პლაზმაში ჰომოცისტეინის მაღალი დონის გავლენის ახალი ასპექტები ანთებითი პროცესის მიმდინარეობაზე პაროდონტის ქსოვილებში, რაც უზრუნველყოფს პათოგენეზური თერაპიის გაუმჯობესებას პაციენტებში პაროდონტიტით ქრონიკულ ჰიპერჰომოცისტეინემიასთან კომბინაციაში.

EARLY POSTNATAL DYSFUNCTIONING OF THE BRAIN MUSCARINIC CHOLINERGIC SYSTEM AND THE DISORDERS OF FEAR-MOTIVATED LEARNING AND MEMORY

Shavgulidze M., Babilodze M., Rogava N., Chijavadze E., Nachkebia N.

Ivane Beritashvili Center of Experimental Biomedicine, Tbilisi, Georgia

The aim of the study was to investigate the early postnatal dysfunctioning of the brain muscarinic cholinergic system as a major factor in the development of cognitive disorders similar with somewhat is noted in animal models of depression and in patients with Major Depressive Disease (MDD). The problem is highly topical because MDD is one of the most common diseases in the world and is a serious cause of human death. The appropriate strategy for treating of MDD is not fully understood yet. This is complicated by the fact that MDD is a complex, multidimensional, heterogeneous disease accompanied by a range of changes, including cognitive impairment, depressed mood, anhedonia, decreased appetite and libido and so on [12,13].

Therefore, it is very topical and important to search for new research approaches to elucidate the pathophysiology of MDD, for which we consider it necessary to thoroughly study the pathophysiological mechanisms of individual symptoms of MDD on adequate, valid, animal models of this disease. Such studies are of great importance in terms of both basic science and clinical medicine.

Such a minding of scientific thinking is very important since it is considered that the above-listed range of symptoms, accompanying MDD, should develop with the participation of various factors, particular specific symptoms may be served by dysfunctions of different neurotransmitters.

In the present study, special attention has been paid to the muscarinic cholinergic system of the brain, as this system is involved in the learning and memory mechanisms. In the central nervous system, acetylcholine promotes many functions such as learning, memory, attention, and motor control [7,14,16]. Muscarinic acetylcholine receptors (MAChRs) have recently been shown repeatedly to play a role in learning and memory [1,3,5]. Therefore, there is a suggestion and we also share this view that dysfunction of the cholinergic system may contribute to the development of cognitive disorders during MDD [2,8].

We hypothesize that for the investigation of the role of dysfunctioning the brain muscarinic cholinergic system as the main factor of cognitive impairment, accompanying various psychoneurological disorders, it is necessary to conduct experiments on animal models where the dysfunctioning of the brain muscarinic cholinergic system will be achieved without any specific (electrolytic, cytotoxic and so on) damaging of nervous cells by exogenous factors. Such a way is a completely new methodological approach, worked out by Nachkebia et.al. [11], which was also used in our study. The method involves dysfunctioning of the brain muscarinic cholinergic system during the early postnatal development (P7-P28) of rat pups and investigation of the long-term effects of this procedure after achieving the adult ages them. In general, the use of such a methodical approach allows obtaining a relatively pure effect of dysfunctioning of the brain muscarinic cholinergic system on the learning and memory in various separate tasks of declarative and non-declarative learning and memory. Studies conducted on such animal models are very important in terms of extrapolation to humans.

The present study examines the processes of learning and long-term retention of information obtained in the two important tasks of non-declarative memory - active avoidance, motivated by foot shock-induced fear, and the elevated plus-maze, based on the natural fear. We thought that the results obtained will answer the important question of whether the tasks of learning and memory motivated by the natural and/or procedural fear, are disturbed in animals that have been subjected, during early postnatal development, to the dysfunctioning of muscarinic cholinergic system.

The urgency of the issue is very high, as the lasting effects of early postnatal dysfunctioning of the brain muscarinic cholinergic system on learning and memory of the non-declarative fear-motivated tasks have been studied by us for the first time.

Material and methods. Experiments were carried out on male white wild rats (n=40). Four groups of animals were used with special procedures for each one.

Experimental group I - rat pups (n=15) received subcutaneously Scopolamine (Scop. n=10) 30 mg/kg two times daily during from postnatal day 7 (P7) to P28; Afterwards they were maintained in home cages under special care. After adult age (2.5-3 month) 5 rats were included in the experiments aimed to investigate the changes of learning and memory in the two-way active avoidance test. The second 5 rats received systemic injection of Scop (15 mg/kg) and 30 min after were included in the experiments aimed to investigate the changes of learning and memory in the two-way active avoidance test. The third 5 rats received systemic injection of Melipramin [(Mel) 15 mg/kg] and 30 min after were included in the experiments aimed to investigate the changes of learning and memory in the two-way active avoidance test.

Experimental group II – rat pups (n=15) received subcutaneously Scop with the same procedure as in group I. In adult age (2.5-3 month) 5 rats were included in the experiments aimed to investigate the level of anxiety and changes of fear-motivated learning and memory in the elevated plus maze test. The second 5 rats received systemic injection of Scop (15 mg/kg) and 30 min after were included in the experiments aimed to investigate the level of anxiety and changes of fear-motivated learning and memory in the elevated plus maze test. The third 5 rats received systemic injection of Melipramin [(Mel) 15 mg/kg] and 30 min after were included in the experiments aimed to investigate the level of anxiety and changes of fear-motivated learning and memory in the elevated plus maze test.

