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INFLUENCE OF HIGH ADRENALIN DOSES ON THE MORPHOFUNCTIONAL STATUS OF RATS EPIPHYSIS UNDER THE CONDITION OF ITS HYPOFUNCTION

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The work highlights the effect of adrenaline on morphology and functional activity of epiphysis in the conditions of its hypofunction. The study was performed on 24 sexually mature males of Wistar rats, with a body weight of 150-220 g. Animals were simulated hypofunction of epiphysis and administered a single dose of adrenaline hydrochloride (0.5 mg/kg) intraperitoneally. It is established that the effect of a high dose of adrenaline is accompanied by the development of destructive processes in the epiphysis cells and is characterized by vacuolization of the cytoplasm with further development of the intracellular structures lysis. These morphological features indicate the development of necrotic processes and, accordingly, the loss of pinealocytes.

Key words: epiphysis, pinealocytes, adrenaline, vacuolization.

The work is a fragment of the research project "Comparative morphology of the cranial dura mater sinuses of vertebrates", state registration No. 0115U000176.

Adrenaline is the main hormone of the adrenal medulla. By its chemical nature, it belongs to catecholamines, which are stress hormones, which, under the influence of adverse environmental factors, mobilize all the forces and resources of the body, changing the morpho-functional state of almost all organs of the visceral systems [5]. Due to the wide range of physiological and pharmacological properties, the adrenaline hormone in therapeutic doses has been successfully used as a drug in various fields of medicine [9, 10-12]. Administration of large doses of adrenaline hydrochloride is recommended for use in many emergency conditions [7, 8]. However, data on the response of the body's functional systems to high, almost toxic doses of adrenaline are scarce in the literature [4]. There is no information at all on the morpho-functional changes in the epiphysis which is responsible for triggering the stress response.

The purpose of the study was to perform histological analysis and to clarify features of the morpho-functional state of the rat epiphysis under its hypofunction and after a single exposure to a high dose of adrenaline hydrochloride.

Materials and methods. The experimental studies involved 24 adult male Wistar rats, with the body weight of 150-220 g in the autumn and winter. Animals were kept under standard vivarium conditions. Hypofunction of the epiphysis was caused by round-the-clock illumination with intensity of 1000-1500 lx with two lamps, which were located on both sides of the cage for 10 days. On the 10th day of the experiment, rats were administered adrenaline hydrochloride intraperitoneally with the single dose of 0.5 mg/kg. The experimental animals were euthanized 2 hours after adrenaline administration, which corresponds to the onset of necrosis [6]. The animals were sacrificed 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 Experiments on Animals" approved by the First National Congress on Bioethics (Kyiv, 2001). The permission to carry out the research was given by the Ethics and Bioethics Commission of the Mykolayiv V.O. Sukhomlinsky National University.

After removal of the epiphysis, together with the adjacent blood vessels, the obtained complex was immersed into a fixing solution of 10% neutral formalin. Using standard methods, the material was embedded in paraffin blocks, of which sections were made 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 the "PrimoStarZeiss" microscope, followed by photographing the microslides with a "Canon" digital SLR camera.

Results of the study and their discussion. Histological examination of the experimental animals slides with a small magnification of the microscope revealed the particulate structure of the organ (fig. 1). Particles detected in the sections are mostly round-shaped. There are different numbers of pineal cells in each epiphysis segment, mostly from 2 to 4.

Analysis of the epiphysis parenchyma structural elements state in the animals which were under the adrenaline exposure conditions indicates specific changes. We have established a general clarification of histological sections. In most cases, they look pale and slightly basophilic. And only in certain places small aggregates of parenchyma cellular elements can be found, having a more intense basophilic staining.

