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Age - and sex-specific changes in astroglial glutamine synthetase activity in rats with neurodegenerative pathology

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One of the trigger mechanisms for the development of neurodegeneration is glutamate excitotoxicity. The ATP-dependent enzyme glutamine synthetase, localized in astrocytes, regulates glutamate homeostasis by catalyzing the synthesis of glutamine from glutamate and ammonia. The purpose of the study was to identify age- and sex-specific changes in glutamine synthetase activity in a streptozotocin-induced model of Alzheimer-type neurodegeneration. The experiments were carried out on 60 old (at the age of 24 months) Wistar rats of both sexes, divided into groups: 1) control (intact) rats; 2) sham-operated rats; 3) rats with intraventricular injection of streptozotocin. using intraventricular injection of streptozotocin. In males, enzymatic activity decreased in the cortex, cerebellum, hippocampus, and hypothalamus by 40%, 33%, 36%, and 26%, respectively, compared with control rats. In females, the decrease was more pronounced, which amounted to 47% in the cortex, 39% in the cerebellum, 43% in the hippocampus, and 32% in the hypothalamus. Thus, the decrease in glutamine synthetase activity is due to age-dependent impairment of glutamate neurotransmission, reduced compensatory capabilities of neurons and altered hormonal status.
Key words: old Wistar rats, neurodegeneration, glutamate excitotoxicity, glutamine synthetase.

INTRODUCTION

Age-associated neurodegenerative diseases (Alzheimer's disease, Parkinson's disease, Huntington's chorea, etc.) acquire particular social importance with an increase in human average life expectancy. This is related to the progressive increase in the number of patients and high cost of the treatment [1, 2]. Preserving human cognitive function is apriority for scientists. However, despite significant investments made in the development of methods of early diagnosis and treatment, there is still no approved clinical protocol for adequate treatment of this pathology. This ultimately results in impaired social adaptability, disability and death. This problem is exacerbated by late diagnosis of the disease, when death of nerve cells and increasing atrophy of the affected brain parts are already progressing, as well as depleting the brain compensatory reserves and manifesting the characteristic symptoms of neurodegeneration. In this aspect, it is important to reveal the biochemical mechanisms of pathogenesis and biochemical markers of neurodegeneration, to develop principles of early diagnosis, prevention and treatment aimed at preventing neuronal death [3].

Glutamate-mediated excitotoxicity is considered to be one of the main molecular mechanisms for the development of neurodegenerative processes [4]. Glutamate is the major excitatory neurotransmitter in the mammalian brain and is involved in many cognitive processes and synaptic plasticity. About 60% of brain neurons use glutamate as their main excitatory neurotransmitter. Numerous studies suggest that altered glutamatergic neurotransmission is associated with the development of many neurodegenerative disorders, including Alzheimer's disease, schizophrenia, bipolar affec-

tive disorder, recurrent depressive disorder and others [5, 6].

Glutamine synthetase (EC 6.3.1.2) is an ATP-dependent enzyme localized predominantly in astrocytes, that catalyses the conversion of glutamate to glutamine, thereby controlling the intracellular concentration of glutamate. Alterations in its activity and gene expression, along with excitotoxicity, have been identified in several neurodegenerative diseases [7].

Identifying the basic mechanisms that trigger the disruption of brain glutamate metabolism with age, and developing ways to correct it, has become particularly important because of the increasing number of people suffering from neurodegenerative diseases.

The aim of our work was to determine glutamine synthetase activity in some brain structures - cortex, cerebellum, hypothalamus, hippocampus - of old white Wistar rats of both sexes in a streptozotocin (STZ)-induced Alzheimer's model.

METHODS

The study was carried out according to the Directive of the European Parliament and of the Council of the European Union (2010/63/EU) on the protection of animals used for scientific purposes.

Keeping and caring for animals. For the experiment, white Wistar rats were obtained from the vivarium of the Institute of Physiology named after Academician Abdulla Garayev of the Ministry of Science and Education of the Republic of Azerbaijan. Animals were housed in plastic cages under a normal light cycle and controlled temperature, with free access to food and water.

Experimental model and experimental conditions. The experiments were carried out on 60 old (at the age of 24 months) Wistar rats of both sexes (30 males and 30 females). Sporadic Alzheimer's disease in rats was modeled with intracerebroventricular (ICV) administration of low doses of STZ, this causes insulin resistance in the brain, leading to the appearance of the characteristic symptoms of Alzheimer's disease: excessive accumulation of hyperphosphorylated tau-protein, beta-amyloid, plaque formation, and cognitive deficit [8].

