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MICRO- AND ULTRASTRUCTURE TESTIS OF WHITE RAT IN NORMAL CONDITION AND IN CASE OF STREPTOZOTOZIN INDUCATION DIABETES

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The aim: *to set the peculiarities of bloodstream of the testis of white rat in normal condition and under the condition of experimental [artificial] diabetes course.*

Material and methods. *The research has been conducted on 40 white mature male rats in the age groups 4.5 to 7.5 months with 130 to 150g of body weight. Research material is represented by ultramicroscopic testicle slice. Experimental diabetes modelling was performed through single intraperitoneal injection of Streptozotocin ("Sigma", USA), dissolved in 0.1M citrate buffer, pH=4.5 (7mg per 100g of body weight of animals). The diabetes mellitus progression was controlled basing on glucose concentration in blood, which was measured by glucose oxidase test. In 2,4,6,8 weeks after experiment launch, animals in whose blood glucose concentration was above 13.4 mmol/l, were used for research.*

The selected trend of study is a part of a planned Science Department Anatomy "Structure bodies and bloodstream ontogeny, under the laser irradiation and pharmaceutical, blood circulation disorders, reconstructive surgery and diabetes", state registration 0110U001854, performed at the Lviv National Medical University named Danylo Halytsky according to the state plan and program. University Animal Care and Use Committee Approval: № 8 from 18th of November 2013.

Results. *The first signs of change of angioarchitectonics testicular were found on the second week of experimental diabetes. During the following terms bloodstream undergoes significant changes.Vessels with irregular contours, winding in the lumen of blood clots found, the vessel wall is thickened, sklerozovana and stratified. Around the vessels edema and haemorrhage are present. Available desolated areas of capillaries, in the lumens of some capillaries, the aggregation of red blood cells that cover the lumen of blood vessels are found. The lumen of the capillaries is narrowed. In the remaining capillary endothelial cells have the appearance of "standing column", which is a characteristic feature of the hypoxic state of tissues. Capillary sclerosis, precapillary spaces are dilated.*

Conclusion. *Changing in the diameter of the vessels of microvasculature endothelial is accompanied by swelling and change in the form of their lumen. Adhesion and aggregation of red blood cells are also observed. These changes show that angiopathy is a trigger hypoxia and as a result in 2 weeks of diabetes course micro and ultrastructural organization of the most convoluted seminiferous tubules varies.*

Key words: *testicle, hemomicrocircular channel, animal model, diabetology, experimental streptozotocin diabetes*

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МІКРО- ТА УЛЬТРАСТРУКТУРА КРОВОНОСНОГО РУСЛА ЯЄЧКА ЩУРА В НОРМІ ТА ПРИ СТРЕПТОЗОТОЦИНІНДУКОВАНОМУ ЦУКРОВОМУ ДІАБЕТІ

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Мета *– виявити особливості кровоносного русла яєчка білого щура в нормі та закономірності перебудови в динаміці перебігу експериментального цукрового діабету.*

Матеріали і методи. *Дослідження проводили на 40 білих статевозрілих щурах самцях віком 4,5-7,5 місяців і масою 130-150 г. Матеріал представлений ультрамікроскопічними фрагментами яєчка. Моделювання експериментального діабету було виконано внутрішньоочеревенним введенням ін'єкцій Streptozotocin («Сігма», США), розчиненого у 0,1 М цитрат буфера, pH = 4.5 (7 мг на 100 г маси тіла тварин). Розвиток цукрового діабету під контролем концентрації глюкози в крові, яка була виміряна тестом на оксидазу глюкози. Тварин, у яких рівень глюкози в крові концентрації в 2, 4, 6, 8 тижнів після запуску експерименту перевищив 13.4 ммоль/л, були використані для дослідження.*

Обраний напрям дослідження є частиною планової наукової роботи кафедри нормальної анатомії «Структура органів та їх кровоносного русла в онтогенезі, під дією лазерного опромінення та фармацевтичних засобів, при порушеннях кровопостачання, реконструктивних операціях та цукровому діабеті», номер державної реєстрації 0110U001854, яка виконується у Львівському національному медичному університеті імені Данила Галицького згідно з державним планом і програмою. «Біоетика» протокол №8, від 18 листопада 2013 р.

