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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

EFFECT OF TOLUENE ON THE GABA SYSTEM COMPONENTS IN THE BRAIN STRUCTURES OF RATS AT DIFFERENT STAGES OF DEVELOPMENT

Speciality: 2411.01 – Human and animal physiology

Field of science: Biology

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SUMMARY OF THE DISSERTATION WORK

Relevance and development of the topic. The rapid development of science leads to an increase in the use of various chemicals, especially organic substances. The acquisition and application of new compounds makes it necessary to study the biological effects of these compounds, the methods of their formation and decomposition. Toluene is one of such substances widely used in the chemical industry and in the household[1].

Toluene is a solvent widely used in industry around the world. Toluene is found in high levels in refineries, around petrol stations and in areas with heavy traffic. Toluene-containing products include paints, varnishes, adhesives and some other cleaning products. Thus, many humans and animals are exposed to toluene through water, food, air, and various consumer goods[2].

Toluene enters the body in 3 ways - through inhalation, absorption and skin. Toluene alters the lipid structure of the cell wall and interacts with proteins due to its lipophilic nature[3].

Brain tissue is most vulnerable to the impact of toluene, so it is most damaged[4]. Toluene is not only harmful to respiration, but its chronic use can also cause neurotoxic effects and death. Low levels of toluene effect on the nerves can lead to neurological diseases. There are two types of neurological disorders due to toluene: neurotoxic and neuropathic. Researchers also studied the interactions

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of toluene with neurotransmitters to understand its behavioral, cognitive, and psychiatric effects[5].

Volatiles, including toluene, can cause structural and functional changes by severely damaging the integrity of the central nervous system (CNS) and disrupting the normal course of psychological, emotional, and neurobiological development[6,7]. Studies conducted on rabbits exposed to toluene for 3 hours have shown damage and apoptosis in brain cells [8].

Inhalation of toluene depends on age, pattern of use (duration and frequency)[9,10].

There is a link between neurochemical changes associated with human exposure to toluene [11]. The neurotoxicity of inhalants is associated with their effects on brain neurotransmitters. Single and daily repeated inhalation of toluene significantly alters the levels of brain neurotransmitters [12].

High doses of toluene have also been shown to affect the

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[11] O'Leary-Moore, S.K. Neurochemical Changes after Acute Binge Toluene Inhalation in Adolescent and Adult Rats: A High-Resolution Magnetic Resonance Spectroscopy Study / S.K.O'Leary-Moore, M.P.Galloway, A.P.McMechan [et al.] // Neurotoxicol Teratol., - 2009. Nov-Dec; 31(6), - p. 382-389.doi:10.1016/j.ntt.2009.07.005.

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GABAergic (gamma-aminobutyric acid), glutamate (Glu)ergic, serotoninergic, and dopamine (DA)ergic pathways [13]. In adult animals, after 10 days of exposure to toluene (8000 ppm, 30 min/day), the α1 subunit of the GABA A receptor increases in the striped body[14].

Although the neurobiological effects of toluene and the mechanisms underlying and dependent on behavioral effects have been studied to some extent, regional changes in toluene in neurochemistry have been little studied.

Injection of toluene into the abdomen has been shown to significantly increase the rate of formation of active forms of oxygen (AFO) and decrease glutathione levels in the brain[15].

Melatonin easily penetrates the nucleus and mitochondria and has an antioxidant effect by clearing free radicals[16]. As melatonin is a direct and indirect antioxidant, it has been suggested as a neuroprotector[17]. The neurotoxicity of toluene is minimized by melatonin. Eliminates molecular damage caused by melatonin oxygen products[18,19].

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The development of necessary measures for the correction of pathological processes and disorders in the CNS under the influence of neurotoxicants is of great importance in theoretical and clinical medicine. To this end, it is of great interest to determine whether melatonin has the ability to reverse the changes in GABA exchange in the brain under toluene intoxication.

In view of the above, in our present dissertation it is necessary to study the effect of toluene and melatonin under the condition of toluene intoxication on GABA exchange in different structures of the brain (cortex of the cerebral hemispheres, cerebellum, brainstem and hypothalamus) at different stages of postnatal ontogenesis.

