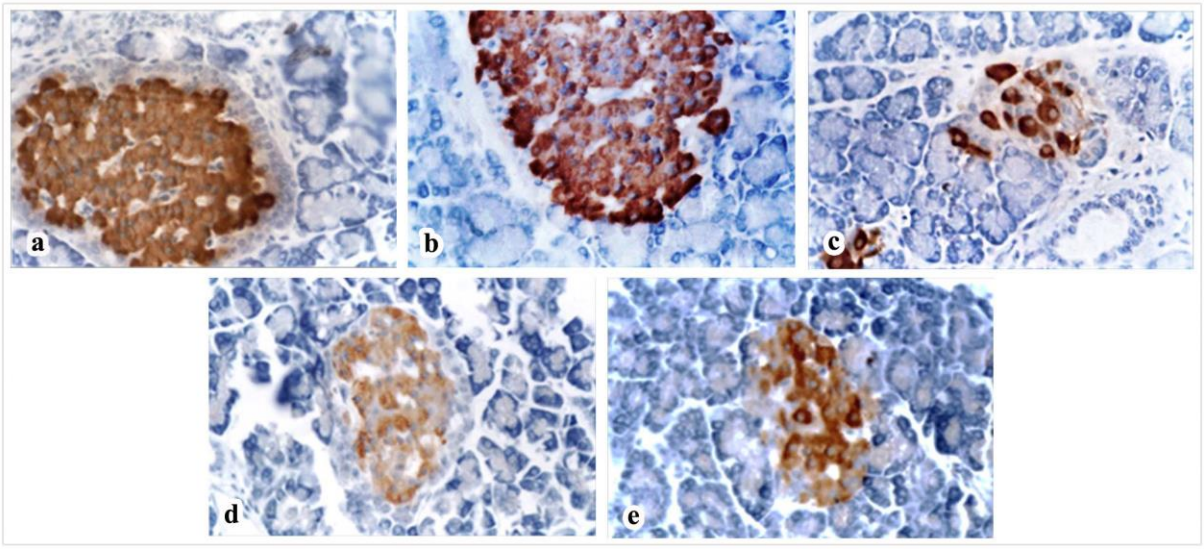

Figure 1. Insulin- and IAPP-immunopositivity of pancreatic islets in control and dexamethasone-treated rats. In the control group, highly immunopositive B-cells in the centre of the islet were surrounded by a mantle zone of non-B-cells. After treatment with dexamethasone (b) core B-cells were weakly immunopositive, whereas the cells in the mantle zone were intensely stained. Clusters of insulin-producing cells were detected outside the islets in dexamethasone-treated rats (c). Immunostaining for IAPP was stronger after dexamethasone treatment (e) than in control animals (d). Magnification x400.

A ring of A-cells immunopositive for glucagon was typically continuous in control rats (Fig. 2a), but not in dexamethasone-treated rats (Fig. 2b). In both groups, D- (Fig. 2c, d) and PP-cells (2e, f) were rare and distributed at the periphery of the islets.