Group III/Control - Rat pups (n=15) received distilled water with the same volume and procedure as the rat pups from experimental groups I and II. After adult age (2.5-3 month) the first 5 rats were included in the experiments aimed to investigate the changes of learning and memory in the two-way active avoidance test. The second 5 rats received systemic injection of Scop (15 mg/kg) and 30 min after were included in the experiments aimed to investigate the changes of learning and memory in the two-way active avoidance test. The third 5 rats received systemic injection of Mel (15 mg/kg) and 30 min after were included in the experiments aimed to investigate the changes of learning and memory in the two-way active avoidance test.

Group IV/Control - Rat pups (n=15) received distilled water with the same volume and procedure as the rat pups from experimental groups I and II. In adult age (2.5-3 month) 5 rats were included in the experiments aimed to investigate the level of anxiety and changes of fear-motivated learning and memory in the elevated plus maze test. The second 5 rats received systemic injection of Scop (15 mg/kg) and 30 min after were included in the experiments aimed to investigate the level of anxiety and changes of fear-motivated learning and memory in the elevated plus maze test. The third 5 rats received systemic injection of Mel (15 mg/kg) and 30 min after were included in the experiments aimed to investigate the level of anxiety and changes of fear-motivated learning and memory in the elevated plus maze test.

Shuttle box for Two-way active avoidance. A shuttle box is an apparatus used in animal learning experiments. Shuttle box is divided by a hurdle into two equally sized compartments, over which a subject can jump to shuttle from one compartment to the other. Each compartment has the independent grid floor. Foot shocks are delivered through the metal bars that form the floor of the box and the animal must move from one compartment to the other in order to avoid an aversive stimulus. The cage contains a general sound generator and light for each compartment.

Two-way shuttle box avoidance procedure. The subject is placed in one of the two compartments. After 3 min has passed, a stimulus (a tone in our experiments) occurs. After the tone has been sounding for 10 seconds the shock is turned on and the subject begins to run in response to the shock. Eventually it shuttles over the hurdle into the other compartment, an act that has two immediate consequences: (1) the shock ends, and (2) the tone ends. After 40 sec the second learning trial starts and the tone again sounds, and 10 seconds later the shock again begins, producing more running and, eventually, a second shuttle, returning the subject to the first compartment. Daily learning session continued until rat's 10 consecutive correct responses (to shuttle into the other compartment in response to tone, without foot shock). Procedure was repeated during the second, third and fourth days, that is until rat's 10 consecutive correct responses from the beginning of daily learning session.

Notice that the subject can avoid shock by shuttling either way - from the left to the right compartment or vice versa. For this reason procedure is called "two-way active avoidance".

The measures recorded, number of trials needed for achievement of daily learning criteria, percentage of correct responses and response latency (latency to avoid or escape), serve as an index of learning and allow memory to be assessed.

Elevated plus maze. The elevated plus maze (EPM) consists of a "+"-shaped maze elevated above the floor by means of four (50 cm height) legs. The maze consists of two open arms (25 x 8 x 0.5 cm) and two closed arms (25x8x12 cm) that extend from a central platform (8x8 cm). Closed arms are surrounded by transparent walls 12 cm high and open arms are surrounded by 0.5-cm ledges and are equipped by 650 light lux centered over the open arms. Each rat was placed in the center platform, facing a closed arm. During the 5-min test, cameras above the maze recorded the mice's movements, the time mice spent in open or closed arms, the time needed for the transfer from the closed to open arms of the maze.

Results were treated statistically by means of Student's t test. * = $p<0.05$, ** = $p<0.01$ were taken as the levels of significance.

Results and discussion. The study of the changes in learning and memory of fear-motivated two-way active avoidance task in animal models that underwent functional dysregulation of the muscarinic cholinergic system during early ontogenesis revealed significant results. In the active avoidance test, the animals learn to avoid an aversive stimulus by changing locations. At the start of the first session, a rat is placed in one of two compartments. After habituation, a stimulus (tone in our case) is resented for a fixed period of time (10 sec) and 3 sec later it is followed by electrical stimulation of foots.

Learning and memory processes in the active avoidance task were assessed by the following indicators: the number of trials required to achieve the learning criterion (10 consecutive correct responses) in each session; the percentage of correct responses in each session. The data obtained in the first session allows to characterize the processes of information acquisition, while the data obtained in subsequent sessions provide us with information on the progress of the consolidation process.

The data presented in Fig.1A show that both, control animals (models with saline) and animals, which were subjected during early ontogenesis to the functional dysregulation of the muscarinic cholinergic system, studied the task of two-way active avoidance in two sessions.

The number of trials required to achieve the daily learning criterion is significantly reduced in experimental models in the course of the first learning session [first day of experiment (Fig.1A)]. Reduction is noted also in the second session (second day of experiments), but difference between control and experimental groups is insignificant.

Against the background of this data, the question naturally arises - is there any stable difference between these two groups in the acquisition and consolidation of the information obtained in the two-way active avoidance task? Comparisons between data sets showed that animals, that underwent functional inhibition of the muscarinic cholinergic system during early ontogenesis, required much fewer trials for achieving the criterion in the both learning session (Fig. 1A), indicating that acquisition of the information found to be easier in experimental models compared with control rats.