Objectives and goals of the research. The main purpose of our research was to study GABA exchange in tissues and mitochondrial fractions of the brain structures (cortex of the cerebral hemispheres, cerebellum, brainstem and hypothalamus) of rats exposed to toluene at different stages of postnatal ontogenesis and the ability of melatonine to restore impaired GABA exchange under toluene intoxication.

Objectives to achieve the goal:

1. To estimate the amount of GABA, Glu and Asp in the tissues of the cortex of the cerebral hemispheres, cerebellum, brainstem and hypothalamus of rats exposed to toluene at the doses of 200, 500 and 1000 mg/kg at different stages of postnatal ontogenesis (1, 3 and 6 months) research of quantity;

2. To study the chronic effect of toluene at doses of 200, 500 and 1000 mg/kg on the activity of glutamic acid decarboxylase (GAD) and GABA transaminase (GABA-T) enzymes in the tissues of different structures of the brain of rats at different stages of postnatal ontogenesis (1-, 3- and 6 months);

3. To study the changes in GABA exchange in mitochondrial fractions of various brain structures of 3-month-old rats exposed to chronic effect of toluene at doses of 200, 500 and 1000 mg/kg;

4. To study the effect of toluene at a dose of 25 mg/kg on the

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amount of GABA, Glu and Asp in the tissue and mitochondrial fraction of brain structures of 3-month-old rats, also GAD and GABA-T enzyme activity for 1, 2 and 3 months 5 days a week.

5. To study whether melatonin can affect the GABA exchange of the brain structures in the conditions of toluene intoxication.

Research methods. All experimental animals were fed with the general diet of the vivarium and kept under constant control. All experiments were carried out in accordance with the principles of protection of animals used for experimental and other scientific purposes in the International Declaration of the European Union. Toluene intoxication model was created on the basis of the R.S. Dyer method. The brain is divided into structures. Amino acids were determined by electrophoresis method. In order to determine the activity of the GAD, I.A.Sytinski, T.N.Priyatkina method was used. GABA-T activity was determined by N.S.Nilova method. Mitochondrial fractions were isolated from the tissue of the brain structures using the Somoquiet al. methods. The accuracy of the dynamics of change of all determined indicators was investigated by the method of calculation of variational statistics.

The main points of the dissertation work:

1. After chronic (for 5 days) exposure to toluene at doses of 200, 500 and 1000 mg/kg, in the brain structures of 1, 3 and 6-month-old rats the amount of GABA and the activity of GAD were higher, while the amount of Glu and Asp and the activity of GABA-T were lower compared to the control. After exposure to high doses of toluene, the change in GABA exchange in the brain structures of these animals was higher compared to lower doses.

2. After 5 days of exposure to toluene at doses of 200, 500 and 1000 mg/kg, the change in GABA exchange in the brain structures of 1-month-old rats was significantly higher compared with 3- and 6-month-old rats. After exposure to toluene at a dose of 25 mg/kg for 5 days a week during the period of 1, 2 and 3 months, the amount of GABA system components, Glu and Asp in the brain structures of 3-month-old rats changed depending on the duration of exposure to this ecotoxicant.

3. After the effect of different doses of toluene at different

stages of postnatal ontogenesis, an increase in the amount of GABA, a decrease in the amount of Glu and Asp, an increase in GAD activity, a decrease in GABA-T activity were more noticeable compared with other structures studied.

4. Melatonin can partially restore impaired GABA exchange in brain structures under conditions of toluene intoxication. The recovery process due to the action of melatonin in the GABA exchange components, which are more susceptible to toluene intoxication, is relatively weak.

