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Characteristics of changes in the structural components of the mucosa and rat laryngeal cartilage at the end of the third week of experimental opioid exposure

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Abstract

In our work we aimed to study the pathomorphological manifestations among the structural components of the laryngeal mucosa and cartilage in the early stages of the experimental opioid effect. This goal was achieved through the use of histological techniques to visualize the structural components of the laryngeal mucosa and cartilage. Histological sections with a thickness of 5 - 7 mm were made. Histological specimens were prepared according to conventional methods using the dye hematoxylin, eosin, azan according to the method of Heidenhain, PAS-reaction according to Mc-Manus and Alcian blue according to Steedman. Microscopic examinations and photographing of the preparations were performed using an MBI-1 microscope and a Nicon D 3100 digital camera.

At the microstructural level after 21 days in rats there was an increase in dyscirculatory changes, which were most pronounced in the vessels of the lamina propria of

the laryngeal mucosa. The epithelial layer of the epiglottis was inhomogeneously thickened, especially on the pharyngeal surface. Small polymorphonuclear infiltrates were found in the laryngeal mucosa of the epiglottis. The vessels of the laryngeal ventricular mucosa of the larynx were dilated and filled with erythrocytes. The development of perivascular edema was registered. In the areas of transudate accumulation, the main substance of the laryngeal mucosa of the larynx was enlightened. Necrotic changes developed in the epitheliocytes of the laryngeal ventricle. Eosinophilic mass accumulated on the surface of the ventricular mucosa.

The results of the study in the future will form a pathomorphological base that can be used for comparative characterization of the dynamics of growth of pathomorphological changes in the structural components of the laryngeal mucosa and cartilage in the early stages and compare these changes with pathomorphological manifestations in the late stages of experimental opioid exposure.

Key words: opioid; laryngeal mucosa; laryngeal cartilage; rat; early terms.

The results of the article correspond to the research plan of Lviv National Medical University named after Danylo Halytsky and are part of the research topic of the Department of Normal Anatomy "Morphofunctional features of organs in pre - and postnatal periods of ontogenesis, under the influence of opioids, supplements, reconstructive surgery and obesity" (state registration number 0120U002129) during 2020 - 2024.

Introduction. It is known that the widespread use of opioids usually leads to clinically significant side effects or toxic effects. In recent years, a large number of studies have been devoted to the problem of uncontrolled use of potent and psychotropic drugs of medical origin [1-13]. In the literature there are studies that describe changes in the ENT organs with prolonged use of these drugs [14 -18].

The aim of the study. The aim of our study was to investigate the effect of opioids on changes in the structural components of the laryngeal mucosa and cartilage at the end of the third week of experimental opioid effects.

Materials and methods of research. The material of the study were sexually mature, outbred rats - males in the amount of 48 animals, weighing 92 g, aged 4.5 months. Animals were injected with nalbuphine intramuscularly once daily for one day (10-11 am) for 21 days. The initial dose of nalbuphine was 8 mg / kg during the first week, 15 mg / kg during the second week, 20 mg / kg during the third week. The created the conditions for chronic opioid exposure [19]. The animals were divided into 2 groups. The 1st group of animals received

nalbuphine for 21 days in one period of time (10 - 11 o'clock in the morning) followed by collection of study material (end of the third week of experimental opioid exposure); the 2nd control group, which for 21 days received injections of saline at home in one period of time (10 - 11 o'clock in the morning). All animals were kept in a vivarium, the experiment was conducted in accordance with the principles of bioethics in accordance with the provisions of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986), Council Directive 86/609 / EEC (1986), Law of Ukraine № 3447-IV "On protection of animals from cruel treatment", general ethical principles of animal experiments, approved by the First National Congress of Ukraine on Bioethics (2001), confirmed by the conclusion of members of the commission on bioethics of Lviv National Medical University named after Danylo Halytsky 10 of December 15, 2019). Before sampling, the animals were removed from the experiment using dibutyl ether. As a material for microstructural study used structural components of the mucosa and cartilage of the larynx of rats, taking into account the preservation of the topographic ratio of structures. Histological sections with a thickness of 5 - 7 mm were made. Histological specimens were prepared according to the generally accepted method using the dye hematoxylin, eosin, azan according to the method of Heidenhain, PAS-reaction according to McManus and Alcian blue according to Stedman [20, 21]. Microscopic examinations and photographs of the preparations were performed using an MBI-1 microscope and a Nicon D 3100 digital camera.