Analysis of the number of correct responses (jump to another section of the active avoidance chamber in response to the tone, without electric shock) in the course of learning sessions showed

that during the first session, the number of correct responses is much higher in animals that were subjected during the period of early ontogenesis to the functional dysregulation of the muscarinic cholinergic system. The difference in the number of correct responses between the two groups, in the second learning session, becomes even higher (Fig. 1B). Thus, based on the data obtained we can conclude that the acquisition and consolidation of the information obtained in the two-way active avoidance reaction is accelerated in experimental models.

In the next series of experiments we explored the effects of anticholinergic drugs and antidepressants on non-declarative learning and memory, in the active avoidance test, motivated by foot shock-induced fear. It appears that intraperitoneal injection of Scop (Fig.2A) delays the learning processes of active avoidance in experimental models, in the first and second session, compared to control models. It is manifested in an increase in the number of trials required to achieve the daily learning criterion. In the third session, the difference in the measures of learning and memory disappears and stable memory in the active avoidance test is formed with the same speed as in controls. Therefore the data indicate that the process of perception and acquisition of the new information is being complicated but the processes of consolidation are not affected in the course of the subsequent learning sessions. The fact that the changes occur only in the initial session and the process is normalized during subsequent ones should indicate that the cause is an increase in foot-shock-induced anxiety, as an acute effect of Scop systemic injection. Our results are supportive of the data by other authors which have shown that unbalanced ACh levels lead to abnormal emotional behaviors [10]. Increased ACh levels by inhibiting acetylcholinesterase (AChE) in the hippocampus of mice cause anxiety and depressive-like behavior ([10].

Previous studies demonstrate that Scop, a nonselective MAChRs antagonist, can produce rapid antidepressant actions (within hours) and is effective even in treatment-resistant major depressive disorder (MDD) patients [4,6,17].

It is possible that mAChRs may be involved in stress-induced anxiety behavior, providing a new indication of the pathophysiological mechanism of anxiety disorders and the potential pharmacological target [9].

Opposite results were obtained in the study of the effects of intraperitoneal injection of the antidepressant Mel on the learning and memory in the active avoidance test. Namely, the number trials sufficient for the achieving of daily learning criteria were diminished significantly in the first learning session and tendency of diminution was retained in the second learning session too (Fig. 2B).

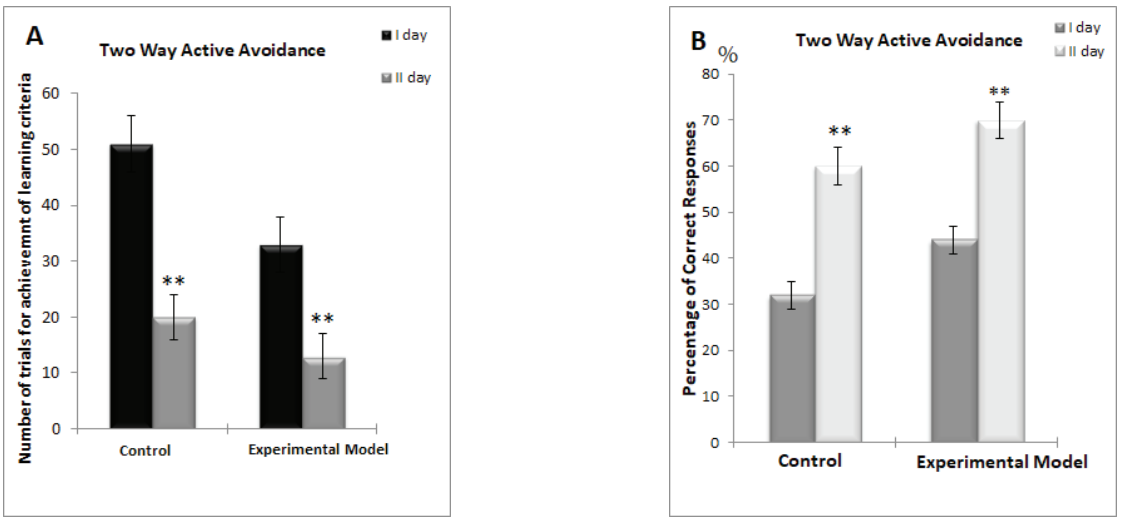

*Fig. 1. Changes in the number of the trials (A) needed for the achievement of learning criterion and percentage of the correct responses (B) in the two-way active avoidance test. ** = p<0.01*