Scientific novelty of research work. For the first time, the levels of the components involved in GABA exchange (the amount of GABA, the activity of GAD and GABA-T enzymes involved in its synthesis and breakdown, the amount of free Glu and Asp) were studied in the tissue of brain structures (cortex of the cerebral hemispheres, cerebellum, brain stem, and the hypothalamus) of the rats exposed to chronic effect (5 days) of different doses of toluene (200, 500, and 1000 mg/kg) at different stages of postnatal ontogenesis (1, 3, and 6 months), also in the mitochondria of the brain structures of 3-month-old rats exposed to toluene at a dose of 200, 500 and 1000 mg/kg for 5 days and to the chronic effect of toluene at a dose of 25 mg/kg (for 1, 2 and 3 months, 5 days a week). Also, for the first time in the study, the effect of melatonin on the amount of GABA, Glu and Asp, as well as on the activity of the GAD and GABA-T enzymes in the conditions of toluene intoxication was studied.

Based on the results of the study, it was found that after chronic (5 days) exposure to toluene at doses of 200, 500 and 1000 mg/kg in the tissues of the studied brain structures of 1, 3 and 6-month-old rats, the amount of GABA was higher, the amount of Glu and Asp was lower, the activity of GAD has increased significantly, while the activity of GABA-T has decreased compared to the control. After chronic (5 days) exposure to toluene at doses of 200, 500 and 1000 mg/kg, the amount of GABA in the mitochondrial fractions of the studied brain structures of 3-month-old rats, the activity of GAD was significantly higher, the amount of Glu, Asp and the activity of GABA-T- was significantly lower.

Changes in the amount of GABA, Glu, Asp, and the activity of GAD and GABA-T enzymes in the tissue and mitochondrial fractions of brain structures after exposure to toluene were dose-dependent. High doses of toluene (1000 mg/kg) resulted in more substantial changes in GABA exchange compared to lower doses (500 and 200 mg/kg).

It was found that toluene caused higher changes in the components of GABA exchange in the brain structures of 1-month-old rats than in 3- and 6-month-old animals.

After exposure to toluene at a dose of 25 mg/kg for 1, 2 and 3 months 5 days a week, the amount of GABA increased, the amount of Glu and Asp decreased, the activity of GAD increased and the activity of GABA-T decreased in tissue and mitochondrial fractions of brain structures of 3-month-old rats and such changes were proportional to the duration of exposure.

After exposure to melatonin, changes in the GABA exchange components in the brain structures of 1-, 3-, and 6-month-old rats are partially reversible due to intraperitoneal injection of toluene. Compared to 3- and 6-month-old rats, 1-month-old rats have a relatively slow recovery process in impaired GABA exchange after exposure to toluene. Also, compared to other structures studied, recovery from changes in GABA exchange in the brain is low.

Theoretical and practical significance of the research. Toluene has been shown to cause changes in the levels of all components involved in GABA exchange. Based on these changes, changes occur in the CNS in excitatory and inhibitory mediators. These changes can lead to imbalances between the mediators studied and, as a result, to diseases associated with impaired GABA exchange. Research shows that toluene has a negative impact on human health, as well as the need to raise awareness about the abuse of inhalants and the need for new treatments to eliminate the harmful effects of this ecotoxicant. For this reason, maximum allowable concentration (MAC) must always be taken into account in case of exposure to toluene. It is also unacceptable for children and adolescents to work in industries where they may be exposed to toluene. The study has highlighted the ability of melatonin to restore

impaired GABA exchange under toluene intoxication conditions and the importance of melatonin in the development of new treatments under appropriate conditions.

Approbation and application. Dissertation materials were discussed at the following scientific meetings: Fundamental and applied problems of neurosciences: functional asymmetry, neuroplasticity, neurodegeneration (Moscow, 2018), Modern problems of biology (Sumgayit, October 23-24, 2018), VI Congress of Biophysicists of Russia (16-21.09.2019, Sochi), XV International Interdisciplinary Congress "Neuroscience for Medicine and Psychology" (Sudak, Crimea, Russia, May 30 - June 10, 2019), Actual problems of modern biology (Baku, February 5, 2019), In scientific seminars of the Department of "Physiology" of ASPU (Baku, 2018, 2019), XVI International Interdisciplinary Congress "Neuroscience for Medicine and Psychology» (Sudak, Crimea, Russia, October 6-16, 2020).

Name of the organization where the dissertation work was carried out: The dissertation work was carried out at the Azerbaijan State Pedagogical University of the Ministry of Education of the Republic of Azerbaijan.