Results of the research. As a result of the collection of experimental material after 21 days in rats exposed to an opioid analgesic at a dose of 20 mg / kg at the microstructural level revealed an increase in dyscirculatory changes, which were most pronounced in the vessels of the laryngeal mucosa. The epithelial layer of the epiglottis was inhomogeneously thickened, especially on the pharyngeal surface (fig. 1).

An excess of acidophilic, inhomogeneously keratinized, slightly fluffy mass was visualized on the surface of the epithelium of the epiglottis in the pharyngeal part. The horny substance also appeared on the apical surface of the epiglottis. Small polymorphonuclear infiltrates were found in the epiglottis mucosa (Fig. 1). The destruction and exfoliation of collagen fibers of the epiglottis mucosa was noted, which was more pronounced in the pharyngeal part (Fig. 2).

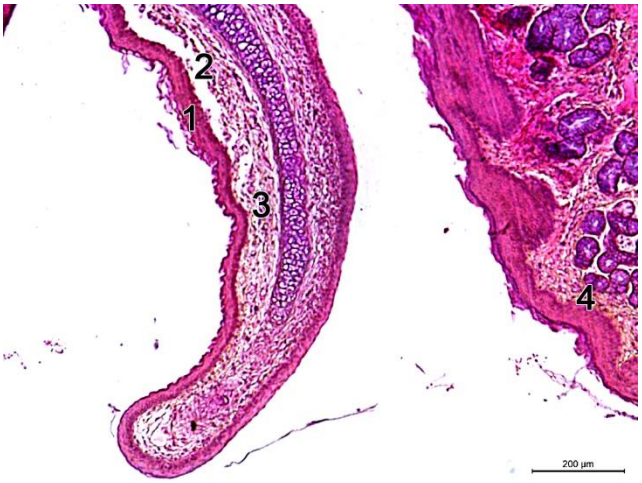

Fig. 1. The laryngeal surface of the epiglottis at the end of the 21st day of experimental opioid exposure. Ehrlich hematoxylin staining and eosin. Photomicrograph. 3b. x 100.

1– inhomogeneous thickening of the epithelial layer of the epiglottis; 2 – stratification of collagen fibers of own plate of a mucous membrane of a pharyngeal surface of an epiglottis; 3– polymorphonuclear infiltration of the own plate of the mucous membrane.

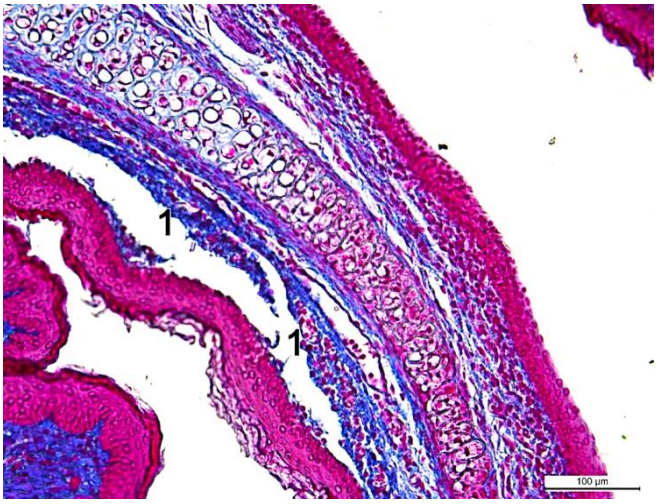

Fig. 2. The laryngeal surface of the epiglottis at the end of the 21st day experimental opioid effects. Coloring Azan by Heidenhain. Photomicrograph. 3bx 200

1– destruction and stratification of collagen fibers own plates of the mucous membrane of the epiglottis

On the surface of the mucous membrane and in the matrix of elastic cartilage epiglottis accumulated a significant amount of PAS-positive substances (Fig. 3). Preserved collagen fibers were inhomogeneously thickened in them PAS-positive substances accumulated (Fig. 4).