Результати*. Перші ознаки змін ангіоархітектоніки яєчка виявлено на 2-му тижні експериментального цукрового діабету. Впродовж наступних термінів кровоносне русло зазнає суттєвих змін. Судини дилятовані, з нерівними контурами, звивисті, в їх просвіті виявлено тромби, стінка судин потовщена, склерозована і розшарована. Навколо судин є набряк і крововиливи, ділянки із запустілими капілярами, у просвітах деяких капілярів виявлено агрегацію еритроцитів, які закривають просвіт судин. Просвіт капілярів звужений. У збережених капілярах ендотеліоцити мають вигляд «стовпчастого стояння», що є характерною ознакою гіпоксичного стану тканини. Капіляри склерозовані, прекапілярні простори розширені.*

Висновки. *Зміна діаметра судин мікроциркуляторного русла супроводжується набряком ендотеліоцитів і зміною форми їх просвіту, спостерігається адгезія та агрегація еритроцитів. Ці зміни свідчать про те, що ангіопатія є пусковим механізмом гіпоксії і як наслідок через 2 тижні перебігу цукрового діабету змінюється мікро- та ультраструктурна організація більшості звивистих сім'яних трубочок.*

Ключові слова: *яєчко, кровоносне русло, діабетологія, експериментальний цукровий діабет*

INTRODUCTION

The most frequent and prognostically unfavourable complication of diabetes mellitus is microangiopathy [1, 3, 4, 7, 11, 18]. The diabetic changes in testicle hemomicrocircular channel links serve as the basis for diabetic development of testicle structures pathological changes [2, 5, 6, 12, 15] In spite of topicality and significance of the issue, professional books contain little information on testicle rearrangement in various pathological conditions [8, 9, 10, 13, 14, 16, 17] and there are practically no data on diabetic testicle hemomicrocircular channel links ultrastructural arrangement.

The first changes in rat testicle hemomicrocircular channel links ultrastructural arrangement are noticed already in 2 weeks run of streptozotocin-induced diabetes mellitus and accumulate throughout next periods of experiment.

MATERIAL AND METHODS

The researches have been performed on 40 white mature male rats in the age groups 4.5 to 7.5 months with 130 to 150g of body weight.

Research material is represented by ultramicroscopic testicle slice. Experimental diabetes modelling was performed through single intraperitoneal injection of Streptozotocin ("Sigma", USA), dissolved in 0.1M citrate buffer, pH=4.5 (7mg per 100g of body weight of animals). The diabetes mellitus progression was controlled according to glucose concentration in blood, which was measured by glucose oxidase test. Animals in whose blood glucose concentration in 2,4,6,8 weeks after launch of experiment was above 13.4 mmol/l were used for research.

Animals were kept and experimented on in compliance with the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Strasbourg, 1985), "General Ethical Principles for Experiments on Animals" adopted by First National Bioethics Congress (Kyiv, 2001). University Animal Care and Use Committee Approval: № 8 of 18 November 2013. The work was performed with the method of electronic microscopy.

The animal was sacrificed by overdose of intraperitoneal narcosis with thiopental sodium (25 mg/kg). Straight after animal death the sampling and standard material processing for electronic microscopy took place. Ultrathin slices were prepared at UZhTP-3 ultramicrotome with glass knives. For the research the slices of silver and lemon yellow colour were taken. The contrast was obtained by treating slices first with 2% uranyl acetate solution, then – with plumbum citrate. Material study and photographic recording were performed with UEMV-100K microscope at accelerating voltage of 75kV and microscope magnification of 1000-124000x.