According to the experiment conditions, animals were divided into three groups: 1) control (intact) rats; 2) sham-operated rats; 3) rats with ICV administration of STZ. STZ - group of rats was injected with streptozotocin (3mg/kg, 5µl, slowly 1µl/min) to the left and right ventricle of the brain using a Hamilton microsyringe under general anesthesia (intraperitoneal injection of Calypsol and Xylazine). Sham-operated rats received ICV injections of the same volume of sterile 0.9% saline. The injecting procedures were performed in a stereotactic setup with precise coordinates.

In 6 months, animals were anesthetized with a mixture of Calypsol and Xylazine and decapitated. Then, the brain was removed from the skull and divided into the cortex, cerebellum, hypothalamus and hippocampus. Homogenate was prepared according to standard methods. The activity of the enzyme glutamine synthetase was measured using the Glutamine Synthetase Aktivity Assay Kit.

The experimental procedures were approved by the Institutes Scientific Council (protocol 3, 27 April, 2021).

Statistical analysis. In statistical processing, Student's t-test was used to assess differences between the control and experimental groups. Data were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Numerous studies of aging processes of the body, as well as age-related pathologies performed on experimental models, indicate that cognitive impairment is associated with disruption of synaptic connections between neurons, impaired brain plasticity and changes in neurotransmitter balance. Particular importance is attached to disorders in glutamate neurotransmission and weakening of adaptive capabilities of the brain

in aging, and as a consequence - the development of neurodegenerative diseases [9].

Sex differences in ageing and age-related neurodegenerative diseases are a continuing area of scientific interest. Significant sex characteristics should be taken into account when drawing up personalized programs of geroprotection, prevention and treatment, and determining measures of psychological assistance [10].

Sex-specific features of age-associated pathologies are related to the influence of sex steroid hormones. Studies have shown the role of glutamate in regulating sexual behavior, as well as the neuroprotective properties of estrogen. Thus, sexually mature female rats have higher glutamate metabolic activity than males, conditioned by the neuroprotective effect of the sex hormone estrogen. With aging, physiological functions decline, hormonal depletion and estrogen deficiency occur [11].

According to our results, the level of glutamine synthetase activity is lower in old control female rats compared to males, which may be due to age-related decrease in estrogen levels and involution of the reproductive system (Fig. 1).

Experimental modelling of neurodegenerative pathology of the Alzheimer's type results in a decrease in glutamine synthetase activity in the brain structures of rats of both sexes. In males, reliable data were obtained for a 40%, 33%, 36%, and 26% decrease in enzyme activity in the cortex, cerebellum, hippocampus and hypothalamus, respectively, compared to old control rats (Fig. 2). In female rats, the decrease was more pronounced, which was in the cortex - by 47%, cerebellum - by 39%, hippocampus - by 43% and hypothalamus - by 32%, compared to the control group (Fig. 3). Sham-operated rats of both sexes revealed not reliable changes in the enzyme activity.

Changes in glutamine synthetase activity are associated with features of glutamatergic neurotransmission in both normal physiological ageing and age-related pathologies. Ageing is characterized by hormonal imbalance and a gradual decline in physiological functions, including changes in the glutamatergic system, which contribute to reduced brain function [12]. While normal ageing is associated with mild to moderate cognitive impairment, it is also a major risk factor for neurodegenerative diseases, which are characterized by progressive cognitive impairment and behavioral disorders [1-3]. In physiological ageing, brain function declines due to a decrease in energy production by the mitochondria of cortical neurons, resulting in a slowing of the rate of the glutamate-glutamine cycle [5]. Glutamatergic transmission, involved in memory, learning, and cognition, becomes impaired with age, contributing to cognitive decline [13].