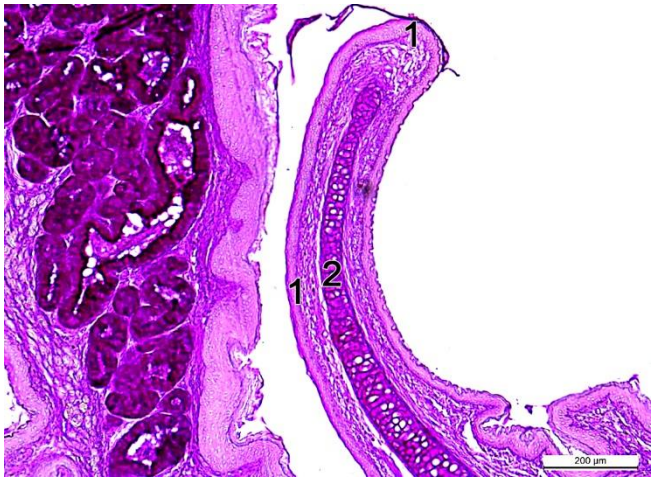

Fig. 3. The laryngeal surface of the epiglottis at the end of the 21st day of experimental opioid exposure. PAS staining is a MacManus reaction. Photomicrograph. 3b. x 100.

1– a significant amount of PAS-positive substances on the surface of the mucous membrane; 2 - a significant amount of PAS-positive substances in the main substance of the elastic cartilage of the epiglottis.

Fig. 4 The laryngeal surface of the epiglottis at the end of the 21st day of experimental opioid exposure. PAS staining is a MacManus reaction. Photomicrograph. Coll. x 200.

1– a significant amount of PAS-positive substances on the surface of the mucous membrane; 2 - a significant amount of PAS-positive substances in the main substance of the elastic cartilage of the epiglottis; 3 - inhomogeneous accumulation of PAS-positive substances in the thickened collagen fibers of the own plate of the mucous membrane

Also a significant amount PAS-positive substances were localized in the cytoplasm of the terminal epithelium secretory glands (Fig. 5). In the matrix of elastic cartilage epiglottis content of acidic glycosaminoglycans decreased (Fig. 6).

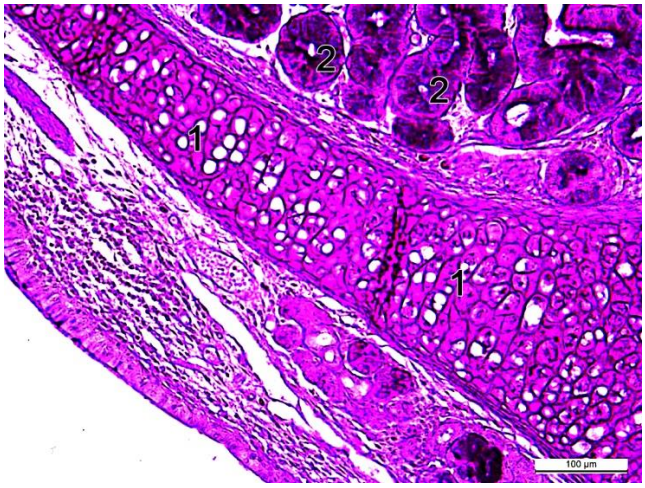

Fig. 5 The laryngeal surface of the epiglottis at the end of the 21st day of experimental opioid exposure. PAS staining is a Mc-Manus reaction. Photomicrograph. 3b. x 200.

