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MORPHOGENESIS OF THE MINOR SALIVARY GLANDS OF THE HARD PALATE DURING TRIPTORELIN ADMINISTRATION IN THE EARLY OBSERVATIONAL TERMS IN RATS

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Minor salivary glands are very sensitive to pathological processes in the body. However, until now, the reactivity of minor salivary glands in response to neuroendocrine changes in the body has not been sufficiently studied and is, therefore, one of the unsolved problems of modern morphology. Triptorelin administration has a negative effect on the structural components of the secretory and excretory ducts of the minor salivary glands of rats. They were manifested by a statistically significant increase in all structural elements with a maximum on the 30th day, without restoring them to the indicators of the control group on the 180th. Comparing the morphometric indicators of the experimental group on the 30th and 180th days, a statistically significant decrease in morphometric indicators was determined. Mucous cell height decreased by 21 %, the outer diameter of the terminal secretory unit – by 22 %, the lumen of the terminal secretory unit – by 27 %; the height of the principal cells of the excretory ducts – by 9 %, the outer diameter of the excretory ducts – by 24 %, the lumen of the excretory ducts – by 27 %. In our opinion, this happened due to decreased compensatory properties of the tissues of the minor salivary glands.

Key words: triptorelin, testosterone, luteinising hormone, minor salivary glands, mucous cells, excretory ducts.

**В.І. Шепітько, Є. В. Стецук, К.В. Штепа, Н.В. Боруґа, О.В. Волошина,
Л.Б. Пелипенко, С.І. Сербін**

МОРФОГЕНЕЗ МАЛИХ СЛИННИХ ЗАЛОЗ ТВЕРДОГО ПІДНЕБІННЯ ПРИ ВВЕДЕННІ ТРИПТОРЕЛІНУ НА РАННІХ ТЕРМІНАХ СПОСТЕРЕЖЕННЯ У ЩУРІВ

Малі слинні залози дуже чутливі до патологічних процесів в організмі, однак до теперішнього часу реактивність малих слинних залоз у відповідь на нейроендокринні зміни в організмі вивчена не достатньо і тому є однією з невирішених проблем сучасної морфології. Введення триптореліну негативно впливає на структурні компоненти секреторних та вивідних протоків малих слинних залоз щурів, які проявились статистично достовірним збільшенням всіх структурних компонентів з максимумом на 30-у добу, без відновлення їх до показників контрольної групи на 180-у. Порівнюючи морфометричні показники експериментальної групи на 30-у та 180-у доби визначили статистично достовірне зменшення морфометричних показників, а саме: висота мукоцитів зменшилась на 21 %, зовнішній діаметр кінцевого секреторного відділу на 22 %, просвіт кінцевого секреторного відділу на 27 %; висота головних клітин вивідних протоків на 9 %, зовнішній діаметр вивідних протоків на 24 %, просвіт вивідних протоків на 27 %, на нашу думку, за рахунок зменшення компенсаторних властивостей тканин малих слинних залоз.

Ключові слова: Трипторелін, тестостерон, лютеїнізуючий гормон, малі слинні залози, мукоцити, вивідні протоки.

The study is a fragment of the research project "Experimental morphological study of cryopreserved placenta transplants action diphereline, ethanol and 1 % methacrylic acid on the morphofunctional status in a number of internal organs", state registration No. 0119U102925.

Saliva is a complex fluid produced by three pairs of major salivary glands and hundreds of minor salivary glands. It contains a wide variety of components and physical and chemical properties that are important for maintaining the health of the oral cavity. Saliva protects the teeth and the oral mucosa and facilitates tongue articulation, which is necessary for chewing and swallowing. In addition, saliva plays a vital role in maintaining a balanced microbiota [10]. Thus, the numerous functions performed by saliva are necessary for the proper protection and functioning of the body as a whole and general health. Many diseases and drugs can affect saliva secretion through various mechanisms, leading to salivary gland dysfunction and related oral problems, including xerostomia, dental caries, and fungal infections [8].

More and more dentists focus on diseases of the salivary glands of the oral cavity, both major and minor. According to statistics, diseases of the salivary glands account for approximately 3–5 % of all diseases of the maxillofacial area. Various pathological factors can cause pathological changes in the salivary glands. Scientific sources describe changes in the structural components of the salivary glands associated with accompanying diseases [7]. An example of this is the reasonably widespread information about changes in the salivary glands about obesity, diabetes, and diseases of the thyroid gland and kidneys. Information on changes in the salivary glands in diabetes, obesity, thyroid gland and kidney diseases is widely presented.

