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Реферати

РЕАКЦИЯ ГЕМОМИКРОЦИРКУЛЯТОРНОГО РУСЛА ПЕЧИНКИ ТА ЗМІНИ В ФУНКЦИОНАЛЬНОМУ СТАНІ ЦИКЛУ ОКСИДУ АЗОТУ ЗА УМОВ МОДЕЛЮВАННЯ АЛКОГОЛЬНОГО ГЕПАТИТУ

Микитенко А.О., Єрошенко Г.А.

Метою роботи було вивчити зміни гемомікроциркуляторного русла печінки та роль NO-ергічної системи в їх розвитку за умов моделювання алкогольного гепатиту. На ранніх термінах моделювання алкогольного гепатиту товщина судинної стінки центральної вени та міжчасточкової артерії і часточкової артеріоли збільшується, а товщина судинної стінки міжчасточкової вени та часточкової венули і підчасточкової вени зменшується, що пов'язано з дисрегуляторними змінами в циклі оксиду азоту в печінці шурів. Дисрегуляторні зміни полягають у збільшенні активності індукцибельної та конститутивних ізоформ NO-синтази на фоні зниження активності аргінази за відсутності статистично значущих змін в активності нітрат- та нітритредуктаз в печінці шурів, яким моделювали алкогольний гепатит.

Ключові слова: печінка, алкогольний гепатит, цикл оксиду азоту, шури.

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РЕАКЦИЯ ГЕМОМИКРОЦИРКУЛЯТОРНОГО РУСЛА ПЕЧЕНИ И ИЗМЕНЕНИЯ В ФУНКЦИОНАЛЬНОМ СОСТОЯНИИ ЦИКЛА ОКСИДА АЗОТА ПРИ УСЛОВИИ МОДЕЛИРОВАНИЯ АЛКОГОЛЬНОГО ГЕПАТИТА

Микитенко А.О., Єрошенко Г.А.

Целью работы было изучить изменения гемомікроциркуляторного русла печени и роль NO-эргической системы в их развитии в условиях моделирования алкогольного гепатита. На ранних сроках моделирования алкогольного гепатита толщина сосудистой стенки центральной вены и междольковой артерии и внутريدольковой артериолы увеличивается, а толщина сосудистой стенки междольковой вены, внутريدольковой венулы и поддольковой вены уменьшается, что связано с дисрегуляторными изменениями в цикле оксида азота печени крыс. Дисрегуляторные изменения заключаются в увеличении активности индукцибельной и конститутивных изоформ NO-синтазы на фоне снижения активности аргиназы при отсутствии статистически значимых изменений в активности нитрат- и нитритредуктаз в печени крыс, которым моделировали алкогольный гепатит.

Ключевые слова: печень, алкогольный гепатит, цикл оксида азота, крысы.

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INFLUENCE OF PINEAL GLAND'S HYPOFUNCTION ON THE STRUCTURE OF VISCERAL ORGANS

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The purpose of this work was to study the morphological and functional changes in the pineal gland, heart, stomach, lungs and intestines of rats in the conditions of the pineal gland's hypofunction. The studies were carried out on 24 sexually mature male Wistar rats, which were kept in standard vivarium conditions with round-the-clock lighting for 30 days. As a result of the performed microscopic studies, it was found that the lack of melatonin is accompanied by erosive gastritis with atrophy of the glands, an increase in proliferative activity and in the number of pathological mitoses in the jejunum of rats, which may indicate the genesis of malignant tumors. Dystrophies, atrophy and hypertrophy of cardiomyocytes, foci of cardiomyocytes' lysis, circulatory impairment and inflammatory changes in lung tissues, which can be considered moderately expressed intestinal pneumonia, were revealed.

Key words: pineal gland, pinealocytes, hypofunction, cardiomyocytes, intestinal pneumonia, gastritis.

The work is a fragment of the research project "Features of metabolism and morphofunctional condition of visceral organs exposed to the influence of environmentally hazardous factors", state registration No. 0118U003395.