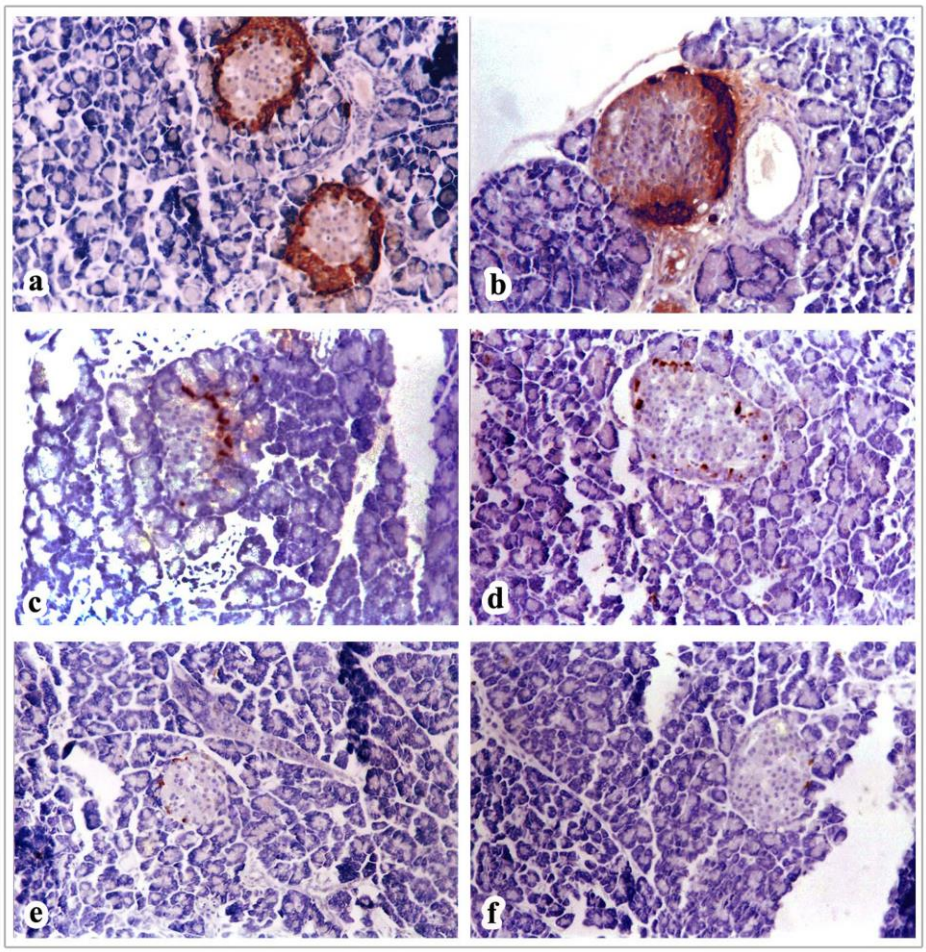

Figure 2. A-, D- and PP-cells of pancreatic islets in control and dexamethasone-treated rats. Strong and diffuse glucagon-immunolabeling throughout the peripheral ring of the pancreatic islet in control animals (a) and discontinuity of immunoreaction in dexamethasone-treated rats in the same area (b); D- (c, d) and PP-cells (e, f) were rare in both control (c, e) and experimental (d, f) groups and were located at the periphery. Magnification x200.

Ultrastructural analysis

Electron microscopic analysis confirmed that B-cells in the control group had a normal ultrastructure characterized by spherical euchromatic nuclei and numerous mature granules with crystalline cores and wide translucent halo (Fig. 3a). In the dexamethasone-treated group, some B-cells were located at the periphery of the islets, in close proximity to the exocrine part of the pancreas (Fig. 3b). The subcellular structure of these B-cells generally corresponded to the subcellular structure of B-cells from control rats. In contrast, the centrally located B-cells (Fig. 3c) had slightly irregular nuclei, dilated cisterns of the endoplasmic reticulum and Golgi complex and swollen mitochondria. Empty granules and immature granules without a halo or with a narrow halo were frequently seen in these cells.