It is known that the palatine glands are located in the mucous membrane of the back half of the hard palate. The terminal secretory units form small lobules separated by thin layers of connective tissue, which contains a significant number of nerve fibers, blood and lymphatic vessels. The excretory ducts open to the surface of the epithelium of the hard palate mucosa [14].

The primary function of these glands is to moisten the oral cavity. They are the minor salivary glands that take part in the formation of local immunity of the oral cavity and are the first to respond to the action of pathogenic factors [4].

It is well known that epidemic parotitis can cause the development of various complications on the part of the male genital organs and causes infertility in 30–40 % of cases, which determines the connection between the salivary glands and the testicles [5]. Minor salivary glands are susceptible to pathological processes in the body; however, until now, the reactivity of minor salivary glands in response to pathological processes has yet to be sufficiently studied and is, therefore, one of the unsolved problems of modern morphology.

The purpose of the study was to determine the changes in the structure of the minor salivary glands of the hard palate with triptorelin administration in the experiment.

Materials and methods. The study material was the glandular part of the hard palate mucosa of 25 male rats. The animals were divided into two groups: I – control – 10 animals; II – experimental – 15 (testosterone blockade of the central genesis, by subcutaneous injection of triptorelin 0.3 mg per 1 kg) – 15 animals were sacrificed on the 30th, 90th and 180th days, 5 animals each. [12]. Rats from the control group received saline injections. The experiment lasted for 180 days. Animals were kept in standard vivarium conditions at the Poltava State Medical University. Experimental animals were sacrificed in strict compliance with the provisions of the “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes” (Strasbourg, 1986), as well as with the “General Ethical Principles of Animal Experiments” adopted by the First National Congress on Bioethics (Kyiv, 2001). Using standard methods, the material was embedded in paraffin blocks, from which sections with a 4 µm thickness were made and stained with hematoxylin and eosin [1]. Histological sections were examined using a Biorex 3 light microscope with a digital microfilter and software adapted for similar studies (serial No. 5604).

The study results were processed using the Microsoft Office Excel software and the Real Statistics 2019 extension. The nonparametric Mann-Whitney test was used to determine the statistical significance of differences between the groups. The difference was considered statistically significant at $p < 0.05$.

Results of the study and their discussion. The minor salivary glands of the control group of animals were located in the thickness of the mucous membrane and the submucosal base of the oral cavity. The terminal secretory units were characterised by bulbous extensions, most occupied by secretory cells. Mucous cells had a pyramidal or prismatic shape with light cytoplasm and were located on the basement membrane, which was visualised. The nuclei of these cells were located in the peripheral parts of the cells. A large number of diffusely located secretory granules were observed in the cytoplasm of mucous cells, most of which were found in the apical part of the cell. Granules were well visualised, varied in size, and

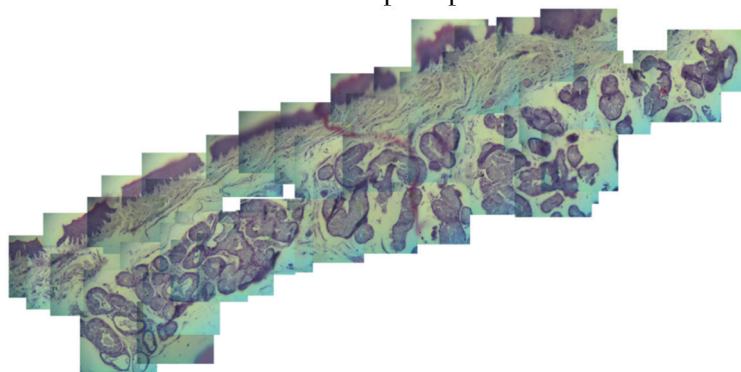


Fig. 1. Reconstruction of a micropreparation of the hard palate, a control group of animals. Hematoxylin-eosin staining. Magnification x10.

light-optical. Cambial cells were found in some places. As for myoepithelial cells, they were poorly visualised and were isolated. They were elongated; the core was flattened and dense with a central location. The excretory duct cells were cuboidal and located on the basement membrane. The nuclei are located in the middle of the cell, dark. Cells of loose connective tissue were located outside the basement membrane (Fig. 1).

When we studied the histological preparations of the minor salivary glands of the hard palate on the 30th day of observation, it was established that on this day of observation, oxidative stress was detected in the tissues of the mucous membrane of the hard palate, which was manifested by changes in the microcirculatory bed of the oral mucosa and the structure of the minor salivary glands (fig. 2A).