Among the physiological characteristics of living organisms, the fundamental one is the rhythm of their activity, which manifests itself in the periodicity of many functions, circadian rhythms, and seasonality [5, 9]. The pineal gland is considered to be the central link that provides the body with information about changes in the light regime. The secretion product of the pineal gland is the hormone melatonin, which regulates the body's biorhythms, both directly affecting cells and by changing the secretion of other hormones and biologically active substances, which concentration varies depending on the time of day [7, 8]. Various

changes in the synthesis of melatonin that go beyond physiological fluctuations can lead to disruption of the body's own biological rhythms between themselves or the rhythms of the body with the environment. The use of artificial lighting at night helps to change both the light regime and the duration of the light effect on the body. Such a violation of the photoperiod is becoming a part of the modern way and pace of life, which is accompanied by serious changes in the health status, which are manifested in the morphological and functional rearrangement of the organs in the visceral systems [2, 3, 4, 6]. In both cases, this is accompanied by the development of morphological and functional changes in tissues and diseases of internal organs.

The purpose of our work was to study the morphological and functional changes in the pineal gland, heart, stomach, lungs and intestines of rats in the conditions of the pineal gland's hypofunction.

Materials and methods. The study was carried out on 24 sexually mature male Wistar rats weighing 240 - 280 g. For the experiments, males were selected, since the level of melatonin in the blood plasma of females depends on the sexual cycle phase. The animals were kept under standard vivarium conditions. The hypofunction of the pineal gland was simulated by round-the-clock illumination with two fluorescent lamps with an intensity of 1000-1500 Lx, which were placed on both sides of the coop. The exposure of the animal organism to light has been carried out for 30 days [1]. The use of this model is due to several reasons. First, the use of other means, such as pinealectomy or blinding of animals, is accompanied by additional stress, which would undoubtedly affect the histological state of the organs under study and the correct interpretation of the results obtained. Secondly, melatonin is synthesized by the pineal gland only under darkroom conditions, and it is generally known that for white rats light intensity of 0.0005 mV / cm² is sufficient to reduce the production of melatonin. Therefore, this model, in our opinion, reproduces the state of hypomelatoninemia.

At the end of the experiment, the experimental animals were euthanized under light ether anesthesia 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 the "General Ethical Principles for Animal Experiments" adopted by the first national congress on bioethics (Kyiv, 2001).

After removing the organs under study, they were immersed in a fixing solution of 10% neutral formalin. Using standard methods, the material was embedded in paraffin blocks, from which sections were cut with the thickness of 4 μm and stained with hematoxylin and eosin. The histological slides obtained in this way were studied at various magnifications with "Carl Zeiss" microscope, followed by photographing the microslides with a "Canon G10 Wide" digital reflex camera.

Results of the study and their discussion. Microscopic analysis of the pineal gland parenchyma cellular composition revealed specific morphological changes. An increase in the number of inactive dark pineal cells, which are distinguished by an intense basophilic color, was established. Such cellular structures are located in the parenchyma of the pineal gland randomly, but in the immediate vicinity of the vascular wall's outer surface. As for light pinealocytes, they constitute the absolute majority, but it should be noted that the number of dark-nucleated light pinealocytes grows.

It was established that in most light cells, the nucleoli practically do not contour. This fact is explained by the excessive filling of the karyoplasm with basophilic substance, in the form of homogeneous structureless masses, which mask the cell nucleolus. This morphological feature indicates the transition of light active pinealocytes to the population of inactive dark cells, which, under conditions of increased functional activity, will pass into an active state. Thus, dark and light pineal cells are morphological reflections of various functional states.

It was revealed that in histological sections of the pineal gland parenchyma of some experimental animals, the phenomena of weakly expressed cytoplasm vacuolization in individual pinealocytes were noted. Such foci of pineal cells vacuolization are located mainly in the periphery, but sometimes they also occur in the central part of the pineal gland (fig. 1).