Numerous studies have demonstrated the effects of sex hormones on synaptic plasticity,

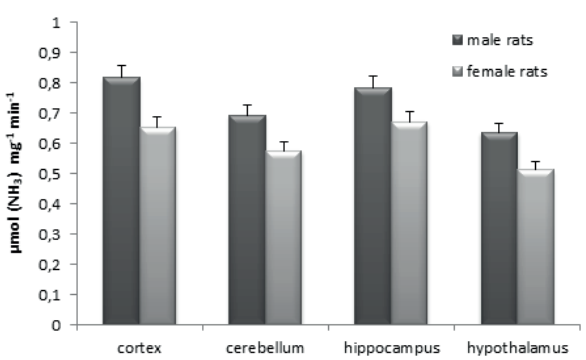

Fig. 1. Glutamine synthetase activity in brain structures of old control male and female rats

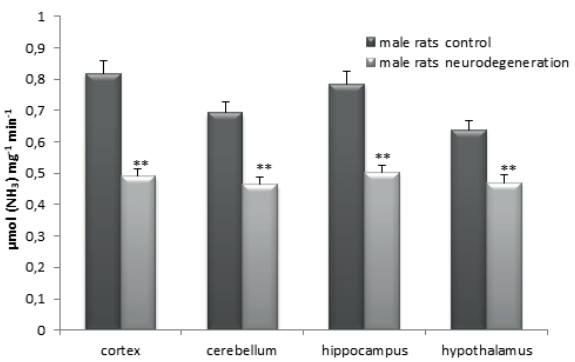

Fig. 2. Changes in activity of glutamine synthetase in brain structures of old male rats in a model of neurodegeneration. *P < 0.05, **P < 0.01

a key mechanism involved in memory, on hippocampus-mediated spatial memory and the glutamatergic neurotransmitter system, as well as the neuroprotective role of estrogen by reducing glutamate-mediated excitotoxicity. The interaction between sex hormones and dominant neurotransmitters, including serotonin, dopamine, GABA and glutamate, is also well established [11, 14].

The age-related decline in estrogen levels in women impairs glutamate neurotransmission by disrupting glutamate-glutamine cycle enzymes and reducing brain glutamate content, highlighting the neuroprotective effects of estrogen. This decline is also associated with reduced memory and concentration [15]. Therefore, women experience a more pronounced age-related disturbance of glutamate metabolism compared to men, caused by the influence of estrogen hormones on glutamate neurotransmission.

Glutamine synthetase is one of the main enzymes of the glutamate-glutamine cycle, regulating glutamate homeostasis and preventing glutamate excitotoxicity. Hydrolysis of glutamine to glutamate with formation of ammonia occurs in neurons with the participation of phosphate-activated glutaminase. However, studies show that modulation of glutaminase by phosphate is impaired in old rats compared to young ones, resulting in reduced brain glutaminase activity [16].

Thus, normal physiological aging is characrterized by a gradual decline in glutamatergic

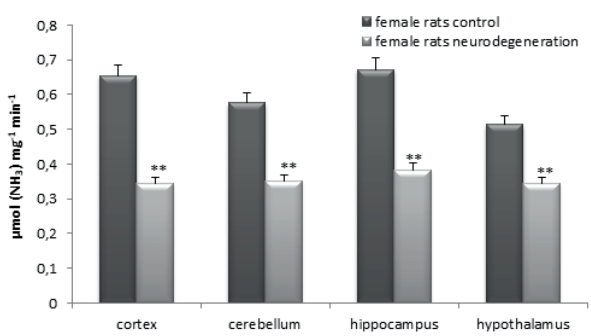

Fig. 3. Changes in activity of glutamine synthetase in brain structures of old female rats in a model of neurodegeneration. *P < 0.05, **P < 0.01

neurotransmission, changes in the glutamate-glutamine cycle, loss of glutamatergic synapses, and consequently cognitive impairment [12, 13]. In addition, hormonal depletion, including age-related sex hormone deficiency, leads to a loss of neuroprotective effects and, consequently, a decline in cognitive activity.

In our experiments, we determined glutamine synthetase activity in the brain of old rats in a neurodegeneration model. Although age-related neurodegeneration is part of physiological aging, its acceleration is a cause of neurodegenerative pathology [2, 3].

In recent years, significant attention has been given to glutamatergic neurotransmission due to its neuroprotective role in supporting cognitive, psychomotor, and emotional functions. Disruption of glutamatergic neurotransmission and the progression of glutamate excitotoxicity play a key role in the pathogenesis of neuronal death in neurodegenerative processes of the brain. This pathobiochemical cascade of cerebral pathology is triggered by high glutamate levels [4-6]. Also, hypoxia-induced glutamate excitotoxicity underlies most neurodegenerative pathologies. The search for ways of preventing neuronal death and support their functional activity under hypoxic stress is also important [17].

Consequently, glutamate and its transporters are involved in both normal brain function and neurodegenerative diseases developing. In this case, there is a disturbance in both the process of glutamate uptake by astrocytes and changes in the activity of enzymes of glutamate metabolism.

