COGNITIVE DISABILITY IN ESTROGENECTOMIZED AND OLD RATS WITH DEVELOPMENT OF DIABETES MELLITUS

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ABSTRACT

Introduction: In recent years, there have been many works devoted to the study of the effects of sex hormones on cognitive function. Clinical studies have found that in menopausal women, the tendency to develop type 2 diabetes will increase, the spatial and short-term working memory worsens, and there is a tendency to develop depression. The findings suggest that estrogens are involved in the mechanisms of insulin resistance in tissues, in the synthesis of mediators in the catecholaminergic systems of the brain, but many questions remain unresolved.

The aim: Therefore, the aim of our study was to establish the effect of estrogens on the indices of spatial memory in ovariectomized and old rats against the background of the development of experimental diabetes.

Materials and methods: The study was conducted on 30 adult rats females 4-5 months and 20 months. The study groups were ovariectomized and reproduced experimental type 2 diabetes mellitus with protamine sulfate. The study of spatial memory was carried out in an eight-sleeved radial labyrinth.

Results and conclusions: Ovariectomy caused the deterioration of spatial memory relative to the living control group, and diabetes mellitus aggravated pathological changes. The changes that occur after ovariectomy suggest estrogen involvement in the regulation of cognitive functions.

KEY WORDS: estrogens, cognitive functions, a spatial memory, diabetes mellitus

INTRODUCTION

SEX HORMONES AND COGNITIVE FUNCTION

In the scientific works of the last years appeared a lot of evidence that sex hormones take active part in the process of the neurogenesis, synaptogenesis have an impact on the energy balance of the neurons through regulation of functions of mitochondria. The high frequency of Alzheimer’s disease (AD) in women during menopause makes scientists think about the influence of estrogens on the development of neurodegenerative diseases [1]. The main target of estradiol is the hypothalamus, forebrain, hippocampus, and nuclei of the seam. One finds estrogen synthase in the brain and there is an evidence that estrogens participate in the regulation of cognitive disorders, bipolar disorders, anxiety, Parkinson disease, Alzheimer’s disease.

The decreased production of sex steroids is associated with impaired processes of forming long-term declarative memory, deterioration of spatial working memory and implementation of executive functions [2]. Cognitive function, which is supported by the prefrontal cortex may be especially dependent on estrogen [3]. Estrogens affect molecular mechanisms of synaptic plasticity. The imbalance of estrogen causes modulation effect, reflected in the changing of cognitive processes. Non-genomic action of estradiol is associated with effects on membrane receptors, which causes membrane hyperpolarisation of neurons, the activation of extracellular regulatory kinase, protein kinases A and C, calcium channels through the activation of the central regulatory pathways that regulate synaptic plasticity. Estradiol launches of NR-2 subunits of NMDA receptors in the hippocampus, which is important for the formation of new synapses [4]. The main manifestations of non-genomic actions of estradiol is its effect on the excitability of the cerebellum, cortex, striatum, hippocampus and hypothalamus, activation of ion channels, the activation of Inositol 3 phosphate, modulation of the entry and exit of calcium, protection of neurons from cellular toxins and free radicals, modulation of the activity of calcium/calcmodulin, activation of kainat current in hippocampal neurons and release of neurotransmitters [5]. Estradiol has a significant influence on synaptic plasticity of neurons in the brain by affecting the functional activity of signal proteins synaptotagmin 1, synaptotagmin 4, synaptein are revealed in cyclical changes synaptotagmin 1 during ovarian cycle in hippocampal region CA1. The deficit in signaltransduction leads to the reduction and subsequent complete cessation of neurogenesis in the hippocampus, further remodeling of astroglia in the CA1 region of the hippocampus, the dentate gyrus [2]. Estradiol provides modulation effect on neurocrine that belong to the family of nerve growth factor. Genomic holinergic and non-genomic mechanisms of estrogens have an effect on neuromediator systems, such as holinergic, adrenergic, serotonin-
ergic, dopaminergic, GABA-ergic, oppoidergic. Estrogen deficiency causes a deficit in the cholinergic system that, according to the latest research is considered as a possible factor in Alzheimer’s disease. Experimental study has shown that estrogens reduce the toxicity of beta-amyloid to neurons by affecting on N-holinoreceptors. The positive effects of estrogen can be explained by antioxidant effects and influence on the expression of neurocrine factor [6]. Hysterectomy influences the noradrenergic system and causes a decrease in levels of noradrenaline, by activation of monoamine oxidase in the hypothalamus and amygdala. Affecting neurotransmitter systems estrogens can affect on the processes of learning and memory. There are well-known periods of a women's life associated with reduced levels of estrogen, when there is the greatest risk of cognitive disorders: puberty, pregnancy, premenopausal, menopause, postmenopausal. The experiments on the animals have shown that hysterectomy made it difficult to save the skills of passive and active avoidance [7].