Published works. 10 scientific articles and 9 theses on the topic of the dissertation were published.

The total number of characters of the dissertation material by indicating the number of characters of each structural unit. The dissertation is written on a computer in 174 pages (237816 characters). It includes the “Chapters of contents” (4386 characters), “Introduction” (15384 characters), “Summary of literature” (60354 characters), “Materials and methods” (11544 characters), “Experimental part” (96224 characters), “Discussion” (62077 characters), “Conclusion” (2154 characters), “Practical recommendations” (390 characters), “List of literature” (45325 characters) and the “List of abbreviations” (998 characters). The dissertation is illustrated with 13 tables and 11 graphs (16089 characters). The bibliography includes 236 sources, 19 in Russian and 217 in English.

MATERIALS AND METHODS OF RESEARCH

264 rats aged 1, 3 and 6 months were used in the experiments. The animals were kept in a room with a constant temperature of 20-25°C. All experimental animals were fed the general diet of the vivarium and kept under constant control[20].

All experiments were carried out in accordance with the principles of protection of animal used for experimental and other scientific purposes in the International Declaration of the European Union[21].

The animals used in the experiments were divided into the following groups: control and experiment. The control animals were divided into 3 subgroups aged 1 month, 3 months and 6 months.

The experimental group consisted of animals exposed to toluene and melatonin under toluene intoxication. To create a model of the effect of toluene on animals, its different doses were injected into the abdomen of animals[22] . Doses of 25 mg/kg, 200 mg/kg, 500 mg/kg and 1000 mg/kg of toluene were used. In experiments, a dose of 10 mg/kg of melatonin was injected into the abdomen of animals under toluene intoxication.

The experimental group was divided into subgroups according to the dose of toluene used: subgroup I - 3-month-old rats exposed to toluene at a dose of 200 mg/kg for 5 days, subgroup II - 3-month-old rats exposed to toluene at a dose of 500 mg/kg for 5 days, subgroup III - 3-month-old rats exposed to toluene at a dose of 1000 mg/kg for 5 days, IV subgroup - 3-month-old rats exposed to toluene at a dose of 25 mg/kg for 5 days a week during 1, 2 and 3 months, subgroup V – animals exposed to melatonin (10 mg/kg) under toluene

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(1000 mg/kg) intoxication. Subgroups I, II, III, and V were divided into subgroups of 1-month, 3-month, and 6-month-old rats each, and subgroup IV was divided into subgroups of 1-month, 2-month, and 3-month rats exposed to toluene 5 days per week.

The brain was divided into structures[23]. In both experimental and control groups of rats, in different structures of the brain (in the cerebral cortex, cerebellum, brain stem and hypothalamus), the components of GABA exchange (the amount of GABA, free Glu and Asp, and the activity of GAD (EC 4.1.1.15) and GABA-T enzymes (EC 2.6.1.19) was studied in tissue homogenate and mitochondrial fraction. After the amount of GABA, Glu and Asp was divided into fractions by high-voltage electrophoresis method[24], the staining intensity painted on paper by chromatography method[25] was assessed by photoelectrocolorimeter (FEK) -56M device. I.A.Sytinsky, T.N.Priyatkina method was used to determine the activity of GAD[26]. The activity of GABA-T was determined using the method of N.S.Nilova[27].

The most adequate and accepted Somogyiet al. method was used to separate mitochondrial fractions from the tissues of the studied structures of the brain[28].

The obtained indicators were processed by the parametric method [29 , 30] . The reliability coefficient of the indicators was

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[27]Нилова, Н.С. Аммиак и ГАМК-трансаминазная активность ткани головного мозга // Докл. АНСССР, - 1966, т.2, - с.483-486.

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determined according to the Fisher Student table.

