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Стаття надійшла: 14.12.2020 р.

DOI 10.26724/2079-8334-2021-4-78-250-254
УДК 572.786-546.175

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ULTRASTRUCTURAL CHANGES OF THE ADENOGIPOPHYSIS INTERMEDIATE PART UNDER LONG-TERM ACTION OF NITRATES AND MEANS OF CORRECTION BY METHYLENE BLUE

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The perpose of the work was to study the ultrastructural changes of melanotropic cells in the intermediate part of the adenohypophysis in 14-, 45- and 90-day-old rats under the action of nitrates and correction with methylene blue. The results of the study showed that the simultaneous action of nitrates and methylene blue had a positive effect on the structure and function of melanotropic cells of the intermediate lobe of the adenohypophysis in all periods of the study. Methylene blue mitigated their toxic effect on melanotropics of the adenohypophysis intermediate and was a kind of protector of nitrates, reducing the manifestation of the depletion stage of the general adaptation syndrome and helping to restore hormonal balance in the intermediate adenohypophysis. The obtained results open the prospect of further study of structural and functional changes patterns in the endocrine glands under nitrate intoxication and provide a basis for developing effective and safe preventive measures to correct endocrine disorders in humans as a result of professional activities.

Key words: nitrate intoxication, simultaneous exposure, melanotropic cells, ultrastructural changes, protective effect.

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УЛЬТРАСТРУКТУРНІ ЗМІНИ ПРОМІЖНОЇ ЧАСТКИ АДЕНОГІПОФІЗА ЗА УМОВ ТРИВАЛОЇ ДІЇ НІТРАТІВ ТА ЗАСОБІВ КОРЕКЦІЇ МЕТИЛЕНОВИМ СИНІМ

Метою дослідження було вивчення ультраструктурних змін меланотропних клітин проміжної частки аденогіпофіза у щурів 14-, 45- і 90- добового віку під дією нітратів та корекції метиленовим синім. Результати дослідження показали, що одночасна дія нітратів і метиленового синього позитивно впливала на структуру і функцію меланотропних клітин проміжної частки аденогіпофіза в усі періоди дослідження. Метиленовий синій на фоні надходження в організм нітратів зм'якшував їх токсичний ефект на меланотропи проміжної частки аденогіпофіза та був своєрідним протектором дії нітратів, знижуючи прояв стадії виснаження загального адаптаційного синдрому і сприяючи відновленню гормональної рівноваги в проміжній частці аденогіпофіза. Отримані результати відкривають перспективу подальшого вивчення закономірностей структурно-функціональних змін залоз внутрішньої секреції в умовах дії нітратної інтоксикації та представляють підґрунтя для розробки ефективних та безпечних профілактичних заходів корекції уражень ендокринної системи, що виникають у людей у результаті професійної діяльності.

Ключові слова: нітратна інтоксикація, одночасний вплив, меланотропні клітини, ультраструктурні зміни, протекторна дія.

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

Currently, in various regions of Ukraine there is significant contamination of soils, groundwater, which in combination with inflated levels of pesticides, radionuclides and other substances poses a real threat to all parts of the ecosystem [4, 8]. However, unlike other xenobiotics, the danger of nitrates on the homeostasis of humans and animals is still not fully understood by society today.

The effects of nitrates are complex and multifaceted. They are manifested in acute poisoning, malignant neoplasms, congenital malformations, infant mortality. This diverse action of nitrates causes significant structural and functional changes in various organs and systems of the body and especially in the endocrine glands, which are known to provide a complex set of compensatory-adaptive processes in the body under any extreme effects [11, 15].

It is known that the pathogenetic basis of eco-dependent diseases are violations in the process of the organism adaptation [14]. The main mechanism of damaging action of nitrates is free radical oxidation

of cell membranes lipids, which leads to the development of metabolic stress in the body, which contributes to disruption of biochemical processes in tissues, enhanced immunotoxic effects and tissue hypoxia [9, 12].