THE ROLE OF SEX HORMONES IN THE DEVELOPMENT OF DIABETES MELLITUS AND COGNITIVE DYSFUNCTION

The incidence of diabetes mellitus is significantly increased in women over the age of 50, and possibly menopause has a definite effect on increasing its prevalence among women of the elderly [8]. The literature describes the regulatory role of the steroid hormones of the ovaries in the utilization of glucose in the brain, as well as the control of estrogens of neurological disorders induced by insulin hypoglycemia in ovariectomized rats [9].

One of the targets of hyperglycemia in the early stages of diabetes, is the central nervous system, which is clinically manifested by cognitive dysfunction. Only recently has it been shown in patients with diabetes, there is an increased risk of not only mild cognitive impairment, but also the probability of progression to dementia. Monitoring of patients with type 2 diabetes (DM) found that they were 2 times more likely to develop AD [6].

The increase in neuro-specific proteins in the blood of patient with diabetes mellitus points to the damage to the nervous tissue and lets life-time assessment of the condition of the central nervous system and the dynamics of neurodegenerative processes be carried out. Increase neurospecific proteins in patients with diabetes does not itself mean that they are markers of cognitive dysfunction.

According to the study one of the main causes of cognitive dysfunction is the chronic hyperglycemia. Most of the indicators that determine cognitive dysfunction have been reduced in patients with diabetes mellitus 1, namely memory functions and attention. There was a significant difference in the level of neurospecific proteins, which correlated with decomposition of carbohydrate metabolism and the presence of cognitive deficits. The most frequent morphological change in the central nervous system was the presence of signs of atrophy of the gray matter of the frontal and parietal lobes, which correlated with hyperglycemia and cognitive dysfunction [3].

A relatively high density of insulin receptors was revealed in the regions of the brain (hippocampus, tonsils, cortex), which support the processes of memory and study. Thus, the brain's resistance to insulin is particularly clearly manifested in Alzheimer's disease and to a lesser extent with moderate cognitive impairment, as evidenced by studies of the hippocampus of patients who died of Alzheimer's disease [10, 11].

It was found that the activation of estrogen receptors: ERα and / or ERβ with their agonists improve memory in the tests of the water labyrinth of Morris and Y-labyrinth, increases neurogenesis of the hippocampus and prevents from apoptotic reactions of the hippocampus [12, 13]. It is important to note that treatment with agonists of pharmacological ER caused a significant increase in the expression of the ERα and ERβ membrane and subsequent activation of BDNF in the hippocampus of mice with type 2 diabetes. ERα and ERβ, cause cognitive impairment in patients with 2 diabetes, and ER activation with ER agonists may be a new and promising strategy for the treatment of diabetic cognitive impairment [14, 15].

The literature about the influence of estrogens on prognosis of diabetes mellitus and cognitive functions in ovarietomized and elderly animals has shown that points of view are not properly studied.

THE AIM

The purpose of our work was to investigate the behavioral reactions of rats in the radial labyrinth, after ovarietectomy and against the background of the development of experimental diabetes mellitus in 4-5 months old and old rats.

MATERIALS AND METHODS

The study was carried out on 30 adult, sexually mature female rats of 4-5 months weighing 200-220 grams and 20 months weighing 300-320 grams. Animals were kept in vivarium under normal light conditions, with free access to water and food on a standard diet. Animals were divided into several groups: a control group of 4-5 months which were subjected to false surgery, the study groups: 4-5 month ovariectomized, and a group of old rats with experimental diabetes mellitus, duration 2 months and a group of 20 months rats, which did not have estral cycles, and a group of old rats with experimental diabetes [16, 17].

