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CYTOTOPOGRAPHIC FEATURES OF PLASMA CELLS IN THE MAJOR SALIVARY GLANDS OF RATS IN NORM AND AT DIFFERENT FUNCTIONAL STATES

The aim of the study was to determine the cytotopographic features of plasma cells in the major salivary glands of rats in norm and in functional states caused by proserin and platyphylline. The study was carried out on 100 white Wistar male rats with body weight (185 ± 20) g. The first experimental group consisted of 40 animals which received platyphylline (cholinolytic) at a dose of 0.3 mg / kg body weight; the second experimental group included 40 animals with proserin introduction (anticholinesterase drug) at a dose of 0.1 mg / kg body weight; the control group included 20 similar intact animals. The animals were euthanized with an overdose of thiopental anesthesia. Platyphylline and proserin effect leads to the increase in the plasmocytes number of the major salivary glands of rats, which is a morphological confirmation of the local protective barrier enhancement in response to stimulation. Stimulation with platyphylline especially leads to the increase in the number of plasmocytes in the periductal connective tissue of the submandibular gland. Proserin causes the increase in the number of plasmocytes in submandibular and sublingual glands predominantly in periacinar interstitium, and in the parotid glands – in periductal stroma.

Key words: salivary glands, plasmocytes, platyphylline, proserin.

Xerostomia, or dry mouth syndrome is a common pathology observed by physicians of various specialties. Underestimation of this condition in patients leads to unsatisfactory treatment results. The syndrome manifests itself in almost 12% of the world population and its prevalence amounts to 25% among the elderly people. Hyposalivation is associated with constantly dry mouth, causes eating, swallowing and speech difficulties [4, 6, 7].

In this case the protective function of saliva is damaged; this function includes moistening of the oral cavity tissues, cleaning the mucous membrane from food debris and desquamated epithelium, biological cleaning of the oral cavity [8]. In addition, the protective function of saliva provides barrier formation with mucins, antibodies and non-immunoglobulin antimicrobial factors. Immunoglobulins A class, produced by stromal plasma cells of the major salivary glands, perform the important protective function, blocking adhesion of the pathogenic microorganisms to the oral mucosa surface [5]. The plasma cells are found in the loose connective tissue of proper layer of the mucous membranes of the hollow organs, omentum, the interstitial connective tissue of the glands (salivary, mammary, etc.), lymph nodes, spleen, bone marrow. They are formed in the lymphoid organs from B-lymphocytes and provide antibodies production in the organism [1].

Research purpose - to determine the cytotopographic features of plasma cells in the major salivary glands of rats in norm and in functional states caused by proserin and platyphylline.

Material and methods. The study was carried out on 100 white Wistar male rats with body weight (185 ± 20) g. The first experimental group consisted of 40 animals which received platyphylline (cholinolytic) at a dose of 0.3 mg / kg body weight; the second experimental group included 40 animals with proserin introduction (anticholinesterase drug) at a dose of 0.1 mg / kg body weight; the control group included 20 similar intact animals. The models affecting the parasympathetic system were used for the rats of the experimental groups applying intra-arterial administration of mentioned drugs diluted with isotonic solution (2.5 ml for 25 minutes).

The animals were euthanized with an overdose of thiopental anesthesia. The study complies with the requirements of the international principles of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasbourg, 1985), the corresponding Law of Ukraine "On the Protection of Animals against Cruelty" (No. 3446-IV as of 21.02.2006, Kyiv), the Ethical Code of Ukrainian Physician and the Code of Ethics for a Scientist in Ukraine.

The parotid, submandibular and sublingual glands were immersed in vials with cold 2.5% glutaraldehyde solution in phosphate buffer at pH 7.4 and stored at refrigerator temperature $+4^{\circ}$ C for 1 day to prepare semithin sections. The right and left parotid, submandibular and sublingual glands were embedded in Epon- 812 according to the generally accepted technique [2].

Material impregnated by Epon-812 was placed in gelatin capsules and filled with resin, followed by polymerization at temperatures ($+35, +45, +60$) $^{\circ}$ C each for 24 hours. The obtained material was

sectioned in ultramicrotome UMTF-7. Staining of the sections was carried out with 0.1% solution of toluidine blue in phosphate buffer at pH 7.8 and polychrome dye [2, 3].