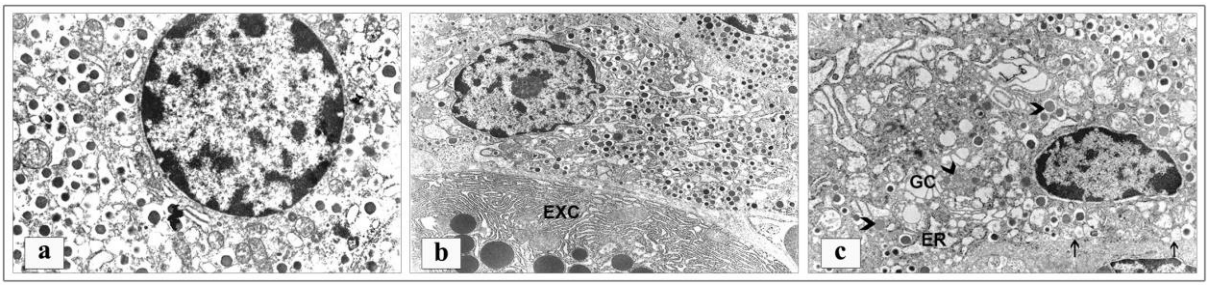

Figure 3. B-cells of pancreatic islets in control and dexamethasone-treated rats. B-cell from the control group (a) surrounded by other endocrine cells, contains numerous fully mature, electron-dense granules. In the dexamethasone-treated group, the B-cell at the periphery of the islet (b) showed normal ultrastructure; EXC - exocrine cell. B-cells at the centre of the islet (c) typically had dilated endoplasmic reticulum (ER) and Golgi complex (GC) and contained many immature (arrowhead) or empty (arrow) secretory granules. x8000 (a), x5000 (b), x6300 (c).

Stereological analysis

The results of the stereological analysis are shown in Table 2. The volume density, mean diameter and profile area of islets were significantly increased in dexamethasone-treated rats. The diameters of pancreatic islets showed great variability in both groups, ranging from 28 μm to 475 μm.

Table 2**.** Stereological analysis of pancreatic islets from control and dexamethasone-treated rats. Results are presented as the mean ± SEM. * $p<0.05$.

	Control	**Dexamethasone**
Volume density (mm^0)	0.005± 0.0005	0.011 ± 0.001*
Absolute volume (cm^3)	0.005± 0.0001	0.007±0.002
Numerical density (N/cm^3)	5423±837	7731± 1061
Total number	4840 ± 522	5007 ± 972
Mean diameter (μm)	102.9 ± 3.74	125.3 ± 4.93*
Mean profile area (μm^2)	6391 ± 369	9062 ± 538*

The frequency distribution of islet diameter and the distribution of total islet volume among islets of different diameter classes are shown in Fig. 4. The distribution of islets by diameter (Fig. 4a) was generally similar in both groups, although in the dexamethasone-treated group small islets (<200 μm) were slightly less frequent than in the control group (83.3% vs. 94%), and those of medium size (200-400 μm) were more frequent than in the control group (16.5% *vs*. 6%). In the dexamethasone-treated group, large islets (>400 μm) were rare (0.2%), whereas they were absent in the control group. Considering the proportion of islets of a given size in the total volume of the endocrine pancreas (Fig. 4b) most of the endocrine volume in the control group consisted of small islets, whereas in the dexamethasone-treated group there was a higher frequency of medium-sized islets, although large islets were also conspicuous.