The 90th day of observation was characterised by constant edema of the mucous membrane with changes in the structure of the microcirculatory channel in the form of stasis. Morphologically: in the

structure of minor salivary glands – changes both in the structure of the terminal units (mucous cell height, outer and inner diameter) compared to the parameters of the control group of animals) and in the structure of the excretory duct. The height of epithelial cells of the outer diameter and the diameter of the internal lumen, but when compared to the indices of the previous group, namely on the 30th day of observation, a gradual decrease of all indices is revealed (Fig. 2B).

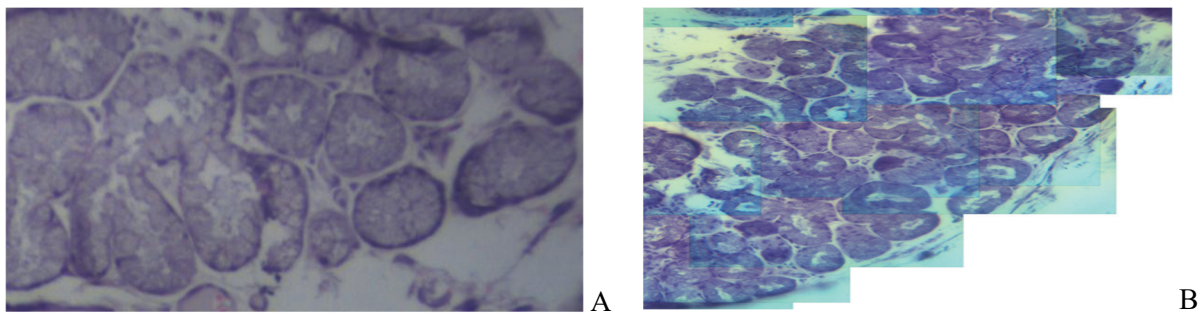


Fig. 2. The hard palate of a rat. 30th day (A) and 90th day (B) of observation after triptorelin administration. Hematoxylin-eosin staining. Magnification x10.

This was manifested by a statistically significant increase in the height of mucous cells, which was $-39.58 \pm 1.49 \mu\text{m}$ – control group $23.12 \pm 0.96 \mu\text{m}$, the size of the outer diameter of $113.1 \pm 1.53 \mu\text{m}$ – control group $64.22 \pm 1.12 \mu\text{m}$ and the lumen of the secretory unit, which was $27.71 \pm 1.04 \mu\text{m}$ – control group $19.97 \pm 1.31 \mu\text{m}$.