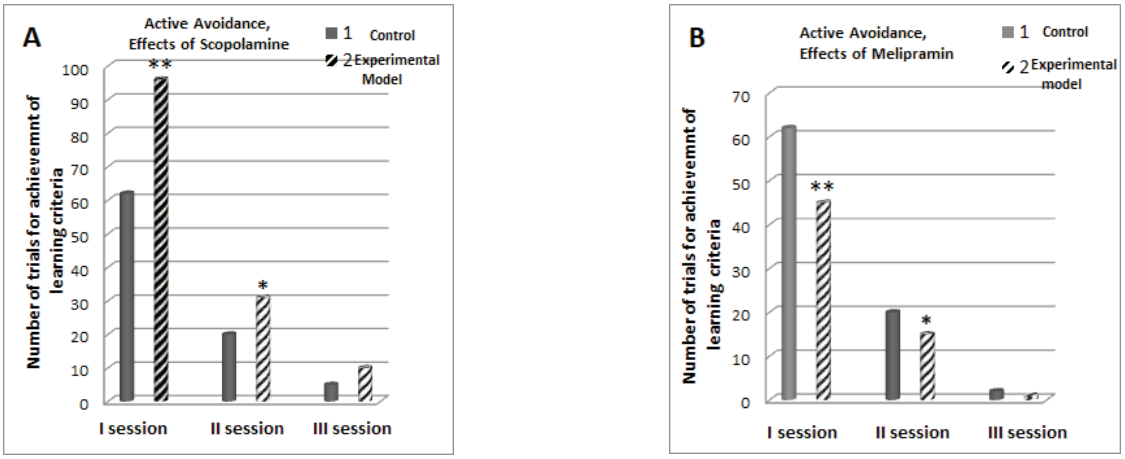

*Fig. 2. Changes in the number of the trials needed for the achievement of learning criteria under the systemic Scopolamine (A) and Melipramin (B) in the two-way active avoidance test in animals subjected to functional dysregulation of the muscarinic cholinergic system during early ontogenesis. *=p<0.05; ** = p<0.01*

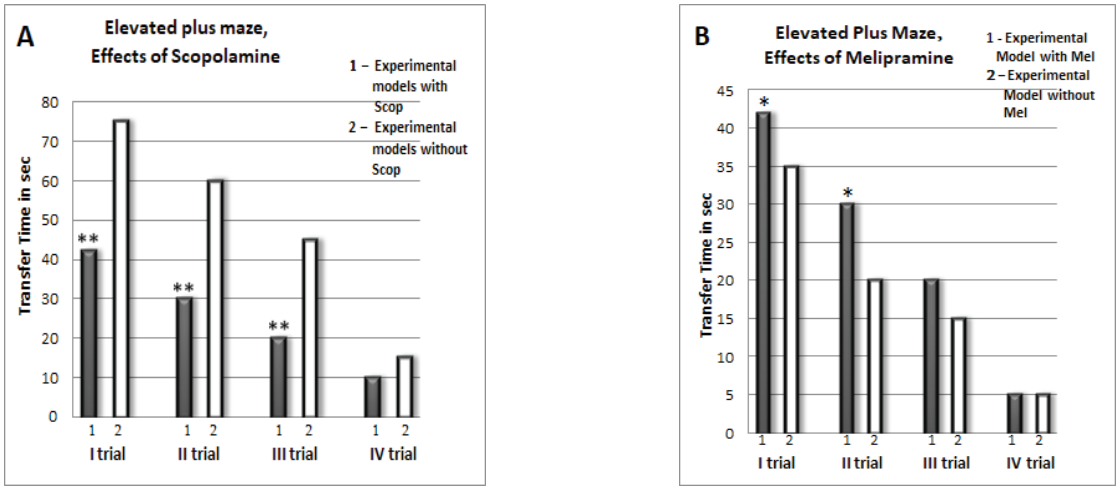

*Fig. 3. Changes in the transfer time from dark to open arms of elevated plus maze, number of the trials needed for the achievement of learning criteria under the systemic Scopolamine (A) and Melipramin (B) in the two-way active avoidance test in animals subjected to functional dysregulation of the muscarinic cholinergic system during early ontogenesis. *=p<0.05; ** = p<0.01*

Despite the facilitation in the acquisition of information about the fear-based threat, at the onset of learning trials with foot shock, fool value fear-motivated memory is formed in three learning sessions in this case too. Systemic injection of Mel, whose acute effect is manifested by an increase of monoamine levels in the brain, stabilizes the active state and suppresses the fear and anxiety response.

To find out if the dysfunctioning of the muscarinic cholinergic system, during the period of early ontogenesis, really have lasting effects on the anxiety in rats special series of experiments was carried out in the elevated plus-maze that is based on the natural fear.

The elevated plus maze is a widely used behavioral test that has been validated to assess the anxiety and anti-anxiety effects in response to the various experimental procedures, and to define brain regions and mechanisms underlying anxiety-related behavior [14]. This test relies on the rodents' natural propensity and comfortability in the dark, narrow areas (closed arms of the maze for example). The longer the time spent by the rodent in the open arms and the shorter is the transfer time to open arms, the lower the level of animal's anxiety.

We studied the level of anxiety and changes of fear-motivated learning and memory in the elevated plus maze test and the effects of systemic injection of Scop and/or Mel in adult rats subjected in early postnatal development to the dysfunction of brain muscarinic cholinergic system.

Experimental models, subjected in early postnatal development to the dysfunction of brain muscarinic cholinergic system, received a systemic injection of Scop and 20 min after were placed in the center of an elevated plus maze. It was found that the transfer latency, or transition time from the closed to the open arm of the elevated plus-maze, was significantly decreased compared to the data of experimental models without Scop (Fig. 3A). That is, the transfer from the dark to the open arms was not complicated compared to the background data of experimental models. The effect was statistically significant and was maintained across all experimental trials but was expressed most highly during the first and second experimental trials.

Systemic injection of Mel increased the transfer latency or transition time from the closed to the open arms of the elevated plus-maze in the rats, subjected in the early period of postnatal development to the dysfunctioning of the muscarinic cholinergic system. It was noted in the first and second trials and then differences disappeared and both groups of animals learn the task by the same speed.

