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Effect of Hyperglycemia on the Excretory Ducts of the Submandibular Gland (Histologic Study)

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Abstract. The paper highlights the peculiarities of histological changes in different subdivisions of the intralobular duct of the submandibular gland in rats in case of experimental hyperglycemia.

Materials and methods. The study included 40 male Wistar rats weighing 230 to 250g. Experimental hyperglycemia was induced by a single intraperitoneal administration of streptozotocin. Biochemical and morphological investigations were conducted; the morphometric analysis was carried out.

Results. Since the 28th day of the experiment, on the background of dynamic increase in the levels of glucose and glycated hemoglobin in the blood, there was observed the development of dystrophic changes in epithelial cells of the granular and striated ducts being accompanied by a gradual decrease in epithelial cell height by 10.28 – 29.46% and 10.77 – 28.28%, respectively. Morphological changes in the intercalated ducts were detected later – since the 42nd day of the experiment and the decrease in their epithelial cell height – by 15.60%, was seen on the 70th day only.

Conclusions. Morphological changes in different subdivisions of the intralobular duct are of dystrophic nature and can be histologically detected since the 28th day of the experiment; they depend on the duration of hyperglycemia and are accompanied by a dynamic decrease in epithelial cell height.

Keywords: hyperglycemia; submandibular gland; excretory ducts.

Problem statement and analysis of the recent research. Diabetes mellitus, due to its morbidity rate as well as the frequency of diabetes-related deaths and disability worldwide is becoming a global epidemic [1-3]. The disease is mainly characterized by chronic hyperglycemia which is caused by insulin deficiency (type I diabetes mellitus) or a combination of factors reducing its activity (type II diabetes mellitus) [4, 5]. Hyperglycemia affects structural components of various organs and systems in different ways, namely through activating the sorbitol pathway and protein kinase C, increasing oxidative stress, reducing the levels of vasodilators, changing Na^+, K^+-ATPase activity or causing non-enzymatic glycation of proteins [6, 7]. The kidneys, micro- and macro-blood vessels, peripheral nerves and retina [8] are progressively damaged; salivary glands, the submandibular gland in particular, are damaged as well: it deteriorates dental status in patients due to the development of hyposalivation and xerostomia [9-11]. It should be noted that the composition of saliva and its buffering properties are changed by the excretory ducts of the gland [12-16]. However, the data on the effects of hyperglycemia on the histological structure of these components of the submandibular gland parenchyma are controversial [17, 18].

Materials and methods

The experiment was carried out in the Educational and Scientific Laboratory of Morphological Analysis and the Center of Bioelementology of the Ivano-Frankivsk National Medical University in accordance with EU Directive 2010/63/EU for animal experiments [19]. The study included 40 male Wistar rats weighing 230 to 250g (the experimental group – 20 animals, the control group – 20 animals) kept under standard vivarium conditions with free access to water. Experimental hyperglycemia was induced by a single intraperitoneal administration of streptozotocin (Sigma, USA) at a dose of 60 mg/kg body weight dissolved in citrate buffer (pH 4.5). Animals of the control group were injected with an equivalent amount of citrate buffer.

The samples were collected in the morning (on an empty stomach) every 2 weeks after streptozotocin administration. Biochemical investigation included the determination of the levels of glucose and glycated hemoglobin in the blood. Morphological investigation of the excretory ducts of the submandibular gland was done on histological sections stained with eosin and hematoxylin [20] and semi-thin sections stained with methylene blue (the fixation of the material in a 2.5% glutaraldehyde solution followed by post-fixation in a 2% osmium tetroxide (OsO_4) solution [21]).

The morphometric analysis (the determination of epithelial cell height in different subdivisions of the intralobular duct) was made on histological sections in ImageJ v. 1.48 [22]. Statistical analysis was conducted using the R software v. 3.0 [23]. The data of descriptive statistics are presented as the mean±standard deviation (Mean±SD). The data of the control and experimental groups were compared using the Mann-Whitney-Wilcoxon test; the difference was considered statistically significant at $p<0.05$.

