SUBTOXIC EFFECTS OF SODIUM FLUORIDE ON THE ULTRASTRUCTURE OF PITUITARY GLAND IN RATS
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The study aimed at investigating the morphofunctional state of the pituitary gland in white rats under the subtoxic exposure to sodium fluoride. Mature male rats (N = 17), weighing 130-150 g, were intragastrically administered with aqueous solutions of sodium fluoride in a dose of 1/10 LD50 ranged from 20 mg / kg of body weight. The duration of the subacute experiment was 60 days. To assess the morphological rearrangement at the subcellular level of organization of the pituitary gland, electron microscopy was performed. The microscopic study revealed changes in the submicroscopic architecture resulted from dystrophic processes caused by the subtoxic exposure to sodium fluoride. Prolonged sodium fluoride intoxication led to a number of changes in the ultrastructure of the pituitary gland, manifested by the development of intracellular oedema, swelling of mitochondria, changes in the density of their matrix, partial reduction and loss of cristae, vacuolization and expansion of the cisterns of the granular endoplasmic reticulum, an increase in the number of primary lysosomes, in the redistribution of chromatin nucleus and a decrease in the number of ribosomes and glycogen granules. Hemocapillaries showed oedema of endothelial cells, uneven thickening of the basement membrane, vasodilatation with the development of stasis and sludge of erythrocytes. As in the vessels of the hypothalamus, the presence of fibrin and a significant number of platelets has been found. These changes indicate a disruption of bioenergetics associated with the mitochondrial apparatus and the development of hypoxic processes, which lead to a decrease in the activity of redox reactions occurring at the level of intracellular membranes and organelles.

Key words: sodium fluoride, brain, pituitary gland, electron microscopy, ultrastructure, intoxication.

The study was conducted in accordance with the plan for scientific research work of Kharkov Medical Academy of Postgraduate Education of the Ministry of Public Health of Ukraine and is a fragment of the research project of the Department of Clinical Pathophysiology, Topographic Anatomy and Operative Surgery «Pathophysiological mechanisms of action of radiotoxins on the body and the principles of early diagnosis and correction» (state registration number 0117U000589, 2017-2021).

Material and methods of the research

The experimental study was conducted on 17 laboratory white male mature rats weighing 130-150 g, aged 3 months. The animals were divided into 2 groups, experimental and control. The experimental group consisted of the rats, which were given intragastrical injections with an aqueous solution of sodium fluoride in a dose of 1/10 LD50 ranged from 20 mg / kg of animal body weight daily. The control group included intact rats kept under standard vivarium conditions and received the same amount of pure water during the period of the experiment. The duration of the subacute experiment was 60 days followed with the animal decapitation. When working with animals, we were guided by the requirements of the "European Convention for the Protection of Vertebrate Animals used in Experiments and Other Scientific Purposes" (Strasbourg, 1986), Law of Ukraine No. 3447-IV of February 21, 2006 "On the Protection of Animals from Cruelty."

To study the pituitary gland of rats with an assessment of its morphological rearrangement at the ultrastructural level of organization, electron microscopy was performed. Samples of the pituitary tissues were fixed in a 2.5% solution of glutaraldehyde, in 0.1 M phosphate buffer pH 7.2 and in 1% osmium tetroxide fixation by G. E. Palade. After dehydration in solutions of increasing concentration of ethanol and absolute acetone, the blocks were embedded into a mixture of epon-araldite resins. Semithin sections of 1–2 μm were cut by ultramicrotome, then stained with methylene blue and studied at the light-optical level. The ultrathin sections were contrasted with...
uranium and lead citrate by Reynolds. Then they were examined under an electron microscope at an accelerating voltage of 75 kV.

**Research results and their discussion**

The ultrastructure of the cells of the central links of the neuroendocrine system is characterized by signs of their decreased functional activity. In the hypothalamus, both in “light” and “dark” neurocytes, the ultrastructure of the nuclear-plasmic reticulum is significantly altered.

In the pituitary gland, most of the “light” neurocytes are characterized by a typical ultrastructure. In individual “dark” neurocytes, all the main organelles are altered (nucleus, nucleolus, cytoplasm). The nuclei of such cells are enlarged, deformed, and often occupy most of the cytoplasm. Small clumps of heterochromatin are distributed evenly throughout the karyoplasm. The karyolemma is convoluted, nuclear pores are well expressed. The nuclei are large, with clear contours, eccentrically located in the nuclei. The cytoplasm is depleted in organelles. In some neurocytes, condensation of both nuclear chromatin and organelles is seen against the background of an increase in the electron density of the cytoplasm. Such cells shrink and become fragmented, losing their connection with the neuroglia. The nuclei acquire an atypical shape due to deep invaginations formed by the nuclear membrane. The electron-dense nuclei are large, located eccentrically, have no fibrilar centers, no granular component. In the cytoplasm, typical organelles are unchanged. The electronic density of the neuropil varies due to an increase in the number of neurofibrillary structures in some nerve fibres and lysis in others. It contains single mitochondria, vacuoles and numerous free ribosomes, lysosomes. Lysosomes are variable in size; some form nucleolus-like bodies. The microstructure of the neuropil is preserved. However, discurricular disorders are expressed in various parts of the hypothalamus and in the adenohypophysis. The wall of blood capillaries becomes wavy in some places, their lumens are significantly expanded. Perivascular oedema, vascular congestion and manifestations of stasis and sludge are quite visible. In the lumen of blood capillaries, in addition to endothelial cells, erythrocytes and pericytes, fibrin and a large number of platelets are detected. Therefore, the wall of the terminal capillaries is more than melysin fibres are concentrated around it. The total amount of myelin fibres is reduced in comparison with that in the intact animals.