RESULTS AND DISCUSSION

Testicle hemomicrocircular channel links are located in connective tissue surrounding winding seminal ducts and in their wall. Arterioles pass between seminal ducts. Arteriolar wall consists of three coats. The cross section of endotheliocytes proves their elongated form with scarce arterioles that are projected to lumen. Plasmolemma forms numerous warts; cytoplasm has average electron optical density and contains considerable amount of organelles and micropinocytic vesicles. Adjacent endotheliocytes come in contact forming desmosomes and interdigitations. Nuclei have elongated form with prevailing uncondensed chromatin, but well defined strip of peripheral condensed chromatin by the nucleomma. Basement membrane has sharp contours and is continuous. The inner elastic membrane is detected from outside. It is thin and has irregular wavy contour. Smooth myocytes of arteriole wall middle coat form one layer. They have electron-light cytoplasm, contain elongated nuclei, and there are clearly seen the places of myofibrils affixed throughout all plasmolemma. The adjacent smooth myocytes have desmosome contacts. Arteriole lumens are filled with blood cells (erythrocytes, thrombocytes). There are longitudinal and latitudinal capillaries seen. The longitudinal capillaries are intertwined with latitudinal ones forming capillary networks in the winding ducts. The part of capillaries has narrow lumens and contains no blood cells, and the other part has broad lumens filled with erythrocytes. On the ultrastructural level the capillary wall is typical and consists of endotheliocytes in the amount of 2-4 located on the basement membrane with sharp even contours. In each endotheliocyte there are the nucleus-containing and peripheral areas. Endotheliocyte nuclei have elongated form, their contours are sharp. Nuclei contain mainly uncondensed chromatin, as well as thin strip of peripheral condensed chromatin. Small amounts of condensed chromatin smoothly lie in karyoplasm. In the area around nucleus there is a granular endoplasmic

reticulum represented by ducts and cisterns, membranes of which hold significant ribosome amount; Goldgi apparatus, and mitochondria. The plasmolemma infrequently forms small microvilli. Endotheliocyte basal surfaces have tightly fit solid basement membrane with pericytes between its layers. Pericyte nucleus has oval form with chromatin proportionally located in nucleoplasm. There are the Goldgi apparatus, granular endoplasmic reticulum, mitochondria and individual free ribosomes and vesicles located by it. Venules in contrast to arterioles have broad lumens of irregular form. The venule wall consists of thin layer of endothelial cells on the basement membrane. Endotheliocyte cytoplasm is electron-light and contains insignificant amount of organelles. Venule lumens are filled with blood elements (fig. 1).

Fig. 1. Electron micrograph from part of blood vessel of testis from a diabetic rat, 2 weeks. (mag. approx 10.000x).

In 2 weeks run of streptozotocin-induced diabetes mellitus in the testicle hemomicrocircular channel links the first signs of angiopathy are found. The endotheliocyte swelling is seen in the capillaries; capillary lumens acquire irregular form. In the capillary lumens the erythrocyte adhesion and aggregation take place. Electron-dense endotheliocyte nuclei are projected into vessel lumen. Endotheliocyte nuclei acquire excessively elongated form; nucleomma forms numerous protrusions and invaginations; chromatin has marginal location. Nucleus-free endotheliocyte areas are thinned. Endotheliocyte cytoplasm electron optical density is increased, number of organelles is decreased. Slits between adjacent endotheliocytes are dilated. In individual mitochondria there

are the matrix clarification and single cristas destruction noticed. Plasmolemma forms infrequent projections into capillary lumen what results in thickening of basement membrane. The basement membrane has no sharp outer contour though preserves continuity and has expressed three-layer composition. Pericytes generally preserve their connection to the basement membrane though are sometimes exfoliated from it. Penetrating through the basement membrane the pericytes have direct contact with endotheliocytes; capillary lumen is narrowed. Bundles of collagen fibers are fluffed, and exfoliated one from another with the amorphous liquid spans. By the hemocapillaries the granule cells and interstice swelling is found. The testicle arteriole lumens are somehow narrowed in this experimental period. Some arteriolar endotheliocytes are damaged; basement membrane is thickened and loses its sharp contours. Inner elastic membrane is thickened as well. Nuclei of smooth myocytes acquire rod-shaped form, their contours are even. Venule walls structure is still preserved, but venule lumens are partially dilated (fig. 2).