On the part of the pineal gland's vascular system, we also revealed characteristic morphological changes. So, in the lumen of intraorgan blood vessels, both subcapsular and those located in the central part of the pineal gland, disorders in the morphofunctional state of the blood formed elements were revealed. Structurally, this is expressed in the adhesion of erythrocytes with the formation of various conglomerates, which almost completely overlap the lumen of the respective blood vessel. The gaps between adjacent conglomerates of glued erythrocytes are filled with eosinophilic substance, which is a coagulated mass of plasma proteins. This ratio between various blood elements leads to impairment of its rheological properties, and to occurrence of hypertrophied endothelial cells in certain areas of the vascular wall (fig. 2). A similar reaction of endotheliocytes is accompanied by thickening of the wall and narrowing of the blood vessel's lumen. This phenomenon is especially pronounced in arterioles and precapillary arterioles.

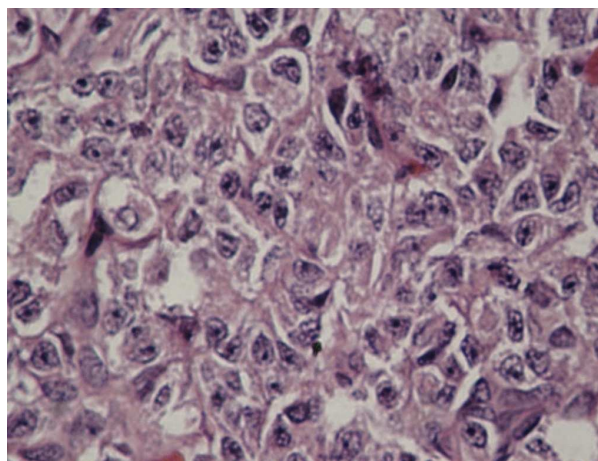


Fig. 1. Micrograph of the cellular composition of the rat pineal gland parenchyma under the conditions of prolongation by light. Magn.: oc. $\times 10$ ob. $\times 40$. Hematoxylin-eosin staining.

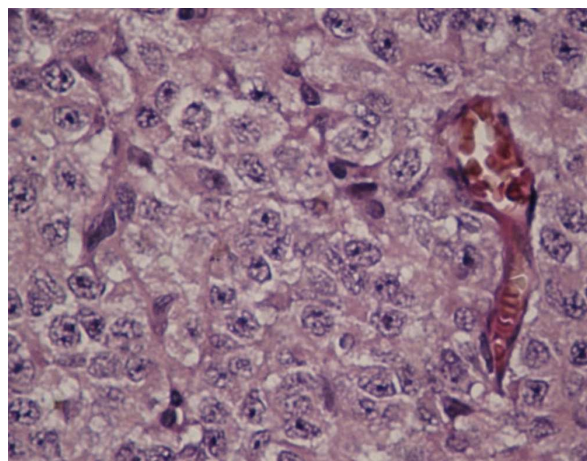


Fig. 2. Micrograph of rat pineal gland parenchyma under conditions of light prolongation. Magn.: oc. $\times 10$, ob. $\times 40$. Hematoxylin-eosin staining.

The histological picture of the heart in experimental animals is characterized by the presence of pronounced cardiomyocytes damage foci in the left ventricular myocardium. In the foci of granulation tissue development and in the lateral zone of normal myocardium, mitosis of connective tissue cells, uneven vascular blood filling, moderate perivascular edema, small hemorrhages, focal swelling and granularity of the intima in small myocardial arteries were often found. Microscopically, dilatation of capillaries and stasis, sludge phenomenon of erythrocytes, edema of interstitial tissue, perivascular hemorrhages, aggregations of leukocytes along the periphery of the ischemic zone were observed. Muscle fibers lose striation, glycogen, but are intensely stained with eosin. In the atrial myocardium, it is possible to reveal dystrophic changes in muscle fibers, areas of their significant deep degradation. Microscopic examination revealed a combination of dystrophy (protein, hydropic, fatty), atrophy and hypertrophy of cardiomyocytes, there were foci of cardiomyocyte lysis and sclerosis.