Therefore, lasting effects of early postnatal dysfunctioning of the brain muscarinic cholinergic system were studied for the first time in the present work. Following new facts deserve special attention: 1) This procedure produce in adult age initial impairment of learning in non-declarative memory test that is based on the enhancement of the level of anxiety in both active avoidance and elevated plus maze tests; 2) Dysfunctioning of the brain muscarinic cholinergic system, in the period of early ontogenesis, does not change in adult animals the consolidation and long-term retention of information obtained in the two important tasks of non-declarative memory, active avoidance, motivated by foot shock-induced fear, and the elevated plus-maze, based on the natural fear; 3) Intraperitoneal injection of Scope in adult animals, subjected during early ontogenesis to the dysfunctioning of the muscarinic cholinergic system, reduce the level of anxiety and facilitates by this way learning and memory of natural fear-motivated non-declarative test.

In conclusion, early postnatal dysfunctioning of the brain muscarinic cholinergic system enhances the level of anxiety in adult age and complicates acquisition of information in fear-motivated non-declarative learning and memory tests, but does not change its consolidation and long-term retention.

REFERENCES

1. Bartko SJ, Winters BD, Saksida LM, Bussey TJ. Different roles for M1 and M2 receptors within perirhinal cortex in object recognition and discrimination. Neurobiol Learn Mem. 2014 Apr;110:16-26. doi: 10.1016/j.nlm.2014.01.002. Epub 2014 Jan 24. PMID: 24462721.

2. Dagytė G, Den Boer JA, Trentani A. The cholinergic system and depression. Behav Brain Res. 2011 Aug 10;221(2):574-82. doi: 10.1016/j.bbr.2010.02.023. Epub 2010 Feb 16. PMID: 20170685.

3. Dasgupta R, Seibt F, Beierlein M. Synaptic Release of Acetylcholine Rapidly Suppresses Cortical Activity by Recruiting Muscarinic Receptors in Layer 4. J Neurosci. 2018 Jun

6;38(23):5338-5350. doi: 10.1523/JNEUROSCI.0566-18.2018. Epub 2018 May 8. PMID: 29739869; PMCID: PMC5990982.
4. Drevets WC, Zarate CA Jr, Furey ML. Antidepressant effects of the muscarinic cholinergic receptor antagonist scopolamine: a review. Biol Psychiatry. 2013 Jun 15;73(12):1156-63. doi: 10.1016/j.biopsych.2012.09.031. Epub 2012 Nov 28. PMID: 23200525; PMCID: PMC4131859.
5. Felder CC, Goldsmith PJ, Jackson K, Sanger HE, Evans DA, Mogg AJ, Broad LM. Current status of muscarinic M1 and M4 receptors as drug targets for neurodegenerative diseases. Neuropharmacology. 2018 Jul 1;136(Pt C):449-458. doi: 10.1016/j.neuropharm.2018.01.028. Epub 2018 Jan 31. PMID: 29374561.
6. Furey ML, Khanna A, Hoffman EM, Drevets WC. Scopolamine produces larger antidepressant and antianxiety effects in women than in men. Neuropsychopharmacology. 2010 Nov;35(12):2479-88. doi: 10.1038/npp.2010.131. Epub 2010 Aug 25. PMID: 20736989; PMCID: PMC3055321.
7. Hasselmo ME. The role of acetylcholine in learning and memory. Curr Opin Neurobiol. 2006 Dec;16(6):710-5. doi: 10.1016/j.conb.2006.09.002. Epub 2006 Sep 29. PMID: 17011181; PMCID: PMC2659740.
8. Maurer SV, Williams CL. The Cholinergic System Modulates Memory and Hippocampal Plasticity *via* Its Interactions with Non-Neuronal Cells. Front Immunol. 2017 Nov 8;8:1489. doi: 10.3389/fimmu.2017.01489. PMID: 29167670; PMCID: PMC5682336.
9. Mei L, Zhou Y, Sun Y, Liu H, Zhang D, Liu P, Shu H. Acetylcholine Muscarinic Receptors in Ventral Hippocampus Modulate Stress-Induced Anxiety-Like Behaviors in Mice. Front Mol Neurosci. 2020 Dec 15;13:598811. doi: 10.3389/fnmol.2020.598811. PMID: 33384583; PMCID: PMC7769836.
10. Mineur YS, Obayemi A, Wigestrand MB, Fote GM, Calarco CA, Li AM, Picciotto MR. Cholinergic signaling in the hippocampus regulates social stress resilience and anxiety- and depression-like behavior. Proc Natl Acad Sci U S A. 2013 Feb 26;110(9):3573-8. doi: 10.1073/pnas.1219731110. Epub 2013 Feb 11. PMID: 23401542; PMCID: PMC3587265.
11. Nachkebia N., Chkhartishvili E., Mchedlidze O., Dzadzamia Sh., Maglakelidze N., Chijavadze E. Malfunctioning of Brain Muscarinic Cholinergic System in the Period of Early Postnatal Development Leads to Behavioral and Sleep Disturbances in Adult Age Rats and Changes in Density of M2/M4 Muscarinic Receptors. // J. Sleep Medicine, 2013. - suppl. 14 S, e216
12. Nestler E.J., Moneggia L., Esch E. J. Neurobiology of depression // Neuron, 2002. - 34: 13–25, 13-25 DOI: 10.1016/S0896-6273(02)00653-0
13. Nestler EJ. Is there a common molecular pathway for addiction? Nat Neurosci. 2005 Nov;8(11):1445-9. doi: 10.1038/nn1578. PMID: 16251986.
14. Thomsen M, Wörtwein G, Fink-Jensen A, Woldbye DP, Wess J, Caine SB. Decreased prepulse inhibition and increased sensitivity to muscarinic, but not dopaminergic drugs in M5 muscarinic acetylcholine receptor knockout mice. Psychopharmacology (Berl). 2007 May;192(1):97-110. doi: 10.1007/s00213-006-0682-y. Epub 2007 Feb 20. PMID: 17310388.
15. Walf AA, Frye CA. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nat Protoc. 2007;2(2):322-8. doi: 10.1038/nprot.2007.44. PMID: 17406592; PMCID: PMC3623971.
16. Wess J, Eglen RM, Gautam D. Muscarinic acetylcholine receptors: mutant mice provide new insights for drug development. Nat Rev Drug Discov. 2007 Sep;6(9):721-33. doi: 10.1038/nrd2379. PMID: 17762886.
17. Wohleb ES, Wu M, Gerhard DM, Taylor SR, Picciotto MR, Alreja M, Duman RS. GABA interneurons mediate the rapid antidepressant-like effects of scopolamine. J Clin Invest. 2016 Jul 1;126(7):2482-94. doi: 10.1172/JCI85033. Epub 2016 Jun 6. PMID: 27270172; PMCID: PMC4922686.