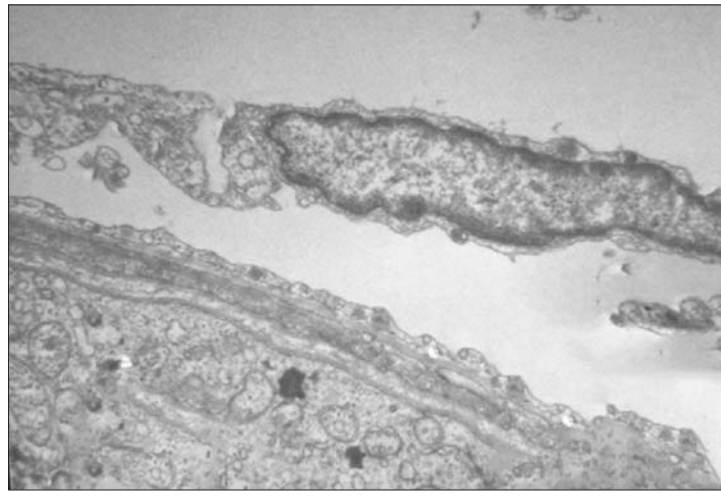

Fig. 2. Electron micrograph from part of blood vessel of testis from a diabetic rat. 8 weeks. (mag. approx 10.000x)

After 4 weeks of experimental diabetes mellitus we've noticed decrease of electron optical density of capillary endotheliocytes, partial exfoliation of endothelium in testicle capillaries due to which the basement membrane is partly bare. The destroyed endotheliocyte nuclei are projected to capillary lumens. The vessel walls swelling is intensified, as well as separation of fibers and thickening of basement membrane. The basement membrane is irregular, has vague contours,

infrequent pores. In pericytes we see marginal location of nuclear chromatin; in mitochondria there is cristas broadening and fragmentation. In testicle arteriole endotheliocytes the nuclear pores are noticed at the nucleus periphery. It is tough to define boundaries between condensed and uncondensed chromatin. Smooth myocytes cytoplasm has average electron optical density; bundles of myofibrils are partly destructurized. Arteriole adventitious coating is also swelling, thickened, with significant amount of amorphous liquid between bundles of collagen fibers. Venule lumens often acquire irregular, sometimes star-shaped form. In small testicle venules the mural thrombi are found.

In 6 weeks after experiment launch we see considerable number of testicle capillaries destroyed, the wall is thickened, the preserved capillary lumens narrowing are seen. In the capillary lumens the thrombocyte adhesion and erythrocytic sludges are discovered. Endotheliocyte nuclei in the preserved capillaries are excessively elongated, swelling, deeply projected to capillary lumen. Small nuclei are not found. In the endotheliocytes cytoplasm we see destruction of Goldgi apparatus structural elements, destruction of mitochondria inner membrane with vacuole formation. Microclasmatosis phenomenon takes place. Plasmolemma is projected into microvessel lumen. The basement membrane is thickened, without sharp contours. Pericyte nuclei acquire elongated form with small invaginations, nuclear chromatin marginalization, mitochondrial cristas broadening and fragmentation. Arteriole lumens are filled with blood elements. Arteriole endotheliocytes are thickened, their cytoplasm has considerable amount of mitochondria and free ribosomes. The cristas destruction is seen in mitochondria. Endotheliocyte cytoplasm is vacuolated, of decreased electron optical density, and their cytoplasm forms warts. Nucleomma forms numerous small projections. The condensed chromatin has fine-granular look. Arteriole middle coat smooth myocyte cytoplasm has average electron optical density and is partly destructurized. Somewhere there are myofibril-affixing areas structurally preserved.

After 8 weeks of streptozotocin-induced diabetes mellitus run we see deep destructive changes in all rat testicle hemomicrocircular channel links. Capillary lumen is decreased. Endotheliocytes in the preserved capillaries acquire columnar standing what is peculiar to tissue hypoxic state. The nucleus-containing endotheliocyte areas are projected deeply into capillary lumen, thus, they have slit-like form. Plasmolemma forms long appendixes, somewhere twisted, that are projected into capillary lumen. Inter-cell slits between adjacent preserved