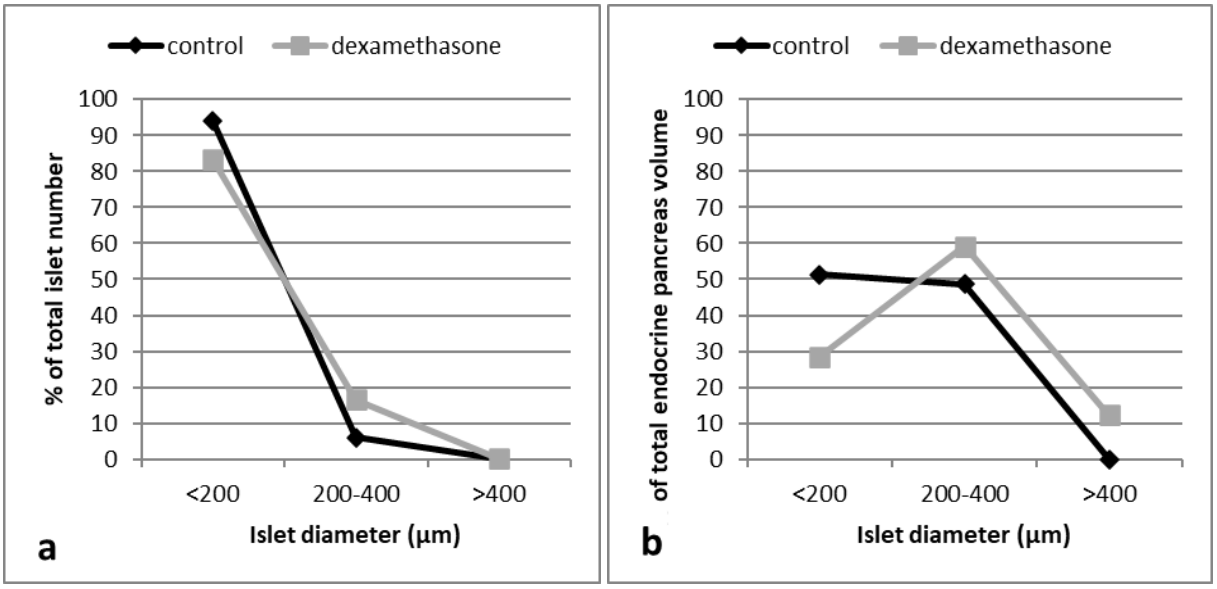

Figure 4. Frequency distribution of islet diameter (a) and the participation of islets of different diameter classes in total endocrine pancreatic volume (b).

Table 3. Volume density, mass, profile area, number of each endocrine cell type per µm^2 of islet and total number per islet in control and dexamethasone-treated rats. Results are presented as the mean ± SEM. * $p<0.05$.

	Control	***Dexamethasone***
	Volume density (mm^0)	
A-cell	0.38±0.027	0.18±0.029*
B-cell	0.60±0.011	0.84±0.011*
D-cell	0.13±0.012	0.09±0.014*
PP-cell	0.20±0.031	0.14±0.030
	Mass (µg)	
A-cell	0.68±0.08	0.94±0.27
B-cell	1.36±0.14	3.02±0.48*
D-cell	0.16 ±0.25	0.25±0.048
PP-cell	0.28 ± 0.03	0.44±0.07
	Profile area (μm^2)	
A-cell	81.9±3.51	87.4±2.39
B-cell	138.5±3.36	217.7±14.48*
D-cell	122.9±4.95	142.6±8.10
PP-cell	78.3±4.3	87.5±4.2
	Number/μm^2 of islet	
A-cell	0.0049±0.00042	0.0022±0.00033*
B-cell	0.0046±0.00069	0.0043±0.00019
D-cell	0.0009±0.00014	0.0007±0.00020
PP-cell	0.0025±0.00037	0.0016±0.00030
	Total number/islet	
A-cell	21.5±1.82	16.5±2.57
B-cell	22.1±2.19	24.6±1.77
D-cell	3.6±0.33	3.8±0.18
PP-cell	11.3±1.76	9.6±1.58

The results of stereological analysis of islets by cell type are shown in Table 3. It was found that the volume density, mass and profile area of B-cells were significantly increased after treatment with dexamethasone. On the other hand, the volume density of A-cells and their number per μm^2 of islets were significantly decreased compared with the control group. Finally, the volume density of D-cells was significantly lower than in the control group. All stereological parameters determined for PP-cells remained within control ranges.

No significant differences were found when comparing the total number of specific cells between the two groups of rats. However, a tendency was observed for the number of B-cells to increase and the number of A-cells to decrease.

DISCUSSION

The aim of this study was to investigate the effects of prolonged treatment with high doses of dexamethasone on the morphological, immunohistochemical, and ultrastructural changes of the endocrine pancreas in male Wistar rats. Dexamethasone is a highly potent synthetic glucocorticoid that was discovered more than 60 years ago (ARTH *et al*., 1958) in the search for anti-inflammatory and immunosuppressive medication with better therapeutic performance and fewer side effects compared with glucocorticoid-based therapeutics already known at that time. Today it is one of the most commonly prescribed medications used for the management of various diseases and conditions, some of which are chronic and require long-term therapy. However, systemic administration of glucocorticoids to human patients, either over a long period of time or in high doses, is associated with an increased risk of developing various side effects including those on the endocrine system and metabolism (ORAY *et al*., 2016). Based on the observed strong association between dexamethasone treatment and insulin resistance, several animal models of insulin resistance have been developed (HOENIG *et al*., 2000; MARTÍNEZ *et al*., 2016). It has been previously shown that dexamethasone can impair pancreatic endocrine function, leading to hyperglycemia and hyperinsulinemia associated with an increase in the proliferation, size and mass of insulin-producing B-cells (RAFACHO *et al*., 2009). However, data on possible changes at the level of whole islands, and other endocrine cell types during dexamethasone treatment are still largely lacking.