In modern theoretical and practical biology and medicine, the key problem of research is to reveal the patterns of ultrastructural organization of the human and animal body at different stages of development and exposure to damaging factors. It is generally accepted that the neuroendocrine system, which is the regulator of all vital processes in the organism, is subject to the primary threat of intoxication and destabilization, the consequences of which are extremely dangerous for vital functions [2].

It is generally accepted that the intermediate part of the adenohypophysis, which produces a number of hormones (melanocytostimulating and lipotropic), regulate the functions of peripheral endocrine glands and some types of metabolism. However, data on ultrastructural changes in the intermediate lobe of the adeno-pituitary gland are lacking.

The issue of finding compensatory reserves of the body and activation of its natural defenses in the critical ecological situation of the environment is extremely relevant today. There are well-known works on the use of pharmacological drugs to correct hypoxic conditions and protect cells and the body as a whole under stress of various origins, including chemical and metabolic [6], but the use of methylene blue (Methylenum coeruleum) to correct the effects of nitrates on the structure and function of the adenohypophysis intermediate lobe were considered only in some studies.

У зв'язку з цим, вивчення морфо-функціональних змін проміжної частки аденогіпофіза у тварин різних вікових груп за умов тривалої дії нітратів та корекції метиленовим синім є не тільки актуальним, але й потребує подальшого вивчення.

Therefore, the study of morpho-functional changes in the intermediate lobe of the adenohypophysis in animals of different ages under conditions of prolonged nitrate action and correction with methylene blue is not only relevant but also needs further studies.

The purpose of the work was to study the ultrastructural changes of melanotropic cells in the intermediate part of the adenohypophysis under the action of nitrates and their correction with methylene blue.

Materials and methods. The study was performed in a 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 equivalent conditions. The keeping and use of animals was carried out in accordance with the developed resolutions "General ethical principles of animal experiments", approved by the VII National Congress of Bioethics 2019.

Prolonged effect of nitrates on the animal body was achieved by daily introduction into the drinking ration, starting from 7 days of rats' postnatal development (after previous water purification) 120 mg/l of sodium nitrate, i.e. at a dose typical of many regions of Ukraine [1]. The total dose of nitrates taken by 14-day-old experimental animals for 7 days was 840 mg, 45-day (for 38 days) – 4560 mg and 90-day (for 83 days) – 9960 mg.

When simulating the action of methylene blue, the animals were orally administered this substance daily in doses: 0.1-0.15 ml of 1 % aqueous solution per 1 kg of body weight.

To study the nature of resistance to methylene blue to nitrates, a study was carried out on the simultaneous action of nitrates and methylene blue (in the doses mentioned above) on the structure and function of melanotropic cells in the intermediate lobe of the adenohypophysis.

For electron microscopic examination of the adenohypophysis intermediate part of intact and experimental rats, the material was fixed in 2.5 % glutaraldehyde solution on phosphate buffer with fixation in 1 % solution of osmium tetroxide according to Caulfield. It was dehydrated in alcohols of increasing concentration and acetone, poured into a mixture of epon-araldite. Ultrathin sections contrasting with 2 % uranium acetate solution and lead citrate were made from the obtained blocks on the LKB III ultrathrome (Sweden). The preparations were examined and photographed under an electron microscope PEM-125K at magnifications from 6000 to 20,000 times. The obtained indices permitted to analyze changes in the cytoarchitectonics of melanotropic cells of the adenohypophysis intermediate part at the electron microscopic level in normal and experimental conditions [3].

Results of the study and their discussion.. The results of the study showed that in 14-day-old intact male rats, the intermediate lobe of the adenohypophysis is presented as a formed functionally active organ, in which two cell types (melanotropic and lipotropic) were observed. In the ultrastructure of the melanotropes' cytoplasm there was a moderate development of organelles, and judging by the number and size of secretory granules, as well as the density of their content, we can assume that all these cells differed in their functional activity. In 45-day-old intact rats, the ultrastructural state of the cytoplasm and nucleus indicated an increase in functional activity. The ultrastructure of the cells in the intermediate pituitary gland

of rats at the age of 90 days differed from that of younger animals only by signs of different functional activity of individual melanotropes.