endotheliocytes are narrow, disorganized, the desmosomes are found in the apical and basement areas of endotheliocyte contacts. Cytoplasm has increased electron optical density; mitochondria are swelling, vacuolated, part of them have destructurized cristas, damaged membranes. In the cytoplasm there are the round electron optically dense corpuscles found. There are practically no micropinocytotic vesicles in the endotheliocytes what prove the transendothelial transport decrease. Endotheliocyte nuclei are elongated, contain condensed chromatin, enlarged nuclear pores. Small nuclei are not found. In the basement membrane there are alternate areas of increased and decreased electron optical density, pericytes are swelling. Cericyte nuclei are electron dense, with destructurized chromatin, small nuclei not found, mitochondria have destroyed cristas. Arterioles in this experimental period are dilated, but their lumens are narrowed on account of high columnar endotheliocytes that are deeply projected into arteriole lumen. Endotheliocyte cytoplasm is dark, structureless, with single mitochondria. Endotheliocyte

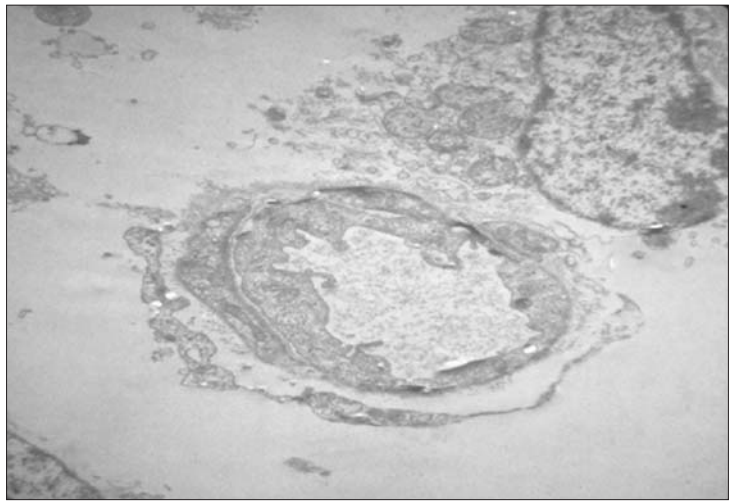

Fig.3. – Electron micrograph from part of blood vessel of testis from a diabetic rat, 10 weeks. (mag. approx 10.000x).

nuclei nucleomma forms numerous long appendixes, chromatin is condensed. Basement membrane is thickened, contains electron optically dense corpuscles, inner membrane is not found. Smooth myocytes cytoplasm is destructurized. Each myocyte is surrounded by electron dense plasmolemma with clearing areas.

After 10 weeks of experimental diabetes run, hemocapillary channel undergoes significant changes. Due to projection of increased in size endotheliocyte nuclei

into capillary lumen the latter acquires irregular slit-like form. The nucleoplasm of endotheliocyte and pericyte nuclei has low electron density, chromatin is condensed by the nucleomma. Perinuclear lumen is narrowed. Endoplasmic reticulum is characterized by dilations, vacuolated cisterns with irregular contours, loosing ribosomes affixed to their surface. Mitochondria are swelling, have cleared matrix and destroyed cristas. Endotheliocyte and pericyte cytoplasm has low electron density, contains small and large vacuoles. Sometimes we see separation of joints between endothelial cells. Basement membrane is thickened, acquires vague contours. Pericytes have irregular form, bigger size, vague contours. Cericyte cytoplasmatic appendixes contain substantial number of vesicles and vacuoles. In the capillary lumens there the erythrocytic sludges, thrombocyte aggregates. Endotheliocyte nuclei have marginally located chromatin and deep karyolemma invaginations. By the nucleus there are the dilated and destroyed cisterns of Goldgi apparatus and granular endoplasmic reticulum, mitochondria with destructive changes. Plasmolemma luminal surface forms numerous invaginations into capillary lumen. Basement membrane is irregularly thickened. Capillary sclerosis occurrences accumulate, pericapillary spaces are dilated (fig. 3).

CONCLUSION

Changing in the diameter of the vessels microvasculature endothelial accompanied by swelling and changes in the form of their lumen, also there are signs of adhesion and aggregation of red blood cells. These changes indicate that angiopathy is a trigger hypoxia and consequently 2 weeks course of diabetes varies micro and ultrastructural organization most convoluted seminiferous tubules.

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