Limited foci of inflammatory infiltration were located subpericardially, and pronounced edema was observed. These changes can be assessed as a consequence of the fibrous pericarditis development. In the atrial myocardium, the occurrence of mitotic figures could be noted. In the muscles of the rat heart, where both muscle and stromal cells proliferate, one can note morphological differences in the mitoses

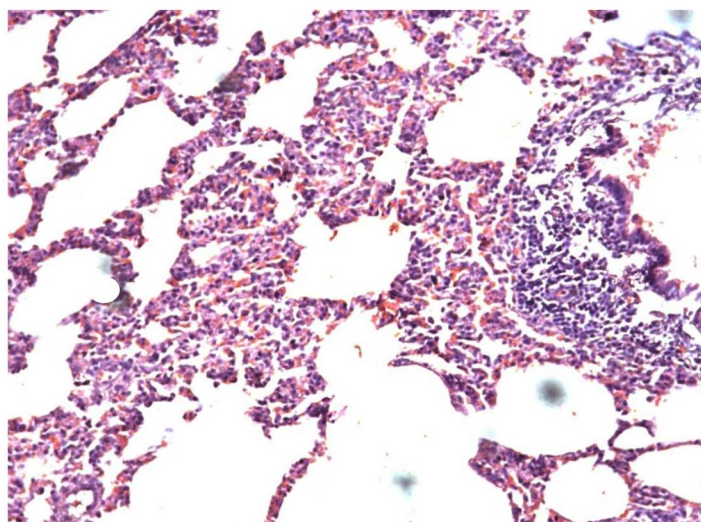


Fig. 3. Interstitial pneumonia in animals with 30-day pineal gland's hypofunction: 1 - capillary plethora; 2 - increased area of distelectases; 3 - bronchial obstruction; 4 - hyperplasia of lymphoid tissue; 5 - emphysema. Paraffin section. Magn.: ob. $\times 10$, oc. $\times 20$. Hematoxylin-eosin staining.

of these cells: connective tissue cells in the state of separation are, naturally, separated, their cytoplasm is basophilic, and the mitotic figure of myocytes is larger, the cytoplasm is lighter in significant areas, chromosomes are located within the muscle fiber. Most cells are in the state of mitosis, with half of all mitotic cells being in metaphase.

Under the prolonged illumination conditions, circulatory disorders in the form of capillary plethora with perivascularitis and interstitial edema were observed in rat lung tissue. In addition, inflammatory changes were found: with an increasing area of distelectases, bronchial obstruction with hyperplasia of lymphoid tissue was growing, bronchitis and interstitial alveolitis increased, which can be considered moderate interstitial pneumonia (fig. 3).

During the morphological study of the rat stomach histological slides, it was found that the mucous membrane is unevenly thinned, has micro-erosions, and is covered with desquamated epithelial cells (fig. 4 A). The proper lamina of the mucosa is expanded, has a moderately pronounced inflammatory infiltration. The stomach glands are of different sizes, most are cystic-enlarged and have atrophic changes. Both the

main and parietal cells have pale, narrow cytoplasm and small hyperchromic nuclei. The submucosal layer is expanded, has a swollen stroma.