In the adenohypophysis, there is an increase in the number of all types of adenocytes, however, many of them are in the process of degeneration, and some parts of the anterior lobe are being destructed. In the adenohypophysis of the experimental rats, interdependent and interrelated changes in the stroma, structures of the vascular bed and adenocytes are observed. Hemocapillaries demonstrate the oedema of endothelial cells, uneven thickening of the basement membrane, vasodilatation with the development of stasis and sludge of erythrocytes. As in the vessels of the hypothalamus, the presence of fibrin and a significant number of platelets is found out. Secretory granules are concentrated near the capillaries. The intensity of damage to adenocytes is different, including the cells of the same type. Most somatotropes preserve their ultrastructure. Individual cells are characterized by a higher electron density of the nucleus and cytoplasm, in which there is an excessive accumulation and stagnation of secretory granules. Vacuolization of the cytoplasm is characteristic of gonadotropes against the background of the destruction processes of most organelles and their 2-3-fold decrease.

In degranulating adenocytes, the nuclei are round, light, and enlarged. In the karyoplasm, large nuclei are distinguished by their size, the presence of lumps of heterochromatin, tightly adjacent to the inner membrane of the nuclear envelope, is noticed. The cytoplasm is vacuolated, having short single cisterns of granular endoplasmatic reticulum; ribosomes are virtually not differentiated, mitochondria are swollen, with weakly marked or destroyed cristae. The density and area occupied by the secretory granules are significantly reduced.

**Conclusions**

Exposure of the rats to subacute sodium fluoride intoxication for two months leads to morphological restructuring of the central links of the neuroendocrine system at the subcellular level of organization that point out a depression of their functional state. More significant changes were recorded in thyrotropes and corticotropes. The most pronounced shifts were established immediately after the end of the period of intoxication. In the process of readaptation, changes in the hypothalamus and in the adenohypophysis. The wall of blood capillaries becomes wavy in some places, their lumens are significantly expanded. Perivascular oedema, vascular congestion and manifestations of stasis and sludge are quite visible. In the lumen of blood capillaries, in addition to endothelial cells, erythrocytes and pericytes, fibrin and a large number of platelets are detected. Therefore, the wall of the terminal capillaries is more than melysin fibres are concentrated around it. The total amount of myelin fibres is reduced in comparison with that in the intact animals.

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Реферат
СУБТОКСИЧЕСКОЕ ВОЗДЕЙСТВИЕ ФТОРИДА НАТРИЯ НА УЛЬТРАСТРУКТУРУ ГИПОФИЗА КРЫС
Колосник И.Л.

Исследовано морфофункциональное состояние гипофиза белых крыс при субтоксическом воздействии фторида натрия. На половозрелых крысах самцов (N=17), массой 130-150 г, которым ежедневно внутривенно вводились водные растворы фторида натрия дозой 1/10 LD50 из расчета 20 мг/кг массы животных. Продолжительность подострого опыта составляла 60 суток. Для изучения гипофиза крыс с оценкой его морфологической перестройки на субклеточном уровне организации проводили электронномикроскопическое исследование. Проведенное электронно-микроскопическое исследование ультраструктурной организации гипофиза при воздействии фторида натрия выявило изменения субмикроскопической архитектоники, характерные для развития дистрофических процессов. Длительная интоксикация фторидом натрия вызывает ряд изменений ультраструктуры гипофиза, выражающихся в развитии внутриклеточного отека, набухания митохондрий, изменениях плотности их матрикса, частичной редукции и потери крист, вакуолизаций и розширении цистерн зернистого эндоплазматического ретикулума, увеличении количества первичных лизосом, в перераспределении хроматина ядра и уменьшении числа рибосом и гранул гликогена. В гемокапиллярах выявлен отек эндотелиоцитов, неравномерное утолщение базальной мембраны, вазодилатация с развитием стазов и сладжей зиротических процессов. Эти изменения свидетельствуют об нарушении биоэнергетики, связанной с митохондриальным аппаратом и развитием гипоксических процессов, которые ведут к снижению активности окисительно-восстановительных реакций, протекающих на уровне внутриклеточных мембран и органелл.

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Колосник І.Л.

Ключеві слова: фторид натрія, головний мозок, гіпофіз, електронна мікроскопія, ультраструктура, інтоксикація.

Досліджено морфофункциональне становище гіпофізу білих щурів під впливом субтоксичних дій фториду натрію. На статевозрілих щурах популяції Вістар (N = 17), масою 130–150 г, яким щодня внутрішньошлунково вводилися водні розчини фториду натрію в дозі 1/10 LD50, з розрахунку 20 мг/кг маси тварин. Продолжительность подострого опыта составляла 60 діб. Для вивчення гіпофізу щурів з оцінкою його морфологічної перебудови на субклітинному рівні організації провели електронномікроскопічне дослідження. Проведене електронно-мікроскопічне дослідження ультраструктурної організації гіпофізу під впливом фторида натрію виявило зміни субмікроскопічної архітектоніки, характерні для розвитку дистрофічних процесів. Длітня інтоксикація фторидом натрію викликає зміни субмікроскопічної архітектоніки які характерні для розвитку дистрофічних процесів. Длительная интоксикация фторидом натрия вызывает изменения субмікроскопічної архітектоніки які характерні для розвитку дистрофічних процесів.