1– a significant amount of PAS-positive substances in the main substance of the elastic cartilage of the epiglottis; 2 - a significant amount of PAS-positive substances in the cytoplasm of the epithelium of the terminal secretory glands.

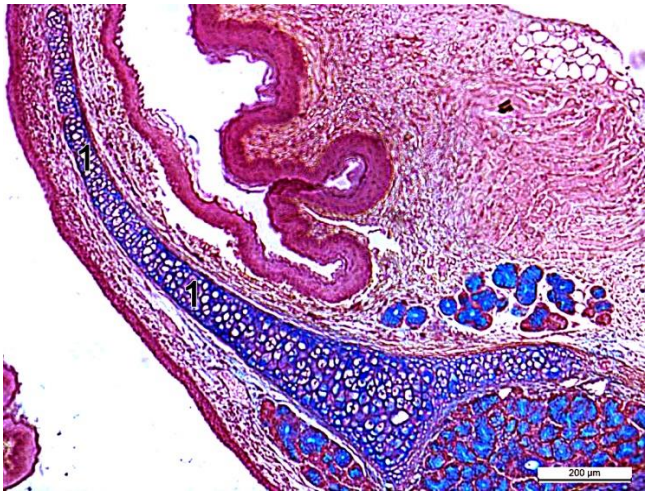

Fig. 6 The laryngeal surface of the epiglottis at the end of the 21st day of experimental opioid exposure. Coloring Alcian blue by Steedman. Photomicrograph. 3b. x 100.

1– decrease in the content of acidic glycosaminoglycans in the matrix of elastic cartilage of the epiglottis.

The vessels of the laryngeal mucosa of the larynx were dilated, full of erythrocytes (Fig. 7, 8). Registered development perivascular edema (Fig. 8). In areas of accumulation of transudate the main substance of the lamina propria of the laryngeal mucosa was enlightened.

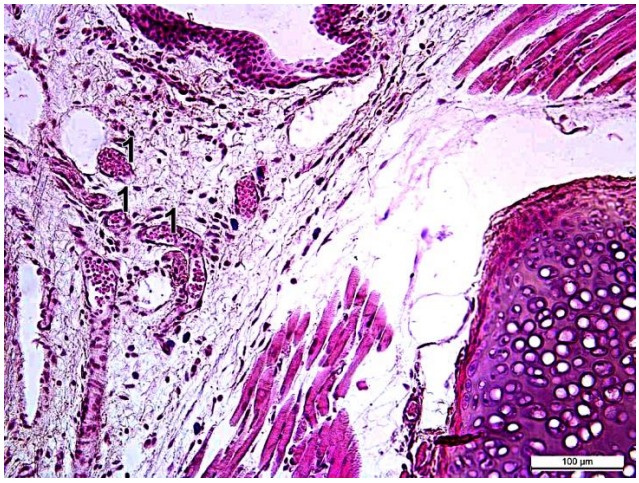

Fig. 7 Ventricular larynx at the end of the 21st day of experimental opioid exposure. Ehrlich hematoxylin staining and eosin. Photomicrograph. 3b. x 200.

1– dilation and overflow of erythrocytes of vessels of own plate of a mucous membrane of a ventricle of a larynx.

Fig. 8 Ventricular larynx at the end of the 21st day of experimental opioid exposure. Ehrlich hematoxylin staining and eosin. Photomicrograph. 3b. x 400.

1– dilation and overflow of erythrocytes of vessels of own plate of a mucous membrane of a ventricle of a larynx; 2– perivascular edema.

As a result, the bundles of collagen fibers in such zones stratified (Fig. 9, 10). The laryngeal mucosa of the larynx was infiltrated mainly by lymphocytes, macrophages and single neutrophils (Fig. 10). Individual collagen fibers of the laryngeal mucosa of the larynx were destroyed.