Ovariectomy was performed using the method of Y.M. Kabak firstly cut of the skin in the lumbar region, then cut into the abdominal cavity, pulled out the ovary through the incision with the oviduct and the upper part of the uterus horn, and then removed and repeated this procedure on the other side [16]. Surgical intervention was performed under general anesthesia of calypsoil, 0.75 mg / kg of body weight was injected intraperitoneally.

Experimental type 2 diabetes mellitus was reproduced by intramuscular injection of 15 mg / kg of protamine sulphate twice daily for 2 weeks. After 2 and 4 weeks after the end of the administration of the protamine, blood serum was examined and the hyperglycemia was detected [18, 19, 20].
The study of spatial memory was carried out in an octagonal radial labyrinth: the length of each of the sleeves was 86 cm, the width - 10 cm, the diameter of the central platform - 34 cm, the height of the walls - 20 cm and height above the floor - 50 cm. Each sleeve is separated from the playground guillotine doorways. At the end of each sleeve there was a feeder with food supply, separated by guillotine doors. The test protocol consisted of 3 stages: Stage I - addictive, carried out on the first day to familiarize the animal with the conditions. It consisted of three phases of 5 minutes duration with a 30 second break. In each phase, the animal had the opportunity to explore the labyrinth without nutritional supply. Phase II consisted of a phase of training and testing phases, each of that lasted for 5 minutes with a 30 second rest. In the training phase, at the end of each sleeve, food reinforcements were placed, with four random sleeves closed. After, in the phase of the break, the animal was placed in the center of the labyrinth, with closed doors. In the testing phase, all eight doors were opened, pre-positioning food supply those sleeves that were previously blocked. In this case, the animal should have visited only those sleeves that were blocked during the training phase. The testing phase was estimated by the number of correct and incorrect inputs in the sleeves.

**Fig. 1.** The result of the neurobehavioral test «Eight-sleeved radial labyrinth» of ovariectomized animals at the age of 4-5 months: the average memory point depends on the number of correct and incorrect entries in the sleeves. Each point means the average of the memory score in the test phase in one day. * - here and in other studies comparing the group of examined animals with the control group to false-operated 4-5 months (p ≤ 0.05).

**Fig. 2.** The result neurobehavioral testing «Eight-sleeved radial labyrinth» the old animals at the age of 20 months.
A mistake was considered to be the entrance to the sleeves, the feeder of which was opened in the training phase, as well as the repeated visits to the sleeves opened in the testing phase. Stage III - Stage II repetition after a break for 60 minutes. As a researched criterion, the average memory point (MS) was used.

The statistical processing of the obtained results was carried out using the Statplus Professional program, a collection of 5.9.8.5 / Core v.5.9.33 methods of nonparametric statistics. The data is presented in the form Me (Q1 ... Q3), where Me is the median, Q1 ... Q3 is the interquartile scale. The Mann-Whitney criterion was used to compare the indices in independent samples, and the comparison of dependent samples was performed using Wilkinson’s criterion. The differences were taken to be significant at the significance level of p≤0.05 [18].

Animal studies have been carried out and in accordance with the principles of humanity laid down in the European Community Directive (2010/63 / EC).

RESULTS AND DISCUSSION
On the first day of testing, after the previous training, ovariectomized 4-month rats showed an average memory score of -0.38 (-0.42 ... -0.14), which statistically improved on the second day of testing (p <0.05 ) and amounted to -0.28 (-0.41 ... -0.18) (Fig. 1). On the fourth day of testing, the ovariectomized animals performed statistically significantly (p = 0.035) more re-entries in the sleeve compared with the second day of testing, as evidenced by the average memory point in the second -0.29 (-0.43 ... -0.12) and the fourth day -0.28 (-0.34 ... -0.16). On the fifth day of testing in ovariectomized animals the value of the average memory score from the fourth -0.30 (-0.38 ... -0.16) until the sixth day of the test -0.31 (-0.47. .. -0.12) did not change statistically significantly (p <0.05).