Results and their discussion. Histological investigation determined that the major salivary glands of rats (parotid, submandibular and sublingual) consisted of lobules with terminal parts and systems of excretory ducts. The intralobular connective tissue of the parotid glands was represented by amorphous substance, collagen fibers and fibroblast processes. At the contact places of 3-4 final sections the fibroblast bodies, collagen fibers and blood vessels of the microcirculatory bed – capillaries and postcapillaries can be revealed. The periductal connective tissue contained postcapillaries and venules. The mast cells with eccentric localization of the nucleus were observed around the terminal parts of the interstitial connective tissue, and in the periductal tissue – with the central one. Plasma cells were rarely detected and isolated.

The intralobular connective tissue of the submandibular glands of rats was represented by collagen fibers and fibroblasts; vessels of microcirculatory bed were defined in the interstitium; macrophages, plasma cells and mast cells were observed perivascularly (Fig. 1).

Among the migrant cells of the interstitial connective tissue in the sublingual salivary glands of rats, macrophages, lymphocytes and plasmocytes were detected. Mainly lymphocytes and macrophages were localized in the interacinar interstitium; plasma cells, macrophages and mastocytes were constantly detected in periductal connective tissue.

Macrophages, lymphocytes and plasmocytes were detected among the migrant cells of the interstitial connective tissue in the sublingual salivary glands of rats. Mainly lymphocytes and macrophages were localized in the interacinar interstitium; plasma cells, macrophages and mastocytes were constantly detected in the periductal connective tissue.

After platyphylline introduction the accumulation of leukocyte type cells, namely, macrophages, plasma cells, leukocytes was observed in the lobules of parotid glands of rats closer to the insertion ducts, unlike the control group of animals. Macrophages and plasmocytes were predominant cells, which morphologically determined the increase in the local protective barrier in response to platyphylline effect (Fig. 2).

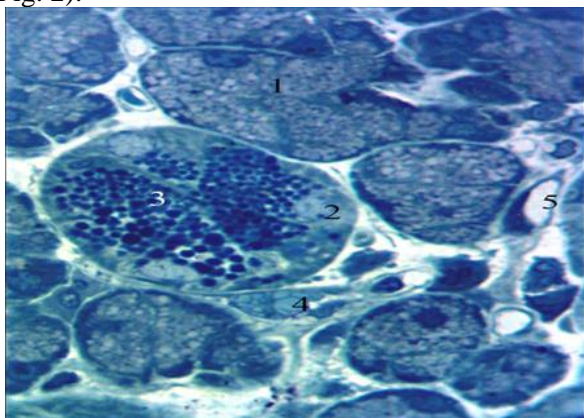


Fig. 1. The submandibular salivary gland of the control group rats. Semi-thin section. Staining with polychrome dye. Mag.: Ob. x 40, Oc. x 10: 1 – terminal part; 2 – granular duct; 3 – granules in cytoplasm; 4 – plasmocyte; 5 – postcapillary.

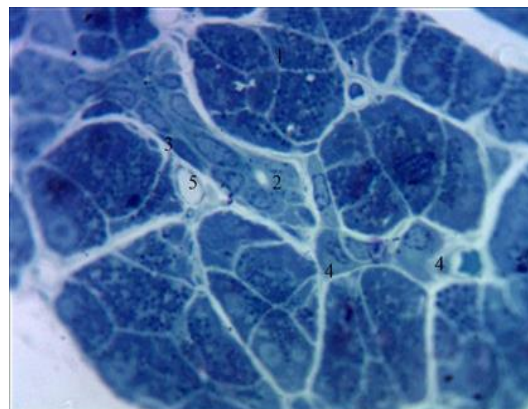


Fig. 2. Leukocyte type cells in periductal connective tissue of the lobule in parotid salivary gland of rat after platyphylline introduction. Semi-thin section. Staining with polychrome dye. Mag.: Ob. x 100, Oc. x 10: 1 – terminal part; 2 – insertion duct; 3 – nucleus of myoepithelial cell; 4 – plasma cell; 5 – postcapillary venule.