Results and discussion

On the 14th day after streptozotocin administration, on the background of dynamic increase in the levels of glucose and glycated hemoglobin in the blood (by 2.83 and 3.32 times compared to the control group; $p<0.05$), at the light-optical level there were no changes in the structure of the excretory ducts. The aforementioned data were confirmed by the investigation of semi-thin sections as well: moderate intensity of staining the epithelial cell cytoplasm in the excretory ducts with methylene blue was found; most epithelial cells of the granular ducts were filled with dark granules; in the striated ducts, basal striation was preserved (Fig.1). The height of epithelial cells in the intercalated and granular ducts remained unchanged compared

On the 28th day after streptozotocin administration, the levels of glucose and glycated hemoglobin in the blood exceeded those in the control group by 3.84 and 4.19 times, respectively ($p>0.05$). Despite this fact, changes in the histological structure of the intercalated ducts were not observed; epithelial cell height did not differ from that in the control group ($p<0.05$). Epithelial cells of the granular ducts were characterized by the presence of fine eosinophilic granules while epithelial cells of the striated ducts

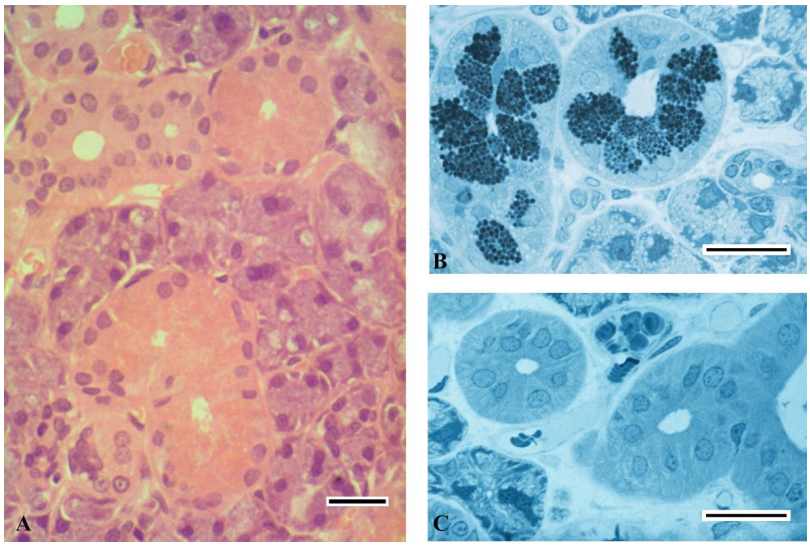

Fig. 1. Relatively unchanged structure of the excretory ducts of the submandibular gland in a rat on the 14th day of the experiment at the microscopic and sub-microscopic levels. A – staining with hematoxylin and eosin; B, C – semi-thin sections stained with methylene blue. Scale bar 20μm

Table 1. Epithelial cell height (μm) in the ducts of the rat submandibular gland during different time periods (Mean±SD)

Time period, day	Animals	Ducts		
		Intercalated	Granular	Striated
14th	Control	4.36±0.87	16.98±2.35	13.97±2.30
	Experimental	4.22±0.70	17.60±2.17	15.18±2.51*
28th	Control	4.34±0.91	17.02±2.26	14.02±2.24
	Experimental	4.21±0.89	15.27±2.90**	12.51±1.48***
42nd	Control	4.35±0.90	16.95±2.55	13.96±2.45
	Experimental	4.43±0.78	13.43±1.59***	11.54±1.75***
56th	Control	4.28±0.86	17.67±2.50	14.53±2.35
	Experimental	4.17±0.93	12.50±1.93***	11.81±2.61***
70th	Control	4.23±0.87	17.65±2.32	14.57±2.60
	Experimental	3.57±0.53***	12.45±2.59***	10.65±2.07***