RESEARCH RESULTS AND DISCUSSION

1. The effect of toluene on the amount of GABA, Glu and Asp in the tissue homogene of brain structures of rats at different stages of postnatal ontogeny

The results of experiments showed that after 5 days of intra-abdominal administration of toluene at doses of 200mg/kg, 500 mg/kg and 1000 mg/kg for 1-day-oldrats, the amount of GABA in the tissue of all studied structures (cortex of the cerebral hemispheres, cerebellum, brainstem and hypothalamus) was 26-39%, 32-51% and 41-71% higher compared to the control (table). Here, the amount of Glu was 16-24%, 20-30% and 26-40% lowerthan the control. Under appropriate conditions, 14-27%, 20-32% and 28-41% decrease was observed in the amount of Asp.

After 5 days of intra-abdominal administration of toluene at doses of 200 mg/kg and 500 mg/kg for 3-months-old rats, the amount of GABA increased in the tissue of cortex of the cerebral hemispheres, cerebellum, brain stem and hypothalamus by 15-26% and 21%-36%, respectively, the amount of Glu decreased by 7-17% and 14-24% and the amount of Asp decreased by 8-19% and 10-25% compared to the control. After 5 days of intra-abdominal administration of toluene at dose of 1000 mg/kg for 3-months-old rats, the amount of GABA increased by 29-53% (table), the amount of Glu decreased by 20-33% and the amount of Asp decreased by 18-37%.

After 5 days of intra-abdominal administration of toluene at a dose of 200 mg/kg, the amount of GABA in the tissue of the brain structures of 6-month-old rats increased by 10-20%. Under appropriate conditions, the amount of Glu decreased by 5-9% and the amount of Asp decreased by 4-12% compared to the control. Changes in the amount of Glu and Asp under appropriate conditions were valid only in the cerebellum ($p<0.05$).

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Table

Changes in the amount of GABA (mkmol/g) in the tissues of various brain structures of 1- and 3-month-old rats as a result of daily intra-abdominal administration of melatonin (10 mg/kg) after chronic (5 days) exposure to toluene (1000 mg/kg)(M±m, n=5).

Brain structure	Condition of experiment	Value	GABA (mkmol/g)	
			1 month	3 months
Cortex of the cerebral hemispheres	Control	M±m	2,48±0,08	2,97±0,12
	Toluene	M±m	3,87±0,11***	4,14±0,19***
		%	156	139
	Toluene+ melatonin	M±m	2,80±0,07*	3,18±0,15
		%	113	107
Cerebellum	Control	M±m	2,15±0,07	2,43±0,08
	Toluene	M±m	3,68±0,15***	3,73±0,11***
		%	171	153
	Toluene+ melatonin	M±m	2,69±0,09**	2,82±0,09*
		%	125	116
Brain stem	Control	M±m	1,81±0,05	2,19±0,08
	Toluene	M±m	2,79±0,11***	3,18±0,10***
		%	154	145
	Toluene+ melatonin	M±m	2,14±0,06**	2,45±0,07*
		%	118	112
Hypothalamus	Control	M±m	2,91±0,11	3,72±0,14
	Toluene	M±m	4,10±0,14***	4,81±0,17**
		%	141	129
	Toluene+ melatonin	M±m	3,35±0,10*	3,98±0,12
		%	115	107

* - p<0,05; ** - p<0,01; *** - p<0,001.

After administration of toluene at doses of 500 mg/kg and 1000 mg/kg for 5 days in the abdomen of 6-month-old rats, the amount of GABA in the tissue of brain structures increased by 16-29% and 24-42%. There was a decrease in the amount of Glu by 8-20% and 14-29%. The decrease in the amount of Asp was 9-18% and 15-25%

After exposure to high doses of toluene in the tissues of the brain structures of animals in all three age groups, the increase in the amount of GABA was higher than the lower dose, and the amount of

Glu and Asp decreased.

Comparing the results according to the stages of postnatal ontogenesis, it was found that after exposure to all three doses of toluene, the amount of GABA in the tissue of all studied brain structures increased significantly in 1-month-old rats compared to 3-month-old rats and 6-month-old rats. Also, GABA amount increased in 3-month-old rats compared to 6-month-old rats. Decreases in the amount of Glu and Asp were lower in 6-month-old rats compared to 1-month-old and 3-month-old rats. This indicator was also lower in3-month-old rats compared to 1-month-old rats.