After platyphylline introduction, macrophages and mastocytes were more often detected in the perivascular connective tissue between the lobules of the parotid gland; plasmocytes and lymphocytes were isolated. Locally there were perivascularly localized groups of leukocyte type cells resembled lymphoid nodules of lymphoid tissue associated with mucous membranes according to the cellular composition (small and medium-sized lymphocytes, macrophages, plasmocytes, mast cells). In the center of the cellular associations, venules with classical wall structure were defined (Fig. 3).

In the submandibular glands the interstitium had signs of hyperhydration after platyphylline introduction. The periprotective interstitium contained plasmocytes, which sometimes formed groups of 2-4 cells. Between adjacent 3-4 terminal parts, the plasmocytes with enlarged cisterns of granular endoplasmic reticulum were visualized perivascularly, in addition to fibroblasts, collagen fibers and macrophages (Fig. 4). Leukocytes, plasmocytes, macrophages and mastocytes were constantly detected in the interacinar interstitium and periductal connective tissue of the sublingual glands after platyphylline stimulation.

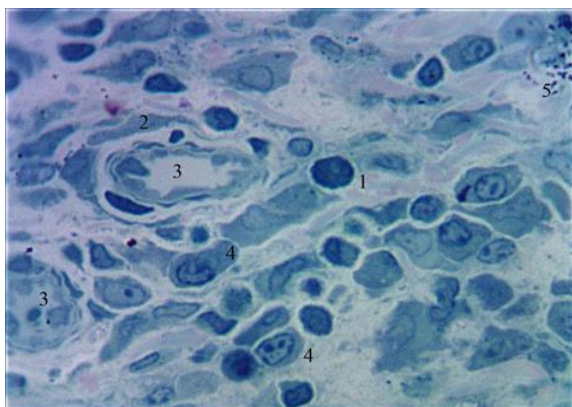


Fig. 3. The accumulation of leukocytes in the interlobular connective tissue of parotid salivary gland of rat after platyphylline introduction. Semi-thin section. Staining with polychrome dye. Mag.: Ob. x 100, Oc. x 10: 1 – lymphocyte; 2 – perivascular fibroblast; 3 – arteriole; 4 – plasmocyte; 5 – mast cell granules in connective tissue.

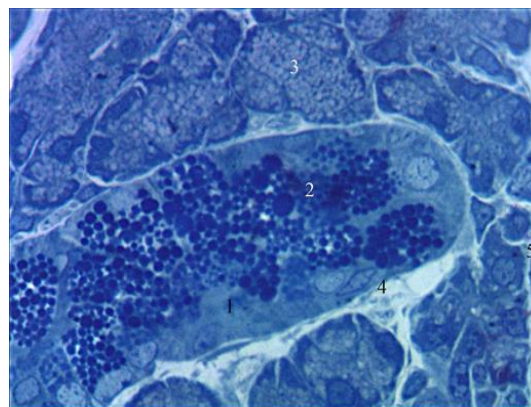


Fig. 4. Granular duct of submandibular salivary gland lobule of rat after platyphylline introduction. Semi-thin section. Staining with polychrome dye. Mag.: Ob. x 40, Oc. x 10: 1 – granular duct; 2 – secretory granules; 3 – terminal part; 4 – myoepithelial cell nucleus; 5 – plasmocyte; 6 – cytoplasm of plasmocyte.

In the II experimental group animals, the layers of intralobular connective tissue of the parotid gland had signs of hyperhydration caused by proserin effect. The periductal interstitium contained diffusely located cells of the leukocyte type forming the chains, sometimes the accumulations of leukocytes – macrophages, lymphocytes, plasmocytes was observed, which could be determined as lymphoid tissue according to the structure, associated with mucous membranes. In the intralobular connective tissue of the submandibular glands after proserin stimulation, the cells of the leukocyte type – lymphocytes, macrophages, plasmocytes both single and in groups from four to twenty cells (mostly perivascularly) were constantly present. In the periductal connective tissue of the sublingual glands, macrophages and plasmocytes were also detected. The periacinar interstitium contained plasmocytes, cisterns of granular endoplasmic reticulum were enlarged.

Thus, the study determined that, the local protective barrier in the major salivary glands of rats was represented by macrophages, plasmocytes and mast cells. Plasmocytes in greater numbers could be revealed in periductal connective tissue of the salivary glands, especially in submandibular and sublingual ones. Platyphylline stimulation caused exertion of local immune response of the salivary glands leading to the increase in immunocompetent cells number in them. The number of plasma cells was also increasing, especially in the periductal connective tissue of the submandibular gland. Stimulation with proserin caused the increase in plasmocytes number of the submandibular and sublingual glands, mainly in periacinar interstitium, and in the parotid glands – mainly in the periductal stroma.