In the current study, it was found that dexamethasone at a dose of 2 mg/kg for 12 days, significantly decreased the final body weight of treated rats. While the control animals gradually gained weight over the course of the experiment, as expected, rats in the dexamethasone-treated group lost weight. This result is consistent with that of MARTÍNEZ *et al*. (2016) who also reported a reduction in body weight in rats treated for 10 days with half the dose of dexamethasone used in our study. This result may be explained by the fact that dexamethasone impairs the regulation of appetite and food intake at the level of the hypothalamus (CHRUVATTIL *et al*., 2017). In addition, dexamethasone suppresses the synthesis of proteins and promotes their breakdown (MALKAWI *et al*., 2018), so the reduction in muscle mass could be another factor contributing to weight loss.

Regarding the weight and volume of the pancreas, this study showed that the absolute pancreatic weight and volume decreased significantly after treatment with dexamethasone, whereas no difference was found for the relative weight of the pancreas. This finding suggests that the decrease in pancreatic weight was in proportion to the weight loss of the whole body. Indeed, both values decreased by approximately 30% compared with control values. Weight changes are closely related to changes in functional capacity and morphological remodeling of the organ. The consistent relationship between body and pancreatic weights suggests that dexamethasone neither suppresses nor stimulates the pancreas as a whole organ. This is understandable since the exocrine pancreas, which consists of acinar, centroacinar, and ductal cells, accounts for approximately 90% of pancreatic mass (PANDIRI, 2014) and is not

considered a primary target for dexamethasone action. On the other hand, the endocrine pancreas accounts for only 1-2% of the total pancreatic mass, so it is unlikely that even appreciable changes in its size would significantly affect the mass of the entire organ.

After treatment with dexamethasone, stereological examination of the endocrine pancreas showed islet hypertrophy and consequently an increase in islet volume density. This increase is accompanied by hypertrophy of B-cells without any change in their number. It is therefore likely that B-cell hypertrophy is a predominant mechanism of islet enlargement under the treatment used in this experiment. Although we observed small clusters of insulin-positive cells in the exocrine tissue of dexamethasone-treated rats, neogenesis of B-cells/endocrine islets, if present, does not appear to be sufficient to reflect the proportion of smaller (newly formed) islets in the total number and volume of islets.

One of the findings of our study is that administration of dexamethasone slightly shifted the islet size distribution toward larger islets and consequently resulted in different participation of small, medium, and large islets in the total pancreatic volume. A decrease in the number of small islets and an increase in the number of medium islets and the occurrence of large islets were observed. Similar results were reported by ALANENTALO *et al*. (2010) during the progression of type 1 diabetes in NOD mice. These results suggest a regenerative process aimed at the formation of larger islets to compensate for the elimination of the small islets. Possible biological processes affecting islet size include development, fusion, growth, and shrinkage due to cell death within islets (JO *et al*., 2012). The decrease in the abundance of small islets suggests that they are more sensitive to the effects of dexamethasone.

Otherwise, pancreatic islets which consists of a few to several thousand cells, exhibit a wide range of sizes. A comparative study of humans, monkeys, pigs, rabbits, and mice found that islets are similar in size, although they differ in overall body and pancreas size and total B-cell mass (KIM *et al*., 2009). This suggests that there is an optimal functional islet size. Some physiological and pathological conditions such as pregnancy, ageing, obesity, and diabetes affect islet size distribution (JO *et al*., 2012), which is related to changes in B-cell mass involved in glucose homeostasis and suggests that islet development is stochastic.

