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ULTRASTRUCTURAL CHANGES OF PINEALOCYTES OF THE PINEAL GLAND UNDER THE ACTION OF METHYLENE BLUE IN RATS OF DIFFERENT AGES

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The purpose of the study was to investigate the ultrastructural changes of the pineal cells of the pineal gland in normal conditions and under the conditions of long-term exposure to methylene blue. In 14-day-old intact rats, the pineal gland is represented as a formed organ. With age, in 45- and 90-day-old animals, the ultrastructural state of the cytoplasm and nucleus of pinealocytes corresponded to increased activity, but was less pronounced than in 14-day-old animals. Under the effect of methylene blue, in the pineal gland of 14- and 45-day-old rats, structural signs of weakly expressed enhancement of the function of light and dark pinealocytes were noted. In 90-day-old animals, under the effect of methylene blue in the pineal gland, along with the enhancement of the function of light pineal cells, signs of normalization of structure and function were noted in dark ones, which is obviously connected with the inclusion of adaptive mechanisms to the effect of methylene blue. The obtained results open the prospect for further study of the patterns of structural and functional changes of the endocrine glands under the conditions of nitrate intoxication and represent the basis for the development of effective measures for the correction of endocrine system lesions that occur in people as a result of professional activity.

Key words: gland, endocrine system, intoxication, nitrate poisoning, correction.

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УЛЬТРАСТРУКТУРНІ ЗМІНИ ПІНЕАЛОЦИТІВ ЕПІФІЗА ЗА УМОВ ДІЇ МЕТИЛЕНОВОГО СИНЬОГО У ЩУРІВ РІЗНОГО ВІКУ

Метою дослідження було вивчення ультраструктурних змін пінеальних клітин епіфіза в нормі та за умов тривалого впливу метиленового синього. У 14-добових інтактних щурів епіфіз представлений як сформований орган. З віком у 45- і 90-добових тварин ультраструктурний стан цитоплазми і ядра пінеалоцитів відповідав підвищеній активності, однак менш виражено, ніж у 14-добових тварин. За умов дії метиленового синього в епіфізі 14-, 45-добових щурів відмічались структурні ознаки слабо вираженого посилення функції світлих і темних пінеалоцитів. У 90-добових тварин при дії метиленового синього в епіфізі поряд з посиленням функції світлих пінеальних клітин, в темних відмічались ознаки нормалізації структури і функції, що очевидно пов'язано з включенням адаптаційно-пристосувальних механізмів до дії метиленової сині. Отримані результати відкривають перспективу подальшого вивчення закономірностей структурно-функціональних змін залоз внутрішньої секреції в умовах дії нітратної інтоксикації та представляють підґрунтя для розробки ефективних заходів корекції уражень ендокринної системи, що виникають у людей у результаті професійної діяльності.

Ключові слова: залоза, ендокринна система, інтоксикація, отруєння нітратами, корекція.

The study is a fragment of the reseach project "Histophysiological state of the endocrine system under the effect of adverse environmental factors", state registration No. 0120U002026.

It is known that the pineal gland, or epiphysis (pineal gland or upper brain appendage) is a small oval glandular formation that belongs to the diencephalon and is located in a shallow sulcus between the upper tubercles of the midbrain above the thalamus. According to data [1], the pineal gland closely cooperates with all diencephalic elements, implementing its humoral connections not only through blood, but also through cerebrospinal fluid. The ventricular system of the brain permits the humoral signals of the

pineal gland to reach the nuclei of the hypothalamus, thereby ensuring the expression of the effects of the pineal gland as an organ of fine endocrine regulation. The main indoleamine-producing hormones of the pineal gland (serotonin and melatonin), as well as the so-called polypeptides, are of primary importance in the maintenance and regulation of important physiological processes in the body, such as biological rhythms, puberty, functioning of peripheral endocrine glands, etc. [4, 5, 6]. Thus, biologically active compounds produced in the pineal gland have not only a central, but also a peripheral effect.