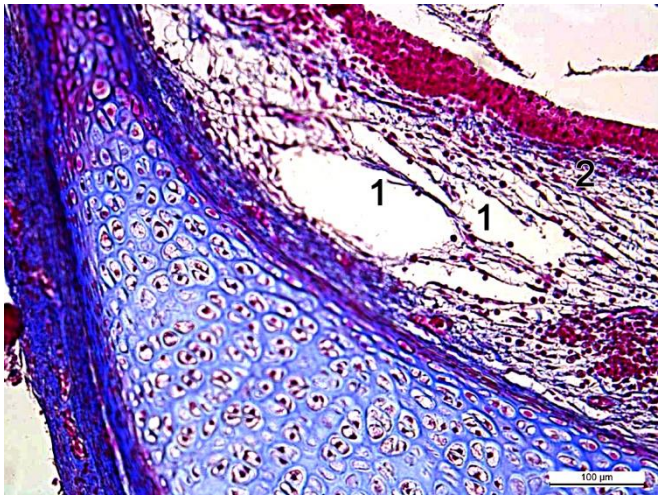

Fig. 9 Ventricular larynx at the end of the 21st day of experimental opioid exposure. Coloring Azan by Heidenhain. Photomicrograph. 3b. x 200

1– stratification of collagen fibers of the laryngeal mucosa of the larynx

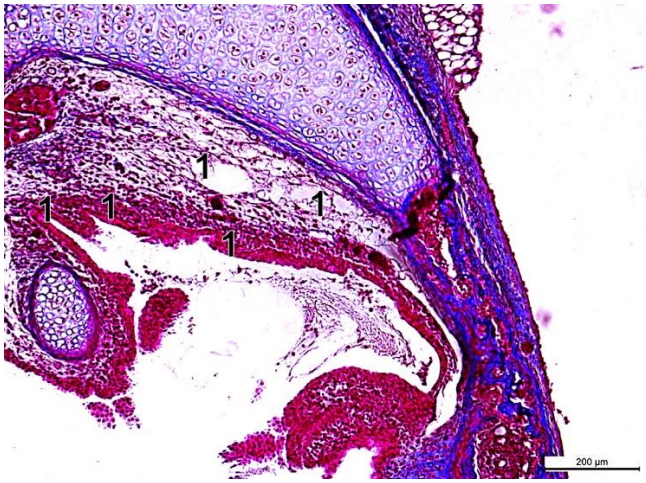

Fig. 10 Ventricular larynx at the end of the 21st day of experimental opioid exposure. Coloring Azan by Heidenhain. Photomicrograph. 3b.x100

1– polymorphonuclear infiltration and stratification of collagen fibers of the ventricle of the larynx and its own plate of the mucous membrane.

Necrotic changes developed in the epitheliocytes of the laryngeal ventricle. Eosinophilic mass accumulated on the surface of the ventricular mucosa (Fig. 11).

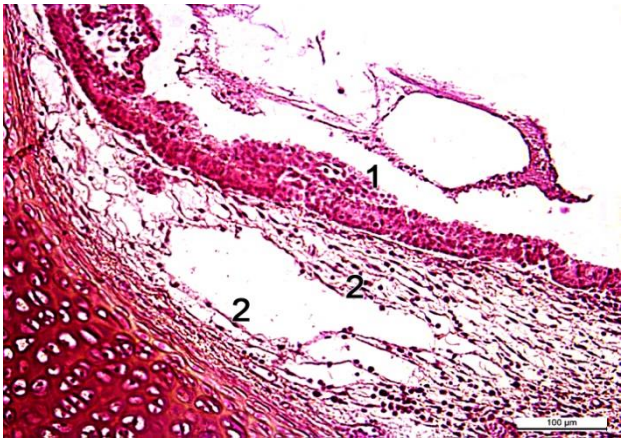

Fig. 11 Ventricular larynx at the end of the 21st day of experimental opioid exposure. Ehrlich hematoxylin staining and eosin. Photomicrograph. 3b. x 200.