SUMMARY

EARLY POSTNATAL DYSFUNCTIONING OF THE BRAIN MUSCARINIC CHOLINERGIC SYSTEM AND THE DISORDERS OF FEAR-MOTIVATED LEARNING AND MEMORY

Shavgulidze M., Babilodze M., Rogava N., Chijavadze E., Nachkebia N.

Ivane Beritashvili Center of Experimental Biomedicine, Tbilisi, Georgia

The aim of the study was to investigate the early postnatal dysfunctioning of the brain muscarinic cholinergic system as a major factor in the development of cognitive disorders similar with somewhat is noted in animal models of depression and in patients with Major Depressive Disease. The present study examined the processes of learning and long-term retention of information obtained in the two important tasks of non-declarative memory - active avoidance, motivated by foot shock-induced fear, and the elevated plus-maze, based on the natural fear.

Experiments were carried out on male white wild rats (n=60). Four groups of animals (15 rats in each) were used with special procedures for each one. Early postnatal dysfunctioning of the brain muscarinic cholinergic system was produced by the new method worked out by Nachkebia and co-authors. Two-way shuttle-box and elevated plus maze devices were used for the investigation of fear-motivated non-declarative memory and anxiety.

The lasting effects of early postnatal dysfunctioning of the brain muscarinic cholinergic system on non-declarative learning and memory and anxiety were studied for the first time in the present work. Following new facts were obtained: 1) This procedure produces in adult age initial impairment of learning in a non-declarative memory test that is based on the enhancement of the level of anxiety in both active avoidance and elevated plus maze tests; 2) Dysfunctioning of the brain muscarinic cholinergic system, in the period of early ontogenesis, does not change in adult animals the consolidation and long-term retention of information obtained in the two important tasks of non-declarative memory, active avoidance, motivated by foot shock-induced fear, and the elevated plus-maze, based on the natural fear; 3) Intraperitoneal injection of Scope in adult animals, subjected during early ontogenesis to the dysfunctioning of the muscarinic cholinergic system, reduce the level of anxiety and facilitates by this way learning and memory of natural fear-motivated non-declarative test.

Early postnatal dysfunctioning of the brain muscarinic cholinergic system enhances the level of anxiety in adult age and complicates acquisition of information in fear-motivated non-declarative learning and memory tests, but does not change its consolidation and long-term retention.

Keywords: muscarinic cholinergic system, early ontogenesis, non-declarative memory, anxiety.

РЕЗЮМЕ

РАННЯЯ ПОСТНАТАЛЬНАЯ ДИСФУНКЦИЯ МУСКАРИНОВОЙ ХОЛИНЕРГИЧЕСКОЙ СИСТЕМЫ ГОЛОВНОГО МОЗГА И НАРУШЕНИЯ ОБУЧЕНИЯ И ПАМЯТИ, МОТИВИРОВАННЫЕ СТРАХОМ

Шавгулидзе М.А., Бабилодзе М.Р., Рогава Н.О., Чиджавадзе Э.О., Начкебия Н.Г.

Центр экспериментальной биомедицины им. И. Бериташвили, Тбилиси, Грузия

Целью исследования явился анализ ранней постнатальной дисфункции мускариновой холинергической системы головного мозга как основного фактора развития когнитивных расстройств, которые отмечаются при большом депрессивном расстройстве.

Изучены процессы обучения и долговременного сохранения информации, полученной при выполнении двух значимых задач недекларативной памяти - активного избегания, мотивированного страхом, вызванного ударом по стопам, и приподнятого крестообразного лабиринта, основанного на естественном страхе.

Эксперименты проведены на белых диких крысах-самцах (n=60), которые распределены в четыре группы (по 15 крыс в каждой) со специальными процедурами. Ранняя постнатальная дисфункция мускариновой холинергической системы головного мозга вызвана применением нового метода, разработанного проф. Н. Начкебия и соавторами. Камера двустороннего активного избегания и приподнятый крестообразный лабиринт использовались для исследования недекларативной памяти, мотивированной страхом и тревогой.