Modern sources of literature mainly contain data devoted to the study of the structure and function of the pineal gland in humans and animals under conditions of the body's exposure to radioactive and electromagnetic radiation, immobilization of animals, changes in the light regime, etc. [2, 3, 7, 9], leaving the issue of the impact on epiphysis of substances used for treatment and prevention of poisoning with nitrates, pesticides and other poisonous substances. Among similar substances, methylene blue, which accelerates the process of restoring methemoglobin, attracts special attention of researchers. It acts as a cofactor of NADPH-methemoglobin reductase.

Methylene blue in vivo turns into a colorless substance leucomethylene blue, which begins to act as an electron donor for oxidized iron, converting it into a reduced form. There are no data on the use of methylene blue to correct the effect of nitrates on the structure and function of the endocrine glands in modern literature. Today in the literature there are data on the effect of methylene blue on the structure and function of the gastrointestinal tract organs, while its long-term effect on the endocrine system, namely on the pineal gland, remains poorly studied, especially in different age periods of postnatal ontogenesis of animals.

In this regard, the study of the morpho-functional changes of the pineal gland's cells in animals of different age groups under normal conditions and under the conditions of long-term exposure to methylene blue is not only relevant, but also requires further study.

The purpose of the study was to investigate the ultrastructural changes of the pineal cells of the pineal gland in normal conditions and under the conditions of long-term exposure to methylene blue.

Materials and methods. The study was carried out in the conditions of the university vivarium on 30 male rats of the Vistar line, divided into three groups, depending on the duration of the postnatal period and experimental feeding: 14, 45 and 90 days. All animals were in equal conditions. Keeping and using animals was carried out in accordance with the developed resolutions "General ethical principles of experiments on animals" adopted by the VII National Congress on Bioethics in 2019.

When simulating the action of methylene blue, animals were orally administered this substance daily in the doses of 0.1-0.15 ml of a 1 % aqueous solution per 1 kg of body weight. For electron microscopic examination of the intermediate lobe of the adenohypophysis of intact and experimental rats, the material was fixed in a 2.5 % glutaraldehyde solution in a phosphate buffer with additional fixation in a 1 % osmium tetroxide solution by Caulfield. The material was dehydrated in alcohols of increasing concentration and acetone. It was embedded in the Epon-araldite mixture. Ultrathin sections were made of the obtained blocks with LKB III ultratome (Sweden) which contrasted with a 2 % solution of uranyl acetate and lead citrate. The specimens were examined and photographed under a PEM-125K electron microscope at magnifications from 6,000 to 20,000 times. The obtained indices permitted to analyze changes in the cytoarchitectonics of melanotropic cells in the intermediate lobe of the adenohypophysis at the electron microscopic level under normal and experimental conditions [10].

Results of the study and their discussion. The performed study showed that in 14-day-old intact rats, two types of pineal cells (light and dark) and neuroglial cells were determined in the pineal gland. In the pineal parenchyma, the number of dark cells significantly exceeded the number of light cells. Moderate development of organelles was noted in the ultrastructure of the pineal cells' cytoplasm. However, the accumulation of free ribosomes and ribosomal complexes in the cells' cytoplasm and the development variability of the endoplasmic reticulum reflected their different functional activity even under normal conditions.

On the 45th day of rats' life, the ultrastructural state of the cytoplasm and nucleus of pinealocytes in intact animals of this age corresponded to increased functional activity, but less it was pronounced than in 14-day-old rats.

In the pineal parenchyma of 90-day-old intact rats, ultrastructural changes of dark pinealocytes were noted, which also showed an increase in functional activity, but less pronounced than in 14-day-old rats. When methylene blue was administered, the pineal gland's ultrastructure in 14-day-old rats

corresponded to the norm, that is, it completely coincided with that of intact animals of this age. Light cells had rather large sizes and processes that penetrated between neighboring dark cells. In the cytoplasm of light cells, there were many small mitochondria with a somewhat compacted matrix and clear cristae. In the center of the cells, one could see a cluster of narrow tubules of the granular endoplasmic reticulum with ribosomes on the surface of their membranes. Medium-sized cisterns without contents were found in the cytoplasm, and single secretory granules were small in size. Intercellular membranes with a narrow slit-like space and single desmosomes were clearly defined between the cells.