After the intra-abdominal administration of toluene at a dose of 25 mg/kg for the period of 1, 2 and 3 months, 5 days a week, the change in the amount of GABA in the brain tissue of 3-month-old rats was 3-10%, 8-17% and 16-28% higher than the control group. The amount of Glu was 5-8%, 7-13% and 12-20% lower than the control in all structures studied under appropriate conditions. The decrease in the amount of Asp was 2-8%, 7-10% and 11-18%.

These results suggest that changes in the amount of GABA, Glu and Asp in the tissue of brain structures after long-term (for 1, 2 and 3 months) intra-abdominal administration of toluene at a dose of 25 mg/kg are time-dependent. Changes in the levels of these amino acids in the studied structures of 3-month-old rats after the intra-abdominal administration of toluene for 1 month period (5 days per week) at a dose of 25 mg/kg are not valid. However, there was an increase in the amount of GABA and a decrease in the amount of Glu and Asp. Although there were changes in the levels of GABA, Glu, and Asp after intra-abdominal administration of toluene at a dose of 25 mg/kg 5 days a week for the period of 2 months, these changes were not valid in the tissues of most of the studied structures (except cerebellum). Significant changes occurred in the amount of amino acids in the tissue of the studied structures of the brain after intra-abdominal injection of toluene at a dose of 25 mg/kg 5 days a week for 3 months.

2. Changes in the activity of GAD and GABA-T enzymes in the tissue homogene of brain structures of rats under the influence of

toluene at different stages of postnatal ontogeny

The activity of GAD enzyme increased by 25-46%, 36-55%, and 48-78% and the activity of GABA-T enzymes decreased by 19-29%, 23-36% and 29-47%, in the brain structures (cortex of the cerebral hemispheres, cerebellum, brainstem and hypothalamus) tissue of 1-month-old rats after 5 days of intra-abdominal administration of toluene at doses of 200 mg/kg, 500 mg/kg and 1000 mg/kg.

As a result of exposure to toluene at doses of 200 mg/kg and 500 mg/kg for 5 days, the activity of GAD enzyme in the tissues ofthe brain structures of 3-month-old rats increased by 11-25% and 16-35%, respectively, and the activity of GABA-T decreased by 10-17% and 14-23%. As a result of exposure to toluene at dose of 1000 mg/kg for 5 days, the activity of GAD enzyme in the tissues of the brain structures of 3-month-old rats increased by 34-58%, respectively, and the activity of GABA-T decreased by 23-37%.

After 5 days of intra-abdominal administration of toluene at a dose of 200 mg/kg, the activity of the enzyme GAD in 6-month-old rats increased by 6-18% in the tissue of brain structures. Under appropriate conditions, the changes in the activity of the GAD in the cerebellum and brain stem were valid, while the changes in the cerebral cortex and hypothalamus were not valid. The activity of GABA-T decreased by 6-12%. Under appropriate conditions, the changes in the activity of GABA-T in the other 3 structures studied, except for the cerebellum, were not valid. After 5 days of intra-abdominal administration of toluene at doses of 500 mg/kg and 1000 mg/kg, the activity of GAD enzyme in brain tissue of the 6-month-old rats increased by 10-24% and 21-42%, while the activity of GABA-T decreased by 9-19% and 15-29%.

After exposure to high doses of toluene in the tissues of the brain structures of animals in all three age groups, the activity of GAD was higher compared to its lower dose, and the activity of GABA-T was further reduced. There were few changes in the activity of GAD and GABA-T in 6-month-old rats compared to 1 and 3-month-old rats. The changes in 3-month-old rats were insignificant compared to 1-month-old rats.