At the age of 20 months, there was a general positive dynamics of the average memory score in the period from the first -0.32 (-0.38 ... -0.21) to the fourth day of testing -0.27 (-0.28 ... 0.14) at p = 0.028 (Fig. 2). The break for the fifth day of testing in the old rats did not statistically change the value of the average memory score from the fourth -0.32 (-0.38-0.14) to the sixth day of the test -0.33 (-0.39 ... 0,11).

Menopause is closely associated with memory loss and cognitive impairment. Estrogen plays an important role in neuroplasticity, for example, an increase in the population of the dendritic CA1 segment in the hippocampus, prolonged potentiation and neurogenesis. In the experiments, the memory and density of dendritic spines are reduced in the prefrontal cortex and the hippocampus after ovariectomy; it is also settled that the ability to recognize the object decreases after 1 week of ovariectomy and spatial memory decreases after 4 weeks [20].

Studies have shown that physical activity and stress influences improve of spatial memory of ovariectomized rats and are associated with increased extragranular aromatisation. This, in turn, affects the expression of estrogen receptors [21]. The data we obtained on ovariectomized animals indicate that on the second day the cognitive function is somewhat improved compared with the first day of testing, which is associated with physical activity in the labyrinth. There is a similar tendency in the old animals on the second day, but indicators of spatial memory are much worse.

Diabetes causes a significant deterioration in memory. So, on the first day of the test, after the previous training, 4-5 month diabetic rats showed an average memory point...
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The combination of ovariectomy and diabetes has caused a significant deterioration of cognitive function. From the first day of testing, after a preliminary training period of 4-5 rats with diabetes and ovariectomy showed an average memory score of -0.67 (-0.73 ... -0.34), which on the second day of testing was statistically improved (p < 0.05) and amounted to -0.65 (-0.72 ... -0.24...), and in the further days of testing has not changed (p < 0.05) and ranged from -0.62 (-0.75 per -0.18...) to -0.65 (-0.63...-0.20) on the last day of testing (Fig. 3).

DISCUSSION
Some authors claim that cognitive impairment in diabetes mellitus is clinically closer to cognitive impairment in vascular diseases of the brain and develops due to the macroand microvascular complications of DM 2. Deterioration of verbal memory and optic-spatial functions are described, in other studies, as the main constituents of cognitive impairments in DM 2 which is more characteristic of AD, and not for vascular dementia. Data of neuroimaging of the life-time of the brain show different variants of changes: a combination of global atrophy, subcortical atrophy, leukoareosis and lacunar, but small heart attacks, isolated heart attacks of the hippocampus, which can cause an «alzheimer-like» clinic with vascular etiology of lesions [19]. In diabetes, neurotropic factors and transcription factors induce structural changes in the neurons of the hippocampus, which are critical for memory and learning. Neurotrophic factors such as neurotrophin 3 (NT-3) and the neurotrophic factor (BDNF) from the brain are powerful regulators of neuronal and synaptic excitability, and when these factors are active, synaptin I and associated with protein growth 43 (GAP-43) plays an important role in neuronal and synaptic plasticity.

In patients with type 2 diabetes, cognitive dysfunction has been detected in the form of memory reduction, attention, and optic-spatial activity interrelated with chronic hyperglycemia. With an increase in the level of HbA1c in patients with type 2 diabetes, BDNF (brain neurotrophic factor) in blood plasma decreases and decreases in cognitive function [4, 22].

CONCLUSIONS
Our data allow us to draw conclusions about the influence of sex hormones on cognitive functions. Ovariectomy caused a deterioration of spatial memory, and the combination of ovariectomy and diabetes deepened the pathological process. In older animals, the study revealed a decrease in spatial memory, and diabetes further aggravated the cognitive function.

Changes that occur after ovariectomy indicate involvement of estrogen in the regulation of cognitive functions in the process and indicate the possibility of using estrogens in the treatment of neurodegenerative changes in premature and age-related menopause and in the against the background of concomitant pathological processes.

REFERENCES
Conflict of interest: The Authors declare no conflict of interest.

Authors' contributions:
According to the order of the Authorship.

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