Впервые изучены длительные эффекты ранней постнатальной дисфункции мускариновой холинергической системы мозга на недекларативное обучение, память и тревогу. Выявлены следующие факты: 1) данная процедура во взрослом возрасте вызывает начальное нарушение обучения в недекларативном тесте памяти, которое основано на повышении уровня тревожности как в тесте активного избегания, так и в приподнятом крестообразном лабиринте; 2) дисфункция мускариновой холинергической системы головного мозга у взрослых животных в период раннего онтогенеза не меняет консолидацию и длительное сохранение информации, полученной в двух значимых задачах недекларативной памяти - активное избегание, мотивированное страхом, вызванным ударом электрошока по стопам, и приподнятый крестообразный лабиринт, основанный на естественном страхе; 3) внутрибрюшинное введение скополамина взрослым животным, подвергшимся в раннем онтогенезе дисфункции мускариновой холинергической системы, снижает уровень тревожности и способствует обучению и запоминанию недекларативного теста, мотивированного естественным страхом.

Ранняя постнатальная дисфункция мускариновой холинергической системы головного мозга повышает уровень тревожности в зрелом возрасте и затрудняет получение информации в недекларативных тестах обучения и памяти, мотивированных страхом, однако не меняет ее консолидации и длительного сохранения.

რეზიუმე

თავის ტვინის მუსკარინული ქოლინერგული სისტემის ადრეული პოსტნატალური დისფუნქცია და შიშით მოტივირებული დასწავლისა და მეხსიერების დარღვევები

მ. შავგულიძე, მ. ბაბილოძე, ნ. როგავა, ე. ჩიჯავაძე, ნ. ნაჭყებია

ი. ბერიტაშვილის ექსპერიმენტული ბიომედიცინის ცენტრი, თბილისი, საქართველო

კვლევის მიზანს წარმოადგენდა თავის ტვინის მუსკარინული ქოლინერგული სისტემის ადრეული პოსტნატალური დისფუნქციის, როგორც ძირითადი ფაქტორის, კვლევა ზრდასრულ ცხოველებში, იმ ტიპის კოგნიტური დარღვევების განვითარებაში, რომლებიც ახდინიშნება ძირითადი დეპრესიული დაავადების დროს.

შესწავლილია არადეკლარაციული მეხსიერების ორ მნიშვნელოვან ამოცანაში მიღებული ინფორმაციის დასწავლისა და გრძელვადიანი შენარჩუნების პროცესები – აქტიური განრიდების ტესტში, რომელიც თათებზე მიყენებული ელექტროშოკისგან გამოწვეულ შიშზეა დამყარებული და აწეულ ჯვარედინ ლაბირინთში, რომელიც ბუნებრივ შიშს ეფუძნება.

ექსპერიმენტები ჩატარდა თეთრ, ველურ, მამრ ვირთაგვებზე (n=60). ცხოველები დაყოფილი იყო ოთხ ჯგუფად (15 ვირთაგვა თითოეულში), სპეციფიკური დიზაინით. თავის ტვინის მუსკარინული ქოლინერგული სისტემის ადრეული პოსტნატალური დისფუნქცია განხორციელდა პროფ. ნ. ნაჭყებიასა და კოლეგების მიერ შემუშავებული ახალი მეთოდური მიდგომით. შიშით მოტივირებული არადეკლარაციული დასწავლის და მეხსიერების და შფოთვის ემოციური ქცევის კვლევა განხორციელდა ორმხრივი აქტიური განრიდების კამერაში და აწეულ ჯვარედინ ლაბირინთში.

წარმოდგენილ ნაშრომში პირველად არის შესწავლილი თავის ტვინის მუსკარინული ქოლინერგული სისტემის ადრეული პოსტნატალური დისფუნქციის შორეული ეფექტები არადეკლარაციულ დასწავლასა და მეხსიერებაზე და შფოთვის ემოციურ ქცევაზე. პირველად იქნა ნაჩვენები, რომ 1) აღნიშნული პროცედურა ზრდასრული ასაკის ცხოველებში იწვევს არადეკლარაციული ამოცანების დასწავლის საწყის გაუარესებას, რაც გამოწვეულია შფოთვის დონის მატებით, როგორც აქტიური განრიდების, ისე აწეული ჯვარედინი ლაბირინთის ტესტში; 2) თავის ტვინის მუსკარინული ქოლინერგული სისტემის ადრეული პოსტნატალური დისფუნქცია არ ცვლის შეძენილი ინფორმაციის კონსოლიდაციას და გრძელვადიან შენარჩუნებას ზრდასრულ ცხოველებში არადეკლარაციული მეხსიერების ორ მნიშვნელოვან ამოცანაში – ექსპერიმენტულად გამოწვეულ შიშზე დაფუძნებულ აქტიური განრიდების ტესტში და ბუნებრივ შიშზე დაფუძნებულ აწეულ ჯვარედინ ლაბირინთში; 3) სკოპოლამინის ინტრაპერიტონეალური ინიექცია ზრდასრულ ცხოველებში, რომლებიც ადრეული ონტოგენეზის პერიოდში მუსკარინული ქოლინერგული სისტემის დისფუნქციას ექვემდებარებოდნენ, ამცირებს შფოთვის დონეს და ამ გზით ხელს უწყობს ბუნებრივ შიშზე დაფუძნებული არადეკლარაციული ტესტის დასწავლას და მეხსიერებას.