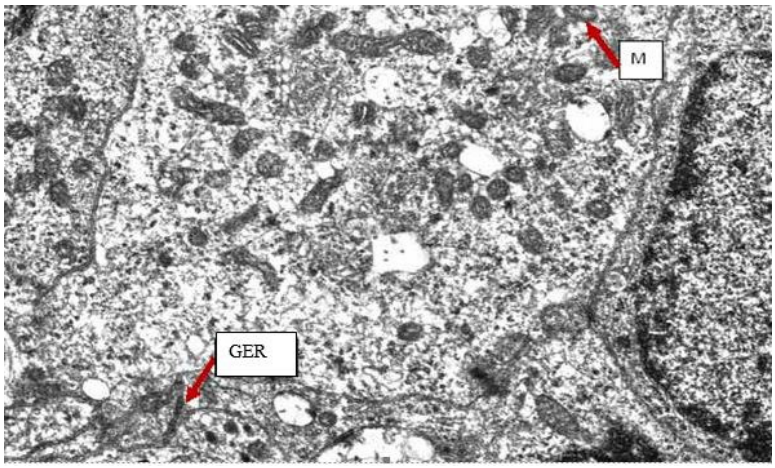

Fig. 1. Epiphysis of a 14-day-old rat after exposure to methylene blue. In the cytoplasm of a light cell, there are numerous mitochondria with a somewhat compacted matrix and clear cristae (M). A cluster of narrow tubules of the granular endoplasmic reticulum (GER). Electrogram. × 9600.

In the ultrastructure of dark cells, small dark mitochondria and single secret granules could be seen. Narrow channels of the granular endoplasmic reticulum were clearly visible. A small amount of parietal heterochromatin and well-defined nuclear pores were observed in the nucleus. Euchromatin was located evenly throughout the nucleoplasm (fig. 1).

Thus, in 14-day-old animals under the influence of methylene blue, many small-sized mitochondria with a slightly compacted matrix and clear cristae were noted in the ultrastructure of light cells. Small dark mitochondria and single secret granules could be seen in the dark cells. The ultrastructure of the pineal gland was characterized by a slight increase in the functional activity of light and dark pinealocytes compared to intact animals.

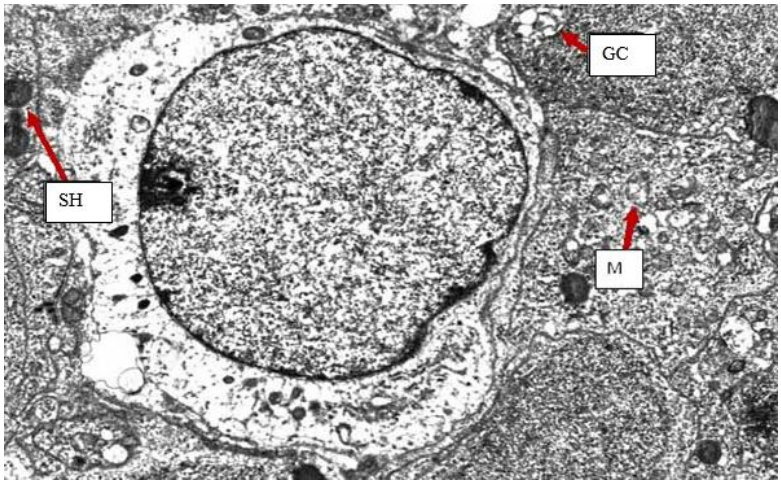

Fig. 2. Epiphysis of a 45-day-old rat after exposure to methylene blue. Numerous mitochondria are slightly swollen, and their cristae are pushed to one of the cell's poles (M). Secretory granules of small size (SH), many ribosomes (P) and polyribosomal complexes. Electrogram. × 4800.