The enzyme glutamine synthetase is mainly localized in astrocytes, whose main function is to protect neurons from excitotoxicity through the uptake of excess ammonia and glutamate and their conversion to glutamine by glutamine synthetase. However, with age, astrocytes contribute to glutamate toxicity in cortical neurons [18].

Studies show that the brain undergoes several changes with age, including increased oxidative stress, altered neurotransmitter

balance, impaired cellular bioenergetics and increased levels of pro-inflammatory factors, all contributing to a progressive decline in brain functional ability [12]. First and foremost, these processes occur in glial cells, whose ageing results in an increased risk of developing various neurodegenerative diseases. Age-related changes in glial cells reduce their ability to maintain the normal functionality of the nervous system [19].

In our study, there was a decrease in the enzyme activity in old rats of both sexes. However, the decrease was more pronounced in female rats. This is probably caused by age-related estrogen decline and involution of the reproductive system. As mentioned above, female hormones provide strong neuroprotection by suppressing glutamate neurotoxicity. Ovarian depletion with age causes frequent depression, dementia and a high rate of neurodegenerative diseases in women [15]. In addition, with increasing age and against the background of many age-related diseases, the content of the adaptation hormone melatonin decreases in women and the level of the neurotrophic factor BDNF decline in men [20].

Thus, our study revealed a decrease in glutamine synthetase activity in old rats of both sexes in a model of STZ-induced neurodegeneration. Moreover, the decline in enzyme activity was more pronounced in females compared to males. This is conditioned by age-related disturbances in glutamate neurotransmission caused by changes in the structure of components of the glutamatergic system, changes in the activity of glutamate-glutamine cycle enzymes, a deficiency of sex steroid hormones, and a decrease in the brain's plasticity and adaptability.

Disruption of glutamatergic neurotransmission and glutamate excitotoxicity play a major role in the pathogenesis of neuronal death in neurodegenerative processes in the brain. In this case, both the process of glutamate uptake by astrocytes and changes in the activity of glutamate-glutamine cycle enzymes are disturbed.

Results of our study confirm the decreased activity of glutamine synthetase in old rats of both sexes in a STZ model of neurodegeneration. Furthermore, the decrease was more pronounced in female rats compared to males, which is due to the age-related decline in estrogen levels and the loss of their neuroprotective effect.

It is possible that the low level of glutamine synthetase activity in chronic neurodegeneration is caused by progressive beta-amyloidosis and increased oxidative modifications of protein molecules. In addition, oxidation of glutamine synthetase as a result of age-related susceptibility of the enzyme to oxidative stress plays a role in promoting neurodegeneration.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

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ВІКОВІ ТА СТАТЕВІ ЗМІНИ АКТИВНОСТІ АСТРОГЛІАЛЬНОЇ ГЛУТАМІНСИНТЕТАЗИ ЗА УМОВ НЕЙРОДЕГЕНЕРАТИВНОЇ ПАТОЛОГІЇ У ЩУРІВ

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Одним з пускових механізмів розвитку нейродегенерації є глутаматна ексайтотоксичність. АТФ-залежний фермент глутамінсинтетаза, що локалізується в астроцитах, регулює гомеостаз глутамату, каталізуючи синтез глутаміну з глутамату та аміаку. Метою нашого дослідження було виявлення вікових та статевих особливостей зміни активності глутамінсинтетази на моделі стрептозотоциніндукованої нейродегенерації альцгеймерівського типу. Для дослідження використано 60 білих старі щури лінії Вістар обох статей, розподілені на три групи: 1-ша – старі інтактні щури, 2-га – псевдооперовані контрольні щури, 3-тя щури з внутрішньошлуночковим введенням стрептозотоцину. У самців знижувалася ферментативна активність у корі, мозочку, гіпокампі та гіпоталамусі на 40, 33, 36 та 26% відповідно щодо значень у старих інтактних щурів. У

самиць зниження активності глутамінсинтетази було більш виражене: в корі – на 47%, мозочку – на 39%, гіпокампі – на 43% та гіпоталамусі – на 32% щодо значень інтактної групи. У хибнооперованих щурів обох статей зміни активності ферменту були недостовірні. Отже, зниження активності ферменту зумовлено віковим порушенням глутаматної нейротрансмісії, пригніченням компенсаторних можливостей нервових клітин, а також зміною гормонального статусу.

Ключові слова: старі щури Вістар; нейродегенерації; глутаматна ексайтотоксичність; глутамінсинтетаза.

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