Under the conditions of long-term action of nitrates in 14-day-old animals in the intermediate part, along with the activation of structures responsible for the synthesis of melanotropic hormone, there were signs of dystrophic changes.

In 45-day-old experimental animals in the ultrastructure of the cells of the intermediate part under prolonged exposure to nitrates an increase of functional activity was also found. At the same time in cytoplasm of separate cells the dystrophic and destructive disturbances which were connected first of all with destruction of cytoplasm of and a kernel cytological structures were defined which expressiveness increased in comparison with 14-day-old animals.

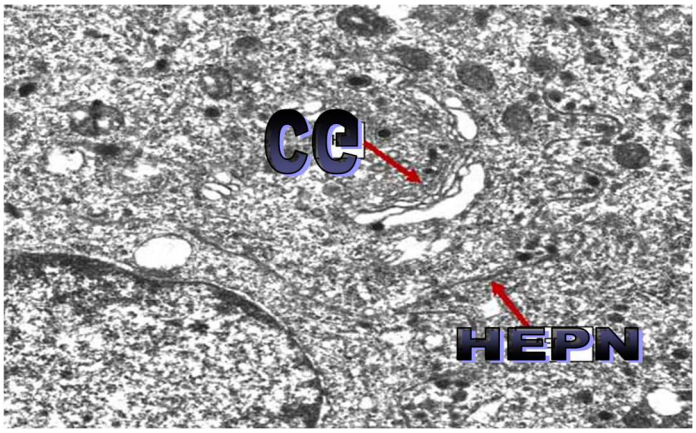

Fig. 1. Electron diffraction pattern. Intermediate part of the adenohypophysis of a 14-day-old rat after simultaneous action of nitrates and methylene blue. Well-developed endoplasmic reticulum (EPR). On the surface of the tubules and vacuoles of the endoplasmic reticulum are small osmophilic ribosomes. Golgi complex with dilated tubules and thickened dictyosomes (CG). × 9000.

In the pituitary gland of 90-day-old experimental rats, ultrastructural data indicated profound destructive and dystrophic changes in organelles. Disorder of protein-synthesizing function, signs of the secretory process inhibition, which were obviously a manifestation of the organ's depletion reaction.

The ultrastructure of melanotropic cells of the adenohypophysis intermediate lobe in 14-day-old rats after the simultaneous action of nitrates and methylene blue was mostly normal. Our attention was drawn to melanotropes, which contained a large number of secretory granules. However, in some cells the granules were completely absent, or a small number of them were determined (fig. 1).

In the cytoplasm of the cells there was a well-developed endoplasmic reticulum, well-developed Golgi complex. Mitochondria had an elongated shape and a dense matrix. Large nuclei were round or slightly oval in shape, the intermembrane gap was evenly expanded in places. Nuclear pores were well observed. There was a moderate amount of parietal heterochromatin in the nucleoplasm. Nucleoli (1–2) were observed with areas of enlightenment and hyperchromia. The intercellular boundaries are clear, the plasmalemmas of the cells were in close contact with each other.