Note: *, **, *** – $p<0.05$, $p<0.01$, $p<0.001$ compared to the control group

were characterized by slightly pronounced basal striation (Fig. 2). Epithelial cell height in these ducts reduced by 10.28 and 10.77% ($p<0.01$ – 0.001) (Table 1). The disruption of the structural organization of the aforementioned subdivisions of the intralobular duct in the early stages of hyperglycemia development was observed by L. S. Cutler et al. as well [25]. At the same time, the authors noted that changes were of a focal nature. In our opinion, changes in the structure and morphometric parameters are caused by both direct effect of hyperglycemia and indirect one due to diabetic microangiopathy [8, 26-28]. The development of the latter is explained by the occurrence of perivascular swelling being observed at the sub-microscopic level. It should be mentioned that the excretory ducts are characterized by low water permeability [29]. Thus, detected dystrophic changes, probably, lead to the alterations in electrolyte composition of secondary saliva [3, 15].

On the 42nd day after streptozotocin administration, the studied biochemical parameters continued to increase in comparison with the control group, namely the level of glucose in blood increased by 4.54 times and the level of glycated hemoglobin increased by 4.79 times ($p<0.05$). At the same time, in the excretory ducts, vacuolization of epithelial cell cytoplasm was observed indicating their damage [30]. In addition, in the granular ducts, a decrease in filling with secretory granules was visually observed which is consistent with the data obtained by L. C. Anderson et al [17]. In comparison with the control group, epithelial cell height in the intercalated ducts remained unchanged

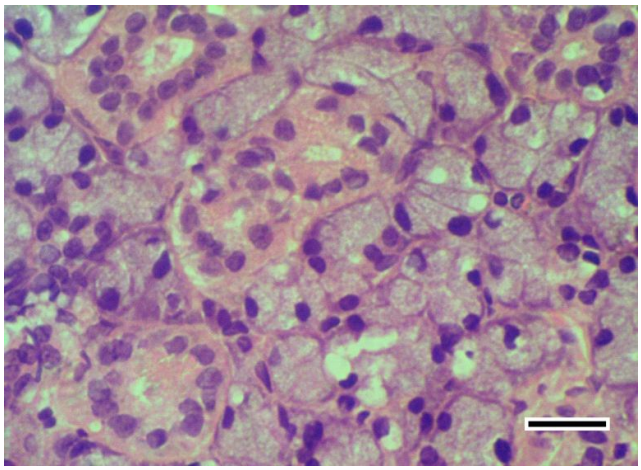

Fig. 2. Histological structure of the intralobular duct subdivisions of the rat submandibular gland on the 28th day after streptozotocin administration. Staining with hematoxylin and eosin. Scale bar 20μm

while epithelial cell height in the granular and striated ducts continued to reduce (by 20.71 and 17.34%, respectively; $p<0.001$) (Table 1).

In the last observation periods (on the 56th and 70th days), the levels of glucose (23.88 – 21.34 mmol/l; $p>0.05$) and glycated hemoglobin (9.52 – 9.94%; $p>0.05$) in the blood remained stable compared to the 42nd day of the experiment. As during the previous observation period, basal striation of the striated ducts was slightly pronounced; in the granular ducts, epithelial cell cytoplasm contained fine eosinophilic granules. However, vacuolization of different subdivisions of the intralobular duct was less intense (Fig. 4). In addition, epithelial cell height in the intercalated, granular and striated ducts reduced by 15.60, 29.46 and 28.28%, respectively ($p<0.001$) compared to the control group (Table 1).

Conclusions

Morphological changes in different subdivisions of the intralobular duct are of dystrophic nature and can be histologically detected since the 28th day of the experiment; they depend on the duration of hyperglycemia and are accompanied by a dynamic decrease in epithelial cell height.

Prospects for further research

An important aspect of understanding salivary gland dysfunction is the establishment of mechanisms of developing detected changes which will serve as a subject for further research.