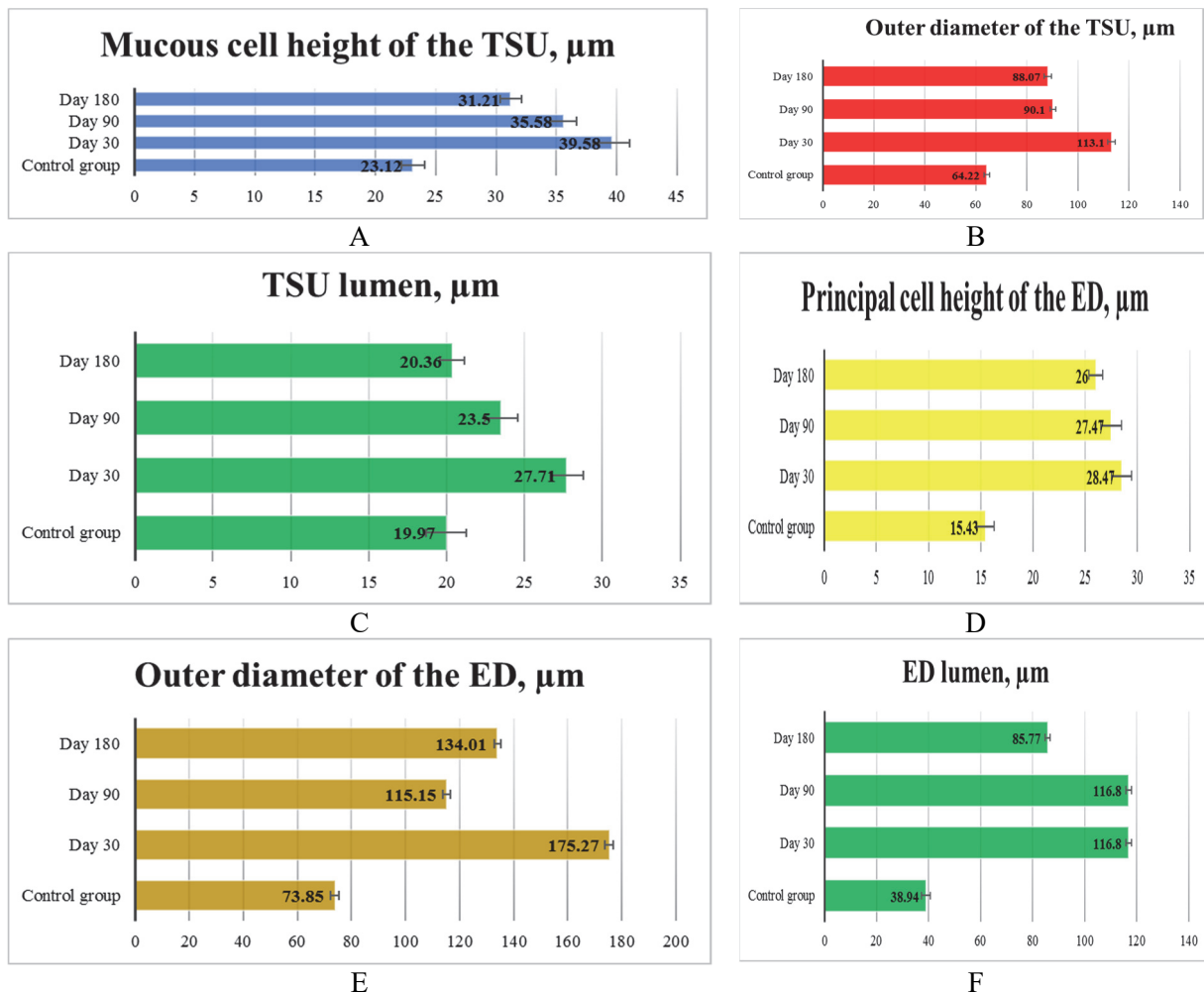


Fig. 3. Observation data at different times: A – mucous cell height of the terminal secretory units, B – outer diameter of the TSU, C – lumen of the TSU, D – height of the principal cells of the excretory ducts, E – external diameter of the ED, F – lumen of the ED.

The 180th day of observation was characterised by changes both in the structure of the microcirculatory bed and in the structure of the minor salivary glands. An increase in the indices of secretory parts and ducts without restoring the indices of the control group. An increase in the number of vacuoles in cells was found, which is the final sign of synthetic activity.

Salivary gland secretion is a neurohormonal mediated reflex, and the volume of saliva secreted depends on the intensity and type of taste and chemosensory, masticatory, or tactile stimulation [3, 9]. Long periods of low blood flow (at rest or without stimulation) are interrupted by short periods of high blood flow stimulated by taste and chewing. The neurohormonal-mediated salivary reflex is modulated by neural signals from other central nervous system centres, most evident as hyposalivation during anxiety. An example of other neurohormonal influences on the salivary reflex is the circadian rhythm, which affects the flow of saliva. Cholinergic parasympathetic and adrenergic sympathetic autonomic nerves cause saliva secretion, transmit signals through muscarinic M3 and adrenergic receptors on

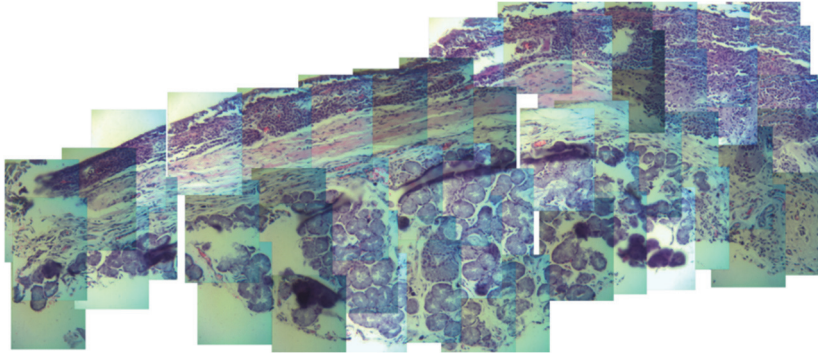


Fig. 4. Reconstruction of the hard palate micropreparation with triptorelin administration, 180 days of follow-up. Hematoxylin-eosin staining. Magnification x10.