After the intra-abdominal administration of toluene at a dose of 25 mg/kg 5 days a week for 1 month, the activity of GAD enzyme increased by 7-14% compared with the control. The activity of GABA-T was slightly lower (3-8%). After the intra-abdominal administration of toluene at a dose of 25 mg/kg 5 days a week for 2 and 3 months, there was an increase in the activity of the enzyme GAD in the tissues of brain structures by 9-20% and 15-29%. The activity of GABA-T decreased by 6-11% and 11-20%. These results showed that changes in the activity of GAD and GABA-T enzymes under appropriate conditions are time-dependent. Based on the results of the experiments, it can be said that intra-abdominal administration of toluene at a dose of 25 mg/kg 5 days a week for 1 month did not cause valid changes in the activity of GAD and GABA-T enzymes in the tissues of the studied structures. Within 2 months, these changes increased relatively, leading to valid changes in some structures (cerebellum and brain stem). As a result of the longer-term effect of toluene (for 3 months), there were significant changes in the activity of the enzymes GAD and GABA-T in the tissues of all the studied structures.

3. Changes in the amount of GABA, Glu and Asp in mitochondrial fractions of brain structures of 3-month-old rats as a result of exposure to toluene

After the intra-abdominal administration of toluene at doses of 200 mg/kg, 500 mg/kg and 1000 mg/kg for 5 days, the amount of GABA increased by 18-32%, 26-45% and 36-60%, respectively, compared with the control in the mitochondrial fraction of brain structures (cortex of the cerebral hemispheres, cerebellum, brainstem and hypothalamus) of 3-month-old rats. Glu content decreased by 9-22%, 18-29% and 25-40%. There was a decrease of 11-21%, 15-31%, 24-43% in the amount of Asp.

Changes in the amount of GABA, Glu and Asp under appropriate conditions depended on the effective dose of toluene. The results showed that there were reliable changes in the amount of these amino acids in all three doses of toluene. In all cases, the amount of GABA increased, while the amount of Glu and Asp

decreased. However, the effects of toluene at higher doses have led to more substantial changes in the amount of these amino acids.

After the intra-abdominal administration of toluene at a dose of 25 mg/kg 5 days a week for 1, 2 and 3 months the amount of GABA increased by 7-15%, 10-20% and 19-34%, respectively, compared to the control in the mitochondrial fraction of the studied brain structures of 3-month-old rats. After the intra-abdominal administration of toluene at a dose of 25 mg/kg 5 days a week for 1, 2 and 3 months the amount of Glu decreased by 4-9%, 8-18% and 15-25% and the amount of Asp decreased by 3-10%, 6-14%, 16-22% compared to the control in the mitochondrial fraction of the studied brain structures of 3-month-old rats. As can be seen, the changes in the amount of the amino acids depended on the duration of toluene effect.

4. Changes in the activity of GAD and GABA-T enzymes in mitochondrial fractions of brain structures of 3-month-old rats as a result of exposure to toluene

After 5 days of intra-abdominal administration of toluene at doses of 200 mg/kg and 500 mg/kg, the activity of the enzyme GAD increased by 16-28% and 26-41%, respectively, and the activity of GABA-T decreased by 12-21% and 18-30% in the mitochondrial fraction of brain structures.After 5 days of intra-abdominal administration of toluene at dose of 1000 mg/kg, the activity of the enzyme GAD increased by 39-59% and the activity of GABA-T decreased by 27-40% in the mitochondrial fraction of brain structures.

The results show that there were more fundamental changes in the activity GAD and GABA-T enzymes in the mitochondrial fraction of the studied structures of the brain after exposure to toluene at a dose of 1000 mg/kg compared to the dose of 500 mg/kg and 200 mg/kg and after exposure to toluene at a dose of 500 mg/kg compared to the dose of 200 mg/kg.

After the intra-abdominal administration of toluene at a dose of 25 mg/kg 5 days a week for 1, 2 and 3 months, the activity of the enzyme GAD increased by 6-17%, 9-26% and 18-30% in the

mitochondrial fraction of brain structures, respectively, and the activity of GABA-T decreased by 6-11%, 9-12% and 14-22% compared to the control. The results of these experiments show that the activity of GAD and GABA-T enzymes in the mitochondrial fraction of brain structures of 3-month-old rats was changed depending on the time of exposure to toluene. The intra-abdominal administration of toluene at a dose of 25 mg/kg 5 days a week for 3 months resulted in more substantial changes in the activity of GAD and GABA-T enzymes in mitochondrial fractions of the studied brain structures compared to 1 and 2 months, and after the exposure of 2 months compared to 1 month.