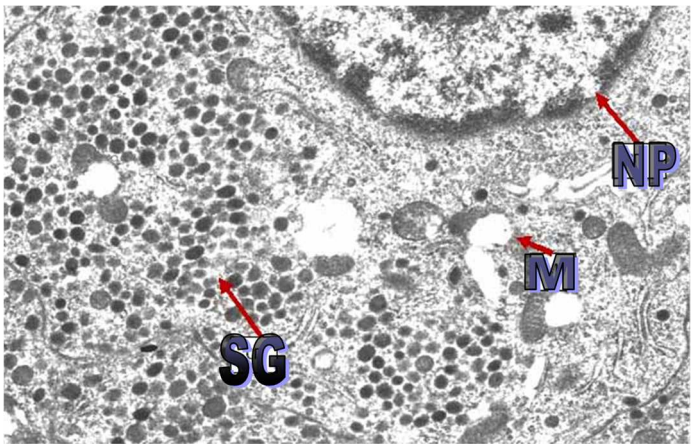

Fig. 2. Electron diffraction pattern. Intermediate part of the adenohypophysis of a 45-day-old rat after the simultaneous action of nitrates and methylene blue. In the cytoplasm of melanotrope is a large number of secretory granules of the same size and electron density (SG). Mitochondria are oval in shape with offset to one of the poles of the cristae (M). Single small clusters of small tanks are without contents. Round nucleus with clear nuclear membranes and a narrow intermembrane gap. In karyolem there are wide nuclear pores (NP). × 9000.

Thus, in the ultrastructure of the melanotropic cells' cytoplasm, activation of membrane organelles (endoplasmic reticulum, Golgi complex) and active processes of secretion granule formation were observed.

The ultrastructure of melanotropic cells in the intermediate lobe of the adenohypophysis of 45-day-old experimental rats after the simultaneous action of nitrates and methylene blue mainly corresponded to control animals. All cell types characteristic of this pituitary gland part were determined. It was possible to detect melanotropes with a large number of secretory granules, which had the same size and electron density (fig. 2).

Cells with fewer secretory granules were identified, but they were fewer in number. In the ultrastructure of the cytoplasm of melanotropes, the pattern of the granular endoplasmic reticulum with thin lumens of the tubular cavities was clearly defined.

In addition to membrane-bound ribosomes, free ribosomes in the form of polymorphic complexes were identified. Mitochondria were slightly oval in shape, but frequently their cristae were shifted to one of mitochondrial poles. Osmophilic bodies occurred singly and were small in size.

Nuclei of predominantly round shape and medium size had clear contours of nuclear membranes with a narrow intermembrane gap. Numerous nuclear pores were determined, in the nucleoplasm heterochromatin prevailed quantitatively over euchromatin and was located in small clusters on the periphery of the nucleus. Nucleoli were small in size. The intercellular membranes were clear and the intercellular space was narrow.

Thus, in 45-day-old experimental animals after the simultaneous action of nitrates and methylene blue in the ultrastructure of melanotropic cells there was an accumulation of secretory granules of the same size and electron density, increased number of organelles and restoration of cytoplasm and nucleus.

The ultrastructural organization of the adenohypophysis intermediate lobe of 90-day-old rats after the simultaneous action of nitrates and methylene blue differed little from the intact animals. Marginal cells with unchanged cytoplasmic structure and absence of secretory granules and two types of melanotropes were detected on the glands. Some melanotropic cells were with a large number of secretory granules, and others – with a moderate and slightly enlightened content of granules (fig. 3).

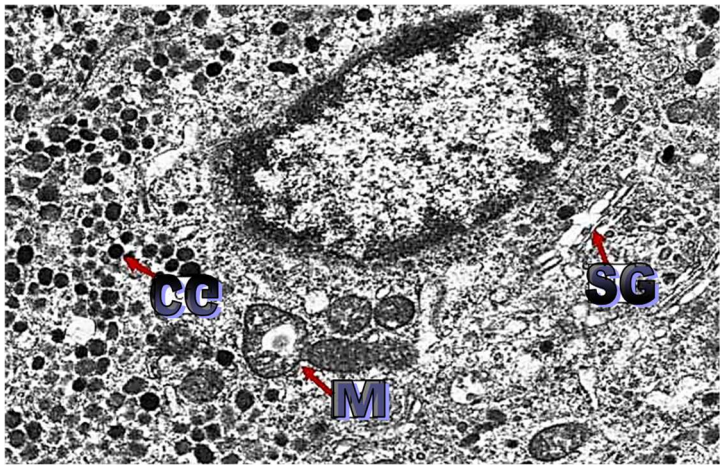

Fig. 3. Electron diffraction pattern. Intermediate part of the adenohypophysis in a 90-day-old rat after simultaneous action of nitrates and methylene blue. Melanotrope. In the cytoplasm there is a large number of secretory granules (SG). Oval-shaped mitochondria with areas of enlightenment (M). Dilated cavities of the Golgi dictyosome complex (CG). × 9000.