1– inhomogeneous eosinophilic mass on the surface of the laryngeal mucosa; 2– edema of the main substance of the laryngeal mucosa of the larynx.

This mass contained desquamated epitheliocytes, lymphocytes, a significant amount of PAS-positive substances (Fig. 12, 13, 14), as well as acidic glycosaminoglycans (Fig. 15). A significant amount of PAS-positive substances was localized in the matrix of thyroid cartilage (Fig. 14).

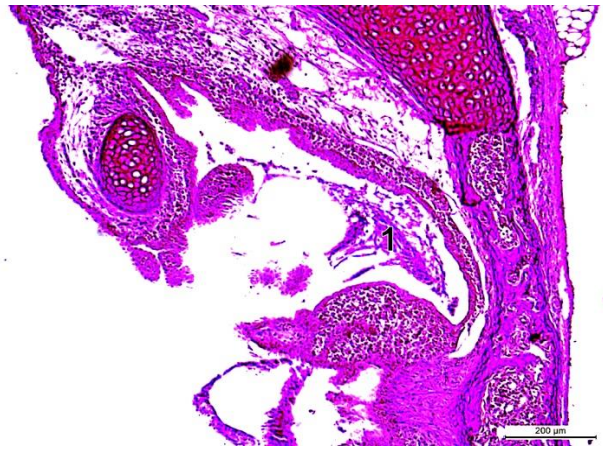

Fig. 12 Ventricular pa subchondral area of the larynx at the end of the 21st day of experimental opioid exposure. PAS staining is a Mac-Manus reaction. Photomicrograph. 3b. x 100.

1– accumulation of PAS-positive substances in the ventricle of the larynx and in the subchondral area.

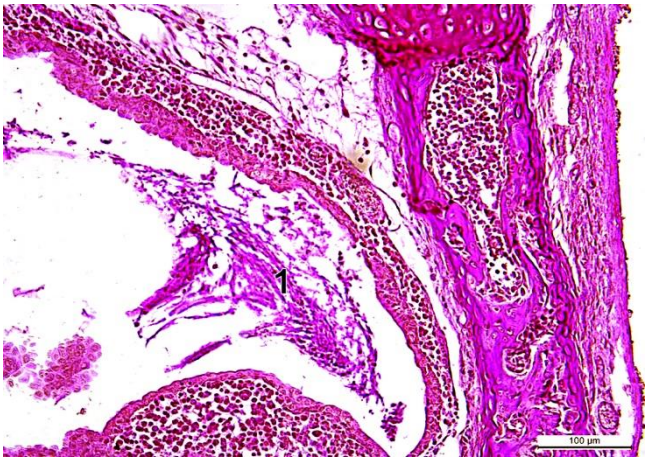

Fig. 13 Ventricular larynx at the end of the 21st day of experimental opioid exposure. PAS-staining is a Mac-Manus reaction. Photomicrograph. 3b. x 200.

1– accumulation of PAS-positive substances in the ventricle of the larynx.

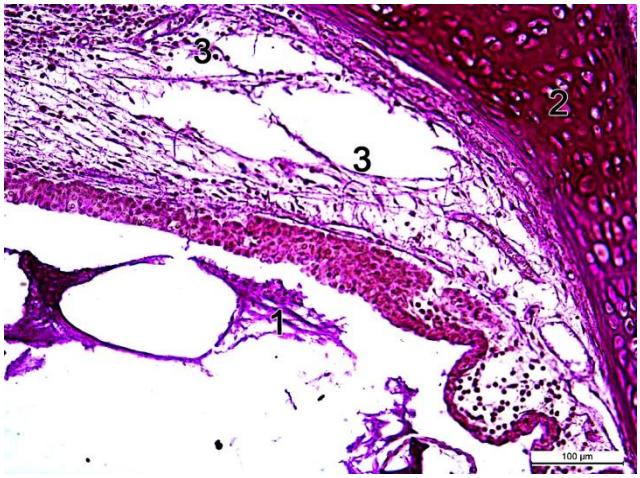

Fig. 14 Ventricular larynx at the end of the 21st day of experimental opioid exposure. PAS-staining is a Mac-Manus reaction. Photomicrograph. 3b. x 200.