Conclusions

1. Platyphylline and proserin effect leads to the increase in the plasmocytes number of the major salivary glands of rats, which is a morphological confirmation of the local protective barrier enhancement in response to stimulation.
2. Stimulation with platyphylline especially leads to the increase in the number of plasmocytes in the periductal connective tissue of the submandibular gland.
3. Proserin causes the increase in the number of plasmocytes in submandibular and sublingual glands predominantly in periacinar interstitium, and in the parotid glands – in periductal stroma.

Prospects for further studies include quantitative analysis of the plasmocytes number in the major salivary glands in norm and after stimulation.

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Реферати

**ОСОБЛИВОСТІ ЦИТОТОПОГРАФІЇ
ПЛАЗМОЦИТІВ У ВЕЛИКИХ СЛИННИХ
ЗАЛОЗАХ ЩУРІВ У НОРМІ ТА ПРИ РІЗНИХ
ФУНКЦІОНАЛЬНИХ СТАНАХ**

**Ященко А.М., Єрошенко Г.А., Коптев М.М.,
Шевченко К.В.**

Метою роботи було встановлення особливостей цитотопографії плазмоцитів у великих слинних залозах щурів у нормі та при функціональних станах, обумовлених дією прозерину та платифіліну. Із урахуванням норм біоетики дослідження виконувалося на 100 дорослих білих щурах-самцях лінії Вістар. І експериментальну групу склали 40 тварин, яким вводили платифілін (0,3 мг/кг); 40 щурів II-ої експериментальної групи підпадали під вплив прозерину (0,1 мг/кг); до контрольної групи увійшли 20 інтактних тварин. Щурів виводили з експерименту шляхом передозування тіопенталового наркозу. Проведене дослідження свідчить, що стимуляція платифіліном призводить до напруження місцевого імунного захисту слинних залоз, спричиняє збільшення представництва в них імунокомпетентних клітин. Кількість плазмоцитів при цьому особливо зростає в перипротоковій сполучній тканині піднижньощелепної залози. Стимуляція прозерином викликає збільшення кількості плазмоцитів у піднижньощелепних та під'язикових залозах переважно у периацинарному інтерстиції, а в привушних – здебільшого у перипротоковій стромі.

Ключові слова: слинні залози, плазмоцити, платифілін, прозерин.

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**ОСОБЕННОСТИ ЦИТОТОПОГРАФИИ
ПЛАЗМОЦИТОВ В БОЛЬШИХ СЛЮННЫХ
ЖЕЛЕЗАХ КРЫС В НОРМЕ И ПРИ РАЗЛИЧНЫХ
ФУНКЦИОНАЛЬНЫХ СОСТОЯНИЯХ**

**Ященко А.М., Єрошенко Г.А., Коптев М.Н.,
Шевченко К.В.**

Целью работы было установление особенностей цитотопографии плазмоцитов в больших слюнных железах крыс в норме и при функциональных состояниях, обусловленных действием прозерина и платифиллина. С учетом норм биоэтики исследование выполнялось на 100 взрослых белых крысах-самцах линии Вистар. I экспериментальную группу составили 40 животных, которым вводили платифиллин (0,3 мг / кг); 40 крыс II-ой экспериментальной группы подвергались влиянию прозерина (0,1 мг / кг); в контрольную группу вошли 20 интактных животных. Крыс выводили из эксперимента путем передозировки тиопенталового наркоза. Проведенное исследование показывает, что стимуляция платифиллином вызывает напряжение местной иммунной защиты слюнных желез, приводит к увеличению представительства в них иммунокомпетентных клеток. Количество плазмоцитов при этом особенно возрастает в перипротоковой соединительной ткани поднижнечелюстной железы. Стимуляция прозеринном вызывает увеличение количества плазмоцитов в поднижнечелюстных и подъязычных железах преимущественно в периацинарном интерстиции, а в околоушных – в основном в перипротоковой строме.

Ключевые слова: слюнные железы, плазмоциты, платифиллин, прозерин.

Рецензент Білаш С.М.