salivary acinar cells, and lead to the secretion of salivary fluid and proteins [11]. Oral saliva contains a number of established and potential disease biomarkers derived from epithelial cells, neutrophils, microbiome, gingival crevicular fluid, and serum. For example, cortisol levels are used to assess stress, matrix metalloproteinases-8 and -9 are promising markers of caries and periodontal disease, and a panel of mRNAs and proteins has been proposed as a marker for oral squamous cell carcinoma. Understanding the mechanisms by which components enter saliva is essential to validating their use as biomarkers of health and disease. Thus, from the literature data, on the 12th day of ethanol exposure, signs of a decrease in secretion by seromucous acinar cells were found, which is confirmed on electronograms by compaction of nuclei, a decline in the number of secretory granules, and the appearance of cytoplasmic emissions on intercellular surfaces. The cells of the striated ducts have acquired a typical prismatic shape. The decrease in the number of mitochondria and the folds of the basal labyrinth indicates a reduction in the processes of saliva modification by epithelial cells. As a result of the decrease in hydration of the interstitium caused by hypersalivation, in the early stages of the experiment, the vessels of the blood microvascular system of the interstitial connective tissue expanded in volume and blood cells were visualised [9]. On the 30th day of the experimental model of chronic ethanol intoxication, the formation of adaptive and compensatory changes in the parenchymal elements of the submandibular glands in response to the long-term effect of ethanol is noted. In the serous-mucous cells of the acini, a thickening of the basal parts of the cytoplasm, which is expressed by dark areas on the electrogram, was found. Nuclei moved to the center and were compacted. Granules were polymorphic, sometimes rod-shaped, electron-light, sometimes fused areas. The changes mentioned above in the acini indicate a compensatory restructuring after hypersalivation in the early stages of the experiment and restructuring of the secretory apparatus, mainly in the direction of carbohydrate synthesis. The luteinising hormone controls the male body's fertility by regulating testosterone production. Violation of the synthesis of luteinising hormone when triptorelin is administered leads to testosterone deficiency. Testosterone deficiency, in turn, leads to changes in the oxidative metabolism of tissues [2]. Necrotizing sialometaplasia is a benign, self-limiting inflammatory disease of the salivary glands that occurs in men with reduced serum testosterone concentrations, primarily affecting the minor salivary glands of the palate. This lesion can mimic a malignant neoplasm, both clinically and histopathologically, manifesting as submucosal edema or a palatal ulcer [6]. According to well-known researchers, the salivary glands have a similar structure and the spatial structure of their excretory ducts in both humans and rats [14]. As it turned out, secretory cells in the differentiation process are located within the terminal units of the glands. The wall of all ducts, except the main excretory duct and its terminal units, is formed by two layers of cells with signs of secretory activity. From the side of their basal part of the plasmalemma, there is a layer of myoepithelial cells, the contractile elements of which are well detected during immunohistochemical studies. Myoepithelial cells perform both a support function and can actively or passively affect the size of the end sections and ducts' lumen when the hydraulic pressure increases [13]. Epithelial cells of the striated ducts had numerous folds of basal striation oriented perpendicular to the basal cell surface and were narrow and high with numerous vacuole-like structures in the cytoplasm. Clear catenate slits are visible in the intercellular spaces. Almost a third of the cells were noted in granular ducts in the presence of large electron-dense granules and optically bright areas in the basal parts starting from the basement

membrane. Heterogeneity of the cytoplasm and the appearance of irregularly shaped nuclei indicate the occurrence of apoptosis. The detected changes in the epithelium of the ducts indicate its partial depletion of cells with the appearance of signs of dystrophy and the full use of their adaptation to support the salivary process during long-term exposure to ethanol. An increased number of macrophages and lymphocytes in the periductal connective tissue was found, which indicates their active role in providing a local protective barrier [14]. It should be noted that our determination of changes in the structure of the minor salivary glands of the hard palate does not contradict the literature data and confirms the mediated pathological effect of triptorelin, which causes hormonal dysregulation in the hypothalamus-pituitary-testis-small salivary glands system and leads to quantitative and qualitative changes in them.

Conclusion

Triptorelin administration has a negative effect on the structural components of the secretory and excretory ducts of the minor salivary glands of rats. They were manifested by a statistically significant increase in all structural elements with a maximum on the 30th day, without restoring them to the indicators of the control group on the 180th. Comparing the morphometric indicators of the experimental group on the 30th and 180th days, a statistically significant decrease in morphometric indices was determined. Mucous cell height decreased by 21 %, the outer diameter of the terminal secretory unit – by 22 %, the lumen of the terminal secretory unit – by 27 %; the height of the principal cells of the excretory ducts – by 9 %, the outer diameter of the excretory ducts – by 24 %, the lumen of the excretory ducts – by 27 %. In our opinion, this happened due to decreased compensatory properties of the tissues of the minor salivary glands.

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