In the electron microscopic examination of the pineal gland in 45-day-old rats after administration of methylene blue, the ultrastructure of pineal cells mostly corresponded to the norm. In the center of the sections, light cells with a clear structure of the cytoplasm were found. Mitochondrial matrix was light, fine-grained. Mitochondria were slightly swollen, and their cristae were pushed to one of the cell's poles.

There were small secretory granules, many ribosomes and polyribosomal complexes. The Golgi complex consisted of dictyosomes, the cavities of which were slightly expanded, and at the lower pole of the complex, accumulations of fine granularity were noted, it is obvious that these were secret granules synthesized and not formed in dictyosomes. Processes of light cells were filled with small granules of secretion.

Thus, in 45-day-old animals after exposure to methylene blue, ultrastructural studies of light cells showed that the mitochondrial matrix was light, fine-grained. Single mitochondria were slightly swollen, and their cristae were pushed to one of the cell's poles of the. Small secretory granules occurred. There were no significant deviations from the norm in the ultrastructure.

During the electron microscopic examination of the pineal gland in 90-day-old rats after the administration of methylene blue, in the ultrastructure of the light cells' cytoplasm, many small mitochondria of mainly rounded shape were noted, but elongated ones with condensed cytoplasm and

transversely located cristae were also found. Many free ribosomes were found in the cytoplasm. The endoplasmic reticulum was weakly expressed, and the Golgi complex was sometimes absent. The boundaries between the cells are clear. The nucleus was determined to be round in shape, small in size, in the nucleoplasm of which euchromatin prevailed near the membrane, and the nucleolus was placed in contact with it. Many nuclear pores were observed in the caryolemma.

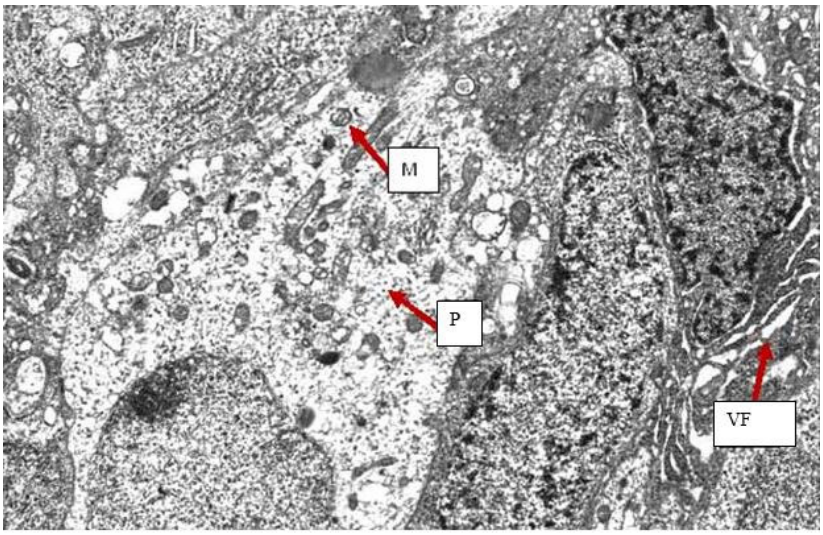

Fig. 3. Electrogram. Epiphysis of a 90-day-old rat after exposure to methylene blue. In a bright cell, there are many small mitochondria of rounded and elongated shape (M). A large number of free ribosomes (P). In dark pinealocytes, the cytoplasm is condensed and there are clusters of vacuolar formations (VF) with missing contents. ×4800.

In dark cells, the cytoplasm was condensed, and secretory granules were absent. Accumulation of vacuolar formations with the absence of contents in their cavities was determined, often vacuoles or cisterns of the granular endoplasmic reticulum were filled with osmiophilic contents. Fibroblasts with an elongated, heterochromatin-rich nucleus were often located near the dark cells. The cytoplasm of these cells contained numerous expanded channel endoplasmic reticulum without content (fig. 3).