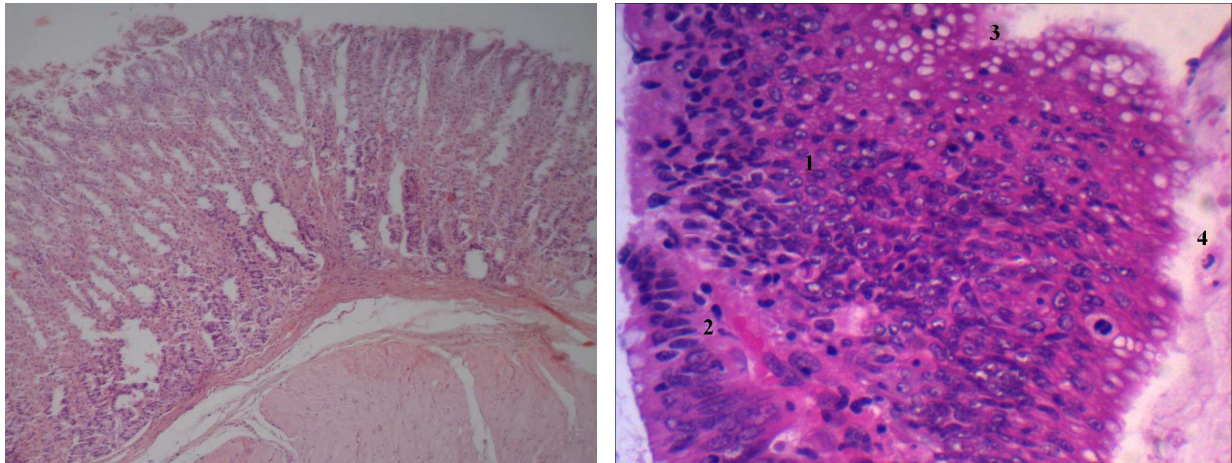


Fig. 4 A. Micrograph of rat stomach tissues with prolonged hypofunction of the pineal gland. Paraffin section. Magn.: ob. \times 10, oc. \times 10. Hematoxylin-eosin staining.

B. Distribution of cellular elements of rat jejunal epitheliocytes with a lack of melatonin. Magn.: ob. \times 10 oc. \times 15. Hematoxylin-eosin staining.

The muscle layer is thickened, muscle fibers are swollen, unevenly hypertrophied. In the submucosa, muscular and subserous membranes, moderately pronounced inflammatory, polymorphocellular infiltration is observed. The samples show chronic active erosive gastritis with glandular atrophy and thinning of the mucous membrane.

When studying histological preparations of the jejunum, it was established that intestinal crypts and villi had a somewhat smoothed appearance due to an increase in the number of mitotic undifferentiated cells. To a lesser extent, goblet cells produced mucus. At high magnification of the microscope, we noted the following features of the distribution of endocrinocytes: the cells had a well-developed cytoplasm, almost all of its surface was occupied by well-contoured nuclei. Nuclear-cytoplasmic ratio in some endocrinocytes was 0.8-0.9. Heterochromatin of the nuclei had a lumpy character and was located diffusely throughout the nuclear envelope. Nucleoli in the amount of 1-2, and sometimes more, were mainly localized along the periphery of the nuclei. In all fields of view of the microscope, cells were found that were in different phases of mitosis and were characterized by high proliferative activity (fig. 4 B).

Based on the distribution data of epithelial cells' proliferative activity, the mitotic index is 22.31 ‰. A characteristic feature is that the overwhelming majority of cells were in a state of mitotic division, a large number of them being represented by pathological mitoses. Thus, the main pathology (16.7%) was predominantly K-mitosis with chromosome agglomeration, characterized by blockade of cell division in the metaphase as a result of damage to the mitotic apparatus. It is represented by hyperspiralized and thickened chromosomes, which form a dense conglomerate - a clump. The second place was occupied by a pathology of chromosome separation (9.6%), characterized by an uneven distribution of chromosomes in mitotic centers and a delay in their entry into daughter cells. In addition, a small number of bridges were observed, being the result of chromosome fragmentation with formation of a bridge, delaying and blocking the formation of cytotomy.

Numerous studies have shown that the experimental model of pineal gland hypofunction is accompanied by a decrease in melatonin levels in the blood plasma [9]. The results of our data indicate that in response to the effects of round-the-clock lighting morphofunctional destructive changes take place not only in the pineal gland, but also in the studied visceral organs, which according to literature sources indicate the development of specific pathological changes of inflammatory nature and consequent reduction of the visceral organs' functional activity [1, 6, 8].