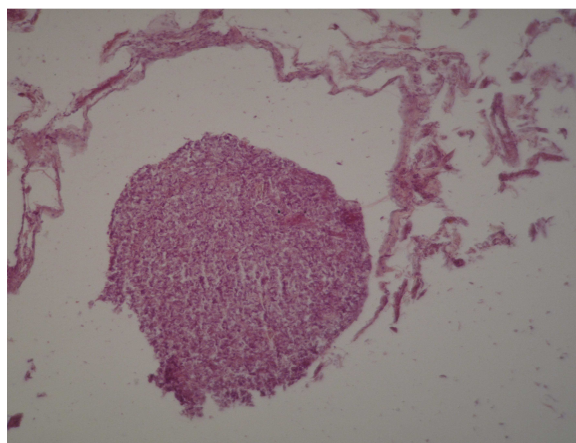


Fig. 1. Micrograph shows the particle structure of the rat epiphysis parenchyma: Approx. oc. $\times 10$, ob. $\times 10$. Staining: hematoxylin and eosin.

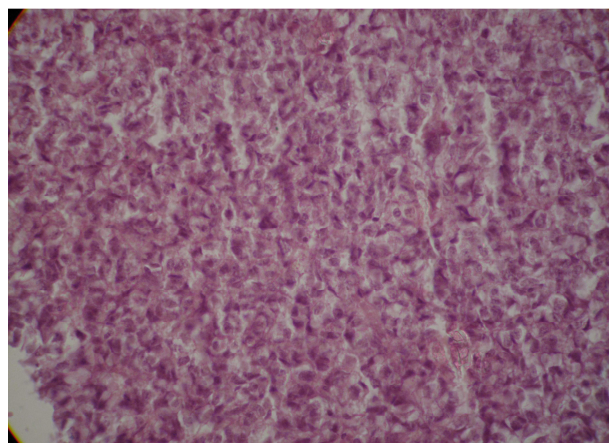


Fig. 2. Micrograph of the cellular composition in the central region of the rat epiphysis parenchyma. Approx.: oc. $\times 10$, ob. $\times 40$. Staining: hematoxylin and eosin.

Clarification of histological slides occurs for two reasons. First, this can be due to the increase in the number of active light pinealocytes. Secondly, by reducing the amount of heterochromatin in the nuclei of pineal cells.

It is established that in histological sections the absolute majority are light pinealocytes, which are large cells the major volume of which cytoplasm is occupied by the nucleus. It should be noted that the cytoplasm of light pineal cells looks transparent, virtually unstructured, except in some cases where a small amount of the basic substance can be seen. The nuclei of the light pinealocytes in the sections are predominantly round-shaped. Only occasionally there occur oval nuclei. It is established that the nuclei of light pinealocytes are characterized by unequal degree of basophilicity, which depends on the amount of basophilic substance in the karyoplasm. Thus, along with very light cells (light nucleus cells), we found more intensely colored (dark nucleus cells) light pinealocytes, which indicates a different degree of the light cells' functional activity (fig. 2). The nucleoli of light pinealocytes are round-shaped and mostly occupy a central position in the karyoplasm. Along with the described structure of the light cells nuclei, nuclei devoid of nucleoli are found quite frequently. With regard to dark pinealocytes, it is established that their number in histological sections is rather limited and they are located mainly in the central regions of the organ's parenchyma (fig. 2). This morphological feature indicates an increase in the functional activity of the epiphysis and the transition of inactive dark cells to the light population. Dark pinealocytes are much smaller in size than light cells. The nuclei of the dark pinealocytes are characterized by a very dark karyoplasm filled with a dense, unstructured mass of condensed chromatin. Due to this, the nucleoli are virtually undetermined in such cells.

It is revealed that in histological sections of epiphysis parenchyma, phenomena of light cells cytoplasm vacuolization of different degree are observed. Some of them are characterized by an enlarged light cytoplasm; in place of other cells, vacuoles of irregular shape and different caliber filled with cytoplasmic fluid can be seen, indicating the death of the cell and the gradual loss of the cellular composition in the epiphysis parenchyma.