Thus, in the pineal gland's parenchyma of 90-day-old rats after prolonged exposure to methylene blue, there were signs of some increase in the functional activity of type I cells.

In 14-day-old animals, after the effect of methylene blue in the epiphysis, at the ultrastructural level, many small-sized mitochondria with a somewhat condensed matrix and clear cristae were noted in light cells. Small dark mitochondria and single secret granules could be seen in the dark cells. The ultrastructure of the epiphysis was generally normal.

Ultrastructural studies of 45-day-old animals after exposure to methylene blue showed that in light cells, the mitochondrial matrix was light and fine-grained. Single mitochondria were slightly swollen, and their cristae were pushed to one of the cell's poles. Small secretory granules occurred. There were no significant deviations from the norm in the ultrastructure, but the structural and functional state of type I cells indicated weak signs of the increased function.

Thus, in the parenchyma of the pineal gland in 90-day-old rats after long-term exposure to methylene blue, there were signs of some increase in the functional activity of type I cells, while in dark ones there were signs of normalization in structure and function.

Thus, under the conditions of exposure to methylene blue in the pineal gland of 14-, 45-, and 90-day-old rats, structural signs of weakly expressed enhancement of light and dark pinealocytes' function were noted.

Studies of the pineal cells of the pineal gland in intact animals showed that characteristic morpho-functional changes in the development of pineal cells occurred in different age periods of animals [2, 8].

Thus, in 14-day-old intact rats, two types of pineal cells (light and dark) and neuroglial cells were determined in the pineal gland. In the parenchyma of the pineal gland, the number of dark cells significantly exceeded the number of light cells, which is consistent with research data [5].

Regarding the issue of the relationship between these two types of cells (light and dark), there are different opinions. According to some researchers [4], light cells originate from dark ones, other authors [6] believe that this difference is the result of the display of different phases in their functional state. Others allow them as independent, self-sufficing groups. Light and dark cells are rather descendants of different forms of spongioblasts. Light cells are closer to astroglia, and dark cells are closer to oligodendroglia [1]. Definitive tissue is formed in the process of these structures' interaction.

On the 45th day of rats' life, the ultrastructural state of the cytoplasm and nucleus in pinealocytes of intact animals of this age corresponded to increased activity, but less pronounced than in 14-day-old rats.

On the 90th day of rat development, signs of increased functional activity were noted in the ultrastructure, but weaker than in 14-day-old intact rats. As studies [10] show, one of the cytological signs of an increase in the number of differentiated cells is an increase in the expression of membrane invagination in their nuclei. This regularity was also observed in our study.

Under the conditions of exposure to methylene blue, in the pineal gland of 14-, 45-, and 90-day-old rats, structural signs of weakly expressed enhancement in the function of light and dark pinealocytes were noted, which is obviously related to the inclusion of adaptation mechanisms to the action of methylene blue.

Conclusions

1. In 14-day-old intact rats, the pineal gland is represented as a formed organ in which two types of cells were determined: pineal (light and dark) and neuroglial. With age, in 45- and 90-day-old animals, the ultrastructural state of the cytoplasm and nucleus of pinealocytes corresponded to increased activity, but less pronounced than in 14-day-old animals.

2. In 14-day-old rats, after long-term exposure to methylene blue, in the pineal gland there was a slight increase in the functional activity of light and dark pinealocytes at the ultrastructural level.

3. In 45-day-old rats exposed to methylene blue, the ultrastructural state of the cytoplasm and nucleus of pinealocytes corresponded to increased activity, but less pronounced than in 14-day-old rats.

4. In 90-day-old animals, under the action of methylene blue in the pineal gland, along with the enhancement of the function of light pineal cells, signs of normalization of structure and function were noted in the dark ones, which is obviously connected with the inclusion of adaptive mechanisms to the action of methylene blue.

The obtained results open the prospects for further study of the patterns of structural and functional changes of the endocrine glands under the conditions of nitrate intoxication and represent the basis for development of effective measures for the correction of endocrine system lesions that occur in people as a result of professional activity.

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