In cells of the first type, the granules differed in their size and degree of osmophilicity. Light granules had indistinct outlines, but they often defined a light rim. Mitochondria in light and dark cells were small in size with a compacted matrix. In some mitochondria there were areas of enlightenment (vacuolization) and placement of crystae at one of the poles, as it was in the case with cells of the intermediate pituitary gland in intact rats of the appropriate age. The Golgi complex was defined by the dictyosome dilated cavities, and there were many small vesicular formations near the complex canals that resembled primary lysosomes.

Many ribosomes in the form of polyribosomal complexes were observed. The nuclei were large, round, with a pronounced chromatin, which was located mainly along the inner nuclear membrane. Many nuclear pores could be seen.

Thus, in 90-day-old rats after simultaneous action of nitrates and methylene blue in the ultrastructure of melanotropic cells there was almost complete normalization, which was accompanied by an increase in the number of organelles and restoration of their structure and function.

The results of the study showed that the simultaneous action of nitrates and methylene blue had a positive effect on the structure and function of melanotropic cells in the intermediate lobe of the adenohypophysis.

Thus, after the action of nitrates alone, a decrease in the functional activity of melanotropic cells was observed. Deep destructive and dystrophic changes of organelles took place in the ultrastructure of cells. Vacuoles of different sizes and fragments of destroyed organelles' membranes were found in some cells. There was a disorder of protein-synthesizing function, signs of the secretory process inhibition, which was obviously a manifestation of the organ's depletion beginning.

In 90-day-old rats after the action of nitrates and methylene blue in the ultrastructure of melanotropic cells there were signs of normalization, accompanied by an increase in the number of organelles and positive changes in their structure and function. The intake of nitrates with drinking water in animals caused 14-day-old rats to develop a stress response, these data correlate with the results of other studies [5, 8, 10, 13] which also prove that nitrates, reacting with functional groups of proteins, disrupt their synthesis and pathologically change the structure of mitochondria. In the longer term of nitrates action (in 90-day-old rats) there were changes, the result of which was a deep imbalance in the work of the adenohypophysis intermediate part. Studies have shown that in 14-day-old rats after simultaneous action of nitrates and methylene blue in the ultrastructure of the cytoplasm of melanotropic cells there was

activation of membrane organelles (endoplasmic reticulum, Golgi complex) and active processes of secretion granules, whereas with action of nitrates only activity of melanotropes enhanced more pronouncedly [7]. Under conditions of longer nitrate intake against the background of methylene blue (in 90-day-old rats), the structure of melanotropic cells in the intermediate part of the adenohypophysis was normalized. Thus, methylene blue against the background of nitrates mitigated their toxic effect on melanotropes of the adenohypophysis intermediate part and was a kind of protector of nitrates, reducing the depletion of the general adaptation syndrome in the intermediate part of the adenohypophysis [8].

Conclusions

1. The intake of nitrates in 14-, 45- and 90-day-old rats caused the gradual development of a stress response, accompanied by dystrophic and destructive disorders.

2. The combined action of nitrates and methylene blue in 14-day-old animals reduces the toxic effects and strength of the stress response in the adenohypophysis. In 45-day-old animals after the simultaneous intake of nitrates and methylene blue in melanotropic cells there is an increase in the number of organelles and signs of restoration of the structure in the cytoplasm and nucleus.

3. The use of methylene blue against the background of long-term intake of nitrates in 90-day-old rats reduced the toxic effects of nitrates and brought the structural organization of the adenohypophysis intermediate part to the control values.

The obtained results open the prospects of further study on the patterns of structural and functional changes of the endocrine glands under the conditions of nitrate intoxication and provide a basis for developing effective measures to correct endocrine system lesions in humans as a result of professional activities.

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Стаття надійшла 23.11.2020 р.