It is established that similar vacuolization foci are expressed both in the central part and on the periphery of the organ. It should be noted that almost all pineal cells began to undergo vacuolization processes. Only occasionally, light pinealocytes with basophilic nuclei and non-vacuolated cytoplasm come into the field of view. It was found that in cells subjected to the process of vacuolization, the degree of nuclei basophilia is significantly reduced. Vacuolization of cellular elements causes an increase in the cytoplasm volume and, due to this, the cytolemma stretching. Subsequently, the lysis of intracellular structures develops, primarily the nuclei, which is manifested by the gradual discoloration of the nuclear substance and nucleoli.

Information on the effect of high doses of adrenaline on the morphology and functional status of the epiphysis in available to us domestic and foreign literature sources is almost absent. However, our findings regarding the morphofunctional status of the epiphysis correlate with the results of studies by other authors who have studied changes in the morphology and functional status of the epiphysis in response to the influence of many other endogenous and exogenous factors [1, 3]. Thus, the revealed morphological disorders, according to literature, can be described as manifestations of parenchymal hydropic degeneration, indicating the development of necrotic processes and, accordingly, death of pinealocytes [2, 4].

Conclusions

1. The histological examination revealed a significant decrease in the number of inactive dark pinealocytes, and an absolute predominance of light pineal cells, which indicates a high functional activity of the organ.

2. The presence of two types light pinealocytes is established, which indicates the asynchrony of metabolic processes.

3. It was found that pinealocytes undergo processes of vacuolization with the subsequent development of intracellular structures lysis, indicating the development of hydropic degeneration, cell death and gradual loss of the cellular composition in the epiphysis parenchyma.

Prospects for further research mean that in the future it is planned to study the status of the epiphysis structural elements after a single injection of adrenaline hydrochloride and euthanasia 24 hours after injection, which corresponds to the peak of necrosis.

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Реферати

ВПЛИВ ВИСОКИХ ДОЗ АДРЕНАЛІНУ НА МОРФОФУНКЦІОНАЛЬНИЙ СТАН ЕПІФИЗУ ЩУРІВ ЗА УМОВ ЙОГО ГІПОФУНКЦІЇ

Пшиченко В.В., Черно В.С., Давиденко Р.М., Гаврилюк І.М., Кучер О.О.

В роботі висвітлено дані щодо впливу адреналіну на морфологію та функціональну активність епіфізу за умов його гіпофункції. Дослідження було проведено на 24 статевозрілих самцях щурів лінії Wistar, з масою тіла 150-220 г. Тваринам моделювали гіпофункцію епіфізу та одноразово вводили адреналін гідрохлорид внутрішньобрюшинно у дозі 0,5 мг/кг. Встановлено, що вплив високої дози адреналіну супроводжується розвитком деструктивних процесів у пінеальних клітинах, що характеризуються вакуолізацією цитоплазми з подальшим розвитком лізису внутрішньоклітинних структур. Дані морфологічні особливості свідчать про розвиток некротичних процесів і відповідно загибель пінеалоцитів.

Ключові слова: епіфіз, пінеалоцити, адреналін, вакуолізація

Стаття надійшла 15.02.2019 р.

ВЛИЯНИЕ ВЫСОКИХ ДОЗ АДРЕНАЛИНА НА МОРФОФУНКЦИОНАЛЬНОЕ СОСТОЯНИЕ ЭПИФИЗА КРЫС В УСЛОВИЯХ ЕГО ГИПОФУНКЦИИ

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В работе освещены данные о влиянии адреналина на морфологию и функциональную активность эпифиза крыс в условиях его гипofункции. Исследование было проведено на 24 половозрелых самцах крыс линии Wistar, с массой тела 150-220 г. Животным моделировали гипofункцию эпифиза и однократно вводили адреналин гидрохлорид внутрiбрюшинно в дозе 0,5 мг / кг. Установлено, что влияние высокой дозы адреналина сопровождается развитием деструктивных процессов в пинеальных клетках, характеризующихся вакуолизацией цитоплазмы с последующим развитием лизиса внутриклеточных структур. Данные морфологические особенности свидетельствуют о развитии некротических процессов и соответственно гибели пинеалоцитов.

Ключевые слова: эпифиз, пинеалоциты, адреналин, вакуолизация

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