ადრეული ონტოგენეზის პერიოდში თავის ტვინის მუსკარინული ქოლინერგული სისტემის დისფუნქცია ზრდასრულ ცხოველებში იწვევს შფოთვის გაძლიერებას და ართულებს ინფორმაციის შეძენას შიშით მოტივირებული არადეკლარაციული დასწავლისა და მეხსიერების ტესტებში. ზრდასრულ ცხოველებში შეძენილი ინფორმაციის კონსოლიდაცია და გრძელვადიანი შენახვა უცვლელი რჩება.

МОРФОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ ВНУТРЕННИХ ОРГАНОВ КРЫС ПРИ АЛЛОКСАНОВОМ ДИАБЕТЕ В ЭКСПЕРИМЕНТЕ

Абуладзе К.З., Хвичия Н.В., Папава М.Б., Павлиашвили Н.С., Турабелидзе-Робакидзе С.Д., Саникидзе Т.В.

Тбилисский государственный медицинский университет; Тбилисский государственный университет им. И. Джавахишвили, Грузия

Сахарный диабет (СД) - многофакторное заболевание, характеризующееся гипергликемией [14,17], нарушениями липидного обмена, усилением окислительного стресса, вызванным образованием высокоактивных свободных радикалов, усилением перекисного окисления липидов и нарушением активности ферментативной антиоксидантной системы [8]. Недостаточная детоксикация свободных радикалов может привести к окислительному повреждению мембран, морфологическим изменениям в тканях и, соответственно, нарушению функций многих органов [5,11]. При длительном сахарном диабете наблюдаются изменения в различных органах - гломерулярная нефропатия с тубулярными и интерстициальными аномалиями, патологоаатомические изменения в печени, сердце, легких, тканях нервной системе, гистоморфологические изменения в кишечнике, развитие атеросклероза [9], которые в значительной мере обусловлены интенсификацией некомпенсируемого окислительного стресса в условиях гипергликемии и истощения системы антиоксидантной защиты.

Моделирование диабета на животных является полезным инструментом для получения информации об интимных механизмах патогенеза диабета в организме человека. Экспериментальное моделирование диабета возможно такими химическими веществами, которые избирательно разрушают инсулин-продуцирующие β-клетки в поджелудочной железе посредством редокс-зависимых механизмов [16]. Одним из наиболее часто используемых химических веществ является аллоксан. Этот препарат вызывает диабет за счет внутриклеточной генерации активных форм кислорода (АФК), образующихся в циклической реакции с участием аллоксана и его восстановленного продукта, называемого диалюровой кислотой [2], которая подвергается самоокислению с образованием перекиси водорода, супероксид-анионаи гидроксильных свободных радикалов; последние образуются по реакции Габера – Вейсса, катализируемой металлами. Считается, что эти восстановленные формы кислорода, в частности чрезвычайно реактивный гидроксил-радикал, инициируют атаку на бета-клетки с последующим ингибированием синтеза и секреции инсулина. Быстрое поглощение инсулин-секретирующими клетками является одной из важных характеристик, определяющих диабетогенность аллоксана.

Целью нашего исследования является оценка морфологических изменений внутренних органов при моделировании редокс-индуцированного сахарного диабета с использованием аллоксана на крысах.

Материал и методы. Исследования проводились на двадцати пяти крысах-самцов линии Wistar (280-350 г). Уход за животными осуществлялся в соответствии с руководящими принципами лабораторных исследований на животных, используемых в экспериментальных исследованиях. Животные были размещены в хорошо вентилируемом помещении в стандартных лабораторных условиях (цикл темнота/свет 12:12 ч). Им позволяли акклиматизироваться в течение 3 недель, когда они имели свободный доступ к стандартному корму и воде *ad libitium*. Все процедуры с животными были одобрены Комитетом по уходу и использованию животных Тбилисского Государственного Медицинского Университета.

После периода акклиматизации диабет у крыс вызывали однократным внутривенным введением свежеприготовленного 12% водного раствора аллоксана (в дозе 200 мг/кг массы тела) внутрибрюшинно. Контрольные крысы (5 крыс) получали аналогичный объем физиологического раствора. Уровень глюкозы измеряли через 1, 2, 3, 10, 15, 20, 25, 30 день после введения аллоксана. Крысы с уровнем глюкозы в крови выше 250 мг / дл на 2-й день после введения аллоксана включались в исследование.

На исследуемых животных изучали аллоксан-индуцированные морфологические изменения сердца, печени, почек и аорты, и содержание малонового деальдегида (МДА) в крови.

Морфологические исследования. Образцы аорты предварительно промывались водой, затем подвергались дегидратации этанолом и ксилолом. Ткани органов (аорта, сердце, печень, почки) крыс помещали в 10% раствор формалина. После изготовления парафиновых блоков, срезы толщиной до 5 мкм, окрашивались гематоксилином и эозином. Препараты исследовали под световым микроскопом (модель Leica DM 1000 LED), увеличение 10×0,25.

Определение MDA. MDA в плазме крови определяли с помощью анализа тиобарбитуровой кислоты (TBA) [6].

Статистический анализ полученных результатов прово-