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MORPHOLOGICAL CHANGES OF RETINAL GANGLION NEURONS IN THE LONG TERM OF THE COURSE OF EXPERIMENTAL DIABETES IN CASE OF COMORBIDITIES

МОРФОЛОГІЧНІ ЗМІНИ ГАНГЛІОНАРНИХ НЕЙРОНІВ СІТКІВКИ У ВІДДАЛЕНІ ТЕРМІНИ ПЕРЕБІГУ ЕКСПЕРИМЕНТАЛЬНОГО ЦУКРОВОГО ДІАБЕТУ ЗА УМОВ КОМОРБІДНОЇ ПАТОЛОГІЇ

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Diabetes mellitus (DM) is one of the leading causes of vision impairment and blindness in people aged 20–70 years [4]. The risk of developing blindness in patients with diabetes is 2.5 times higher than in people without diabetes[1] . With DM, the risk of developing cataracts and glaucoma increases, but the greatest threat to vision is damage to the retina, which is

observed in 80% of patients with a disease lasting for more than 10 years. Diabetic retinopathy is diagnosed in 50–90% of patients with diabetes mellitus; it is characterized by a severe progressive course and can lead to blindness[2, 5]. In approximately 5% of cases, signs of retinopathy are detected before the diagnosis of DM, and 10 years after the onset of the disease, pathological changes of the fundus are noted in 40–50% of patients. With a 20-year duration of diabetes, the manifestations of diabetic retinopathy are found in 90% of patients. It is proved that early detection of vision impairment due to diabetes mellitus and treatment of this complication prevents blindness in 90% of patients with diabetic retinopathy [3].

Considering the above, the goal of our study was to establish the morphological changes of retinal ganglion neurons in streptozotocin-induced diabetes under conditions of chronic stress.

Material and Methods. The study used 20 adult white male rats (body weight 180-200 g), which were equally divided into 4 groups: group 1 – rats with simulated SIDM and chronic immobilization stress, group 2 – rats with SIDM, group 3 – rats with immobilization stress, group 4 – intact animals. In groups 1 and 2, SIDM was simulated by a single intraperitoneal injection of streptozotocin "SIGMA" (USA), which was diluted in 0.1 M citrate buffer with a pH of 4.5 (at the rate of 6 mg per 100 g of body weight). In groups 1 and 3, immobilization stress was simulated by placing the animals in a closed plastic container for 5 hours a day. In group 1, SIDM was simulated and starting from the 14^{th} day of the experiment chronic immobilization stress was simulated on a once-only basis. The material was taken on the 56^{th} day from the beginning of the experiment. Histological, electron microscopic, biochemical and statistical research methods were used. Photographs of histological and semithin sections were used for morphometric studies (field of view of the light microscope Leica DM750 was photographed using a digital camera ToupCam 5.2M UHCCD C-Mount Sony). Morphometry was performed using ImageJ version 1.47t. Statistical analysis was performed using the statistical package Stat.Soft.Inc; Tulsa, OK, USA; Statistica 10.

Results. On the 56^{th} day of the experiment, the level of glucose and glycated hemoglobin in the blood of rats in group 1 is the highest, compared to group 4 and is 21.72±3.57 mmol/l ($p<0.001$) and 12.42±1.73% ($p<0.01$), in group 2 – 19.34±2.56 mmol/l ($p<0.001$) and 10.27±2.36% ($p<0.01$), in group 3 – 5.474±0.83 mmol/l ($p>0.05$) and 2.36±0.29% ($p>0.05$), while in group 4 the indicators are the following ones – 4.65±0.58 mmol/l and 2.43±0.19%. The level of cortisol in experimental groups 1-3 was probably higher than that in intact rats and was: in group 1 – 19.75±2.24 ng/ml, in group 2 – 18.31±2.19 ng/ml, in group 3 – 17.32±2.34 ng/ml (in all cases $p<0.01$), in intact animals

(group 4) – 10.06±1.14 ng/ml. Such biochemical changes in groups 1 and 2 indicate the development of decompensated diabetes, and in group 3 – the development of stress.

On the 56th day of the experiment, in groups 1 and 2, at the photooptical level in the retinal ganglion layer, most neurons are shrunken and small with peripheral chromatolysis, which indicates their atrophy and is confirmed by the data of morphometric analysis, namely: the area of the perikaryons of these neurons, of their nuclei and the nuclear-cytoplasmic ratio is probably lower compared to intact indicators (in all cases $p<0.05$). In rats of group 3, peripheral chromatolysis was detected in individual ganglion neurons, however, we failed to detect probable changes on the part of cytokariometric parameters.

At the ultrastructural level in the retinal ganglion layer, changes in neurons are polymorphic ones. In rats of group 3, reverse dystrophic-destructive changes by the type of vacuolar dystrophy are observed in the ganglion neurons, in the others, lipofuscin inclusions and karyopyknosis are detected. Along with the changed neurons, there are single neurons with a normal structure, as in intact animals. In rats of experimental groups 1 and 2, a significant proportion of neurons undergoes balloon dystrophy, colliquative and partial necroses, and apoptosis. Such changes occur against the background of the development of diabetic microangiopathy, as indicated by the data of our previous studies.

Conclusions. Streptozotocin-induced diabetes mellitus by itself and in combination with chronic ischemic stress leads to pronounced atrophic-destructive changes in neurons of the ganglion layer of the retina, which is confirmed by the data of morphometric analysis and micro-ultramicroscopic studies. Such morphological changes occur and proceed against the background of the development of diabetic microangiopathy. Chronic ischemic stress leads to reactive changes on the part of retinal ganglion neurons that are reversible ones.

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