1– accumulation of PAS-positive substances in the larynx ventricle; 2– a significant amount of PAS-positive substances in the matrix of thyroid cartilage; 3– stratification of collagen fibers due to the accumulation of transudate in the main substance of the laryngeal ventricle.

The main substance of the own plate of the mucous membrane of the vocal folds was infiltrated mainly by lymphocytes and macrophages. The epithelial layer of the vocal folds was of inhomogeneous thickness, on its surface accumulated fluffy masses containing PAS-positive substances.

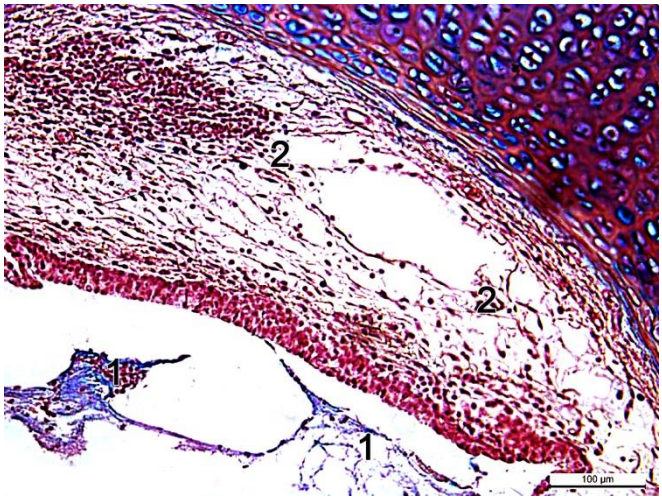

Fig. 15 Ventricular larynx at the end of the 21st day of experimental opioid exposure. Coloring Alcian blue by Steedman. Photomicrograph. 3b. x 200.

1– acidic glycosaminoglycans on the surface of the laryngeal mucosa; 2– stratification of collagen fibers due to the accumulation of transudate in the main substance of the own plate of the mucous membrane.

Acidophilic masses with impurities of lymphocytes and PAS-positive substances, as well as fine-grained and filamentous inclusions of acidic glycosaminoglycans were also visualized on the surface of the subchondral mucosa. Hyperemia, perivascular edema and polymorphonuclear infiltration of the mucosal plate also developed in the subchondral area. However, these dyscirculatory and infiltrative processes were slightly less pronounced than in the ventricles of the larynx.

Conclusions

As a result of the microstructural study of the mucous membrane and cartilaginous corset of the larynx of rats during the 21st day of the experimental opioid effect, the following conclusions can be drawn:

1. Progression of dyscirculatory changes which were most expressed in vessels of own plate of a mucous membrane of a ventricle of a larynx progressed. The epithelial layer of the epiglottis was inhomogeneously thickened, especially on the pharyngeal surface.

2. Small polymorphonuclear infiltrates were found in the own plate of the epiglottis mucosa. There was destruction and stratification of collagen fibers of the lamina propria of the epiglottis mucosa, which was more pronounced in the pharyngeal part

3. A significant amount of PAS-positive substances accumulated on the surface of the mucous membrane and in the matrix of the elastic cartilage of the epiglottis. Preserved collagen fibers were inhomogeneously thickened, they accumulated PAS-positive substances.

4. The vessels of the laryngeal mucosa of the larynx were dilated, full of erythrocytes. The development of perivascular edema was registered. In the areas of transudate accumulation, the main substance of the laryngeal mucosa of the larynx was enlightened.

5. Necrotic changes developed in the epitheliocytes of the laryngeal ventricle. Accumulated on the surface of the ventricular mucosa

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