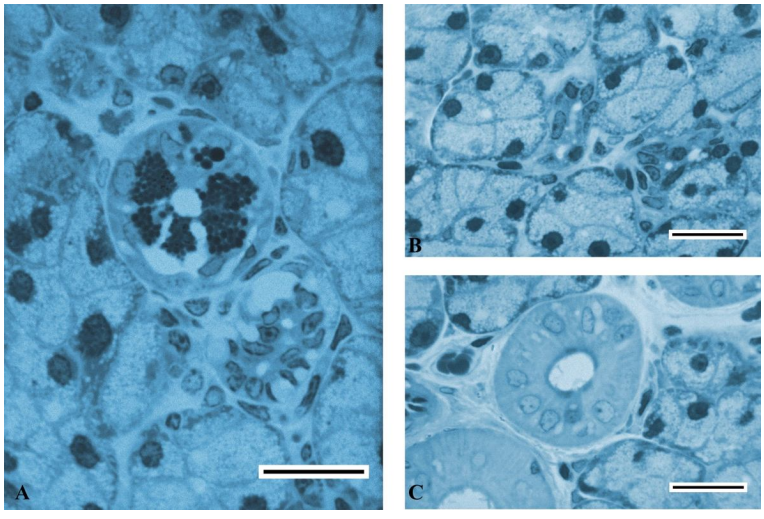

Fig. 3. Vacuolization of the intercalated, granular and striated ducts of the submandibular gland on the 42nd day of the experiment. Semi-thin sections stained with methylene blue. Scale bar 20μm

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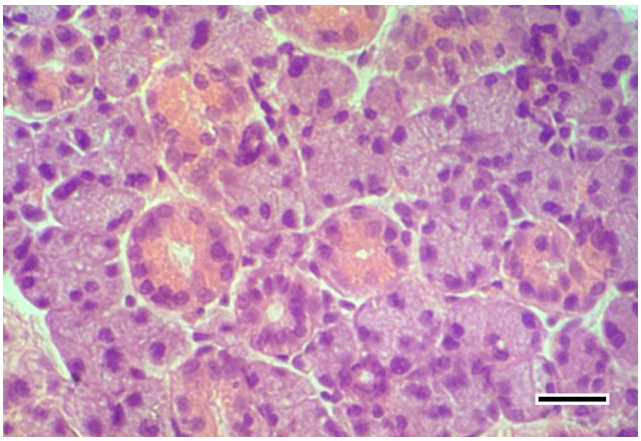

Fig. 4. Structural reorganization of the excretory ducts of the rat submandibular gland of the 70th day of the development of experimental hyperglycemia. Staning with hematoxylin and eosin. Scale bar 20μm

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Вплив гіперглікемії на вивідні протоки піднижньощелепної залози (гістологічне дослідження)

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Резюме. У роботі висвітлено особливості гістологічних змін різних відділів внутрішньочасточкової протоки піднижньощелепної залози щурів при експериментальній гіперглікемії.

Матеріал і методи. Дослідження виконано на 40 щурах-самцях Вістар масою 230 – 250 г. Експериментальну гіперглікемію індукували одноразовим введенням стрептозотоцину. Проводили біохімічне та морфологічне дослідження, морфометричний аналіз.

Результати. На тлі динамічного зростання в крові рівня глюкози та вмісту глікозильованого гемоглобіну виявлено розвиток дистрофічних змін епітеліоцитів гранулярних та посмугованих проток, починаючи з 28-ї доби досліду, що супроводжувалося поступовим зменшенням висоти їхніх епітеліоцитів на 10,28 – 29,46 % та 10,77 – 28,28 %, відповідно. Морфологічні зміни вставних проток виявлялися пізніше – з 42-ї доби, а зменшення висоти їхніх епітеліоцитів – тільки на 70-у добу досліду на 15,60 %.

Висновок. Морфологічні зміни різних ланок внутрішньочасточкової протоки залежать від тривалості гіперглікемії, мають дистрофічний характер, гістологічно виявляються з 28-ї доби досліду та супроводжуються динамічним зменшенням висоти епітеліоцитів.

Ключові слова: гіперглікемія; піднижньощелепна залоза; вивідні протоки.

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