Conclusion

The results of the histological slides histomorphological analysis indicate that the violation of the daily rhythm, that is, the change of day and night, is due to the stay of experimental animals in the conditions of a disturbed photoperiod for a long time, affects the morphological status and functional activity of not only the pineal gland but also nearly all visceral organs in animals.

1. In the pineal gland there is an adaptive morphofunctional restructuring of the parenchyma and the vascular bed in response to the prolonged exposure to light factors.

2. In the myocardium microslides, dystrophic changes and disturbances of the microcirculation bed, increased separation of connective tissue cells with the presence of K-mitosis are observed.

3. In the tissues of the lungs, destructive changes are observed, which are manifested by signs of interstitial pneumonia.

4. Manifestations of the pineal gland's hypofunction were also revealed in histological slides of the rat stomach, in the form of moderately pronounced chronic active erosive gastritis with the glands' atrophy and thinning of the mucous layer.

5. The revealed morphological changes in the jejunum indicate that the lack of melatonin sharply increases the number of pathological mitoses, which may indicate the presence of malignant tumors. At the same time, there is an increase in proliferative activity in the jejunum of rats.

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Реферати

ВПЛИВ ГИПОФУНКЦІЇ ЕПІФИЗУ НА СТРУКТУРУ ВІСЦЕРАЛЬНИХ ОРГАНІВ Пшиченко В.В., Чеботар Л.Д., Ларичева О.М., Цвях О.О., Анасевич Я.М.

Метою даного дослідження було вивчення морфологічних і функціональних змін епіфіза, серця, шлунка, легень і кишечника шурів в умовах гіпофункції епіфізу. Дослідження проведені на 24 статевозрілих самцях шурів лінії Wistar, які перебували в стандартних умовах виварію в режимі цілодобового освітлення протягом 30 днів. В результаті проведених мікроскопічних досліджень встановлено, що нестача мелатоніну супроводжується ерозивним гастритом з атрофією залоз, збільшенням проліферативної активності і кількості патологічних мітозів в порожній кишці шурів, що може вказувати на появу злоякісних пухлин. Виявлено дистрофії, атрофії і гіпертрофії кардіоміоцитів, осередки лізису кардіоміоцитів, порушення кровообігу і запальні зміни в тканинах легень, які можна вважати помірною інтестиціальною пневмонією

Ключові слова: епіфіз, пінеалоцити, гіпофункція, кардіоміоцити, інтестиціальна пневмонія, гастрит.

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ВЛИЯНИЕ ГИПОФУНКЦИИ ЭПИФИЗА НА СТРУКТУРУ ВИСЦЕРАЛЬНЫХ ОРГАНОВ Пшиченко В.В., Чеботарь Л.Д., Ларичева А.Н., Цвях А.А., Анасевич Я.М.

Целью данного исследования было изучение морфологических и функциональных изменений эпифиза, сердца, желудка, легких и кишечника крыс в условиях гипofункции эпифиза. Исследования проведены на 24 половозрелых самцах крыс линии Wistar, которые находились в стандартных условиях вивария в режиме круглосуточного освещения в течение 30 дней. В результате проведенных микроскопических исследований установлено, что недостаток мелатонина сопровождается эрозивным гастритом с атрофией желез, увеличением пролиферативной активности и количества патологических митозов в тощей кишке крыс, что может указывать на появление злокачественных опухолей. Виявлены дистрофии, атрофии и гипертрофии кардиомиоцитов, очаги лизиса кардиомиоцитов, нарушение кровообращения и воспалительные изменения в тканях легких, которые можно считать умеренной интестициальной пневмонией

Ключевые слова: эпифиз, пинеалоциты, гипofункция, кардиомиоциты, интестициальная пневмония, гастрит.

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