Changes in islet size are also related to changes in the vascular organization of the endocrine pancreas, as there are differences in the pattern of vascularization of the islets of different sizes (BONNER-WEIR and ORCI, 1982). Changes in islet size distribution could be an adaptive response to impaired glucose homeostasis after exposure to dexamethasone. Some studies have also reported that the physiological properties of B-cells interacting with each other via gap junctions (PÉREZ-ARMENDARIZ *et al*., 1991) depend on the size of the cell cluster (JO *et al*., 2005) and that the electrically coupled B-cells secrete insulin more effectively than single cells (SHERMAN *et al*., 1988). Based on these facts, changes in the distribution of islets with different diameters would be associated with changes in the function of the endocrine pancreas under the conditions of the applied treatment.

Another finding of this study was that after treatment with dexamethasone, B-cells normally located in the center of the islet also appeared in the mantle zone near the exocrine acinar cells. Because our stereological study did not reveal a significant increase in the total number of B-cells, we speculate that these B-cells in the mantle zone are repositioned core B-cells. Moreover, we have shown here that mantle B-cells in large islets have higher insulin immunopositivity than core B-cells, implying that they are more actively involved in insulin biosynthesis and secretion. Some studies, particularly in mouse models, have found a link between changes in islet cell organization and decreased insulin secretion and have attributed this to a decrease in homologous connections between B-cells (BOSCO *et al*., 1989; GANNON *et al*., 2000). However, in a similar experimental setup, we have already shown that insulin levels were increased in both serum and pancreas (GLIŠIĆ *et al*., 2011), so we can conclude that the relocation of cells in some islets did not negatively affect total insulin production. On the other hand, the differential immunoreactivity of B-cells in the large islets supports

previous data on the functional diversity of pancreatic B-cells (STEFAN *et al.*, 1987; PIPELEERS, 1992; VAN SCHRAVENDIJK *et al*., 1992) and suggests a functional switch between the core and mantle cells.

Regarding the other endocrine cell types, our quantitative analysis showed changes only in the volume density of A- and D-cells (both decreasing) and the number of A cells per μm^2 of islet (decreasing). The results on decreased volume density of A- and D-cells after dexamethasone treatment are consistent with those obtained previously by others in various mouse models of diabetes/insulin resistance (BAETENS *et al*., 1978; DIANI *et al*., 1987) and are in agreement with the increase in the islet size (diameter, profile area) in this group. A decrease in the number of A-cells accompanied by an increase in the number of B-cells, although not statistically significant, could indicate A-to-B-cell transdifferentiation aimed at creating an insulin-producing apparatus in the altered tissue context (LU *et al.*, 2014). A-to-B transdifferentiation may also explain the appearance of insulin-immunopositive cells located in the A-restricted zone at the periphery of islets from dexamethasone-treated rats. According to the results of this study, the applied treatment did not induce a significant response in D- and PP-cells.

In human pathology, IAPP serves as a marker for T2D (WESTERMARK, 1973; CLARK *et al*., 1988). IAPP depositions impairs normal islet architecture and is associated with decreased B-cell numbers (JURGENS *et al*., 2011). IAPP is produced by B and D cells and deposited in the intercellular space (MULDER, 1997; WANG *et al*., 1997). In the present experiment, the islets of the control group of rats showed weak and uniform IAPP immunopositivity, in contrast to strong immunostaining in all islets of the dexamethasone-treated rats. These results are consistent with literature data showing that dexamethasone treatment increases IAPP mRNA, IAPP secretion, and IAPP-like immunoreactivity in the pancreas and plasma (JAMAL *et al*., 1990; MULDER *et al*., 1995). The accumulation of IAPP may be related to the functional depletion of centrally located B-cells in the large islets observed in this study, but had no adverse effect on B-cell number.

In conclusion, the results of this study indicate that treatment with dexamethasone leads to structural and functional changes in the endocrine pancreas, potentially resulting in disturbances of glucose homeostasis. B cells, which most likely reach their functional limits in this experimental context, are the primary targets for dexamethasone effects.

Acknowledgments

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