5. Effect of melatonin on the amount of GABA, Glu and Asp in the brain structures of rats under the condition of toluene intoxication at different stages of postnatal ontogenesis

After exposure to melatonin under the condition of toluene intoxication, 1-month-old rats were found to have a 13% increase in the amount of GABA in the cerebral cortex, 25% in the cerebellum, 18% in the brain stem, and 15% in the hypothalamus compared to the control. Under appropriate conditions, there was 7-16% increase in the amount of GABA in the tissue of the brain structures of 3-month-old rats compared to the control (Table).

As a result of intra-abdominal administration of melatonin under the condition of toluene intoxication, the amount of Glu decreased by 11% in the tissue of cerebral cortex, 19% in the cerebellum, 15% in the brain stem, and 10% in the hypothalamus in 1-month-old rats compared to the control group. Under appropriate conditions, the amount of Glu in the tissue of all structures we studied in 3-month-old rats was 5-11% lower than in the control. At the same time, the level of Asp in different brain structures of 1- and 3-month-old rats decreased by 9-22% and 5-13%, respectively, compared with the control.

After the exposure to melatonin under the condition of toluene intoxication, the amount of GABA in the tissue of the brain structures of 6-month-old rats was slightly higher (2-11%) than in the control group. Under appropriate conditions, a 3-8% decrease in Glu

and a 2-8% decrease in Asp were established.

After the exposure to melatonin under the condition of toluene intoxication, the amount of GABA in the tissues of the studied structures of 1, 3, and 6-month-old rats was significantly reduced compared to the conditions of toluene intoxication and approached the control group. The level of recovery that occurred depended on the age of the animals. In 6-month-old rats the level of recovery of GABA was higher than 1- and 3-month-old rats. In 6-month-old animals, the change in the amount of GABA is not valid in the structures other than the cerebellum compared with the control. The recovery of this amino acid also depends on the structure under study.

After melatonin was injected into the abdomen under the condition of toluene intoxication, due to the effects of toluene it was possible to recover the changes in the amount of Glu and Asp in the brain tissue. After exposure to toluene, melatonin restored higher levels of Glu and Asp in 6-month-old rats. Compared to the other structure studied, both amino acids were restored in the cerebellum at relatively low levels.

The results show a decrease in the levels of GABA, Glu and Asp in the brain structures of rats as a result of melatonin injection under the condition of toluene intoxication. It should be noted that the recovery in the amount of GABA, Glu and Asp in the age groups and brain structures of animals, which are more variable after exposure to this toxicant, are relatively weak after the introduction of melatonin.

6. The effect of melatonin on the activity of enzymes GAD and GABA-T in the brain structures of rats in the conditions of toluene intoxication at different stages of postnatal ontogenesis

In the brain tissue of 1- and 3-month-old rats after exposure to melatonin under the condition of toluene intoxication, the activity of GAD was 14-27% and 9-15%, respectively, higher, and the activity of the enzyme GABA-T was 10-17% and 5-11% lower compared to the control. Under appropriate conditions, in the tissue of the brain structures of 6-month-old rats, there was a slight increase in the

activity of GAD (5-10%), and a 5-9% decrease in the activity of GABA-T compared to the control.

The results show that the effect of melatonin in the conditions of toluene intoxication partially restores the activity of the enzymes GAD and GABA-T in the brain structures of rats. As a result of more serious changes in the activity of these enzymes in the brain under the conditions of toluene intoxication compared to other structures, the recovery process is relatively low even after the action of melatonin. Also, high levels of changes in 1-month-old rats compared to 3- and 6-month-old rats respond to melatonin recoveryat a lower level.

The leading factor in the neurotoxic effect of toluene on the CNS is its effect on the neurotransmitter and receptor system of the brain [31] . Toluene exhibits neurotoxic effects due to increased cholinergic activity associated with GABA receptors and has a non-competitive antagonistic effect against N-methyl-D-aspartate (NMDA) receptors[32].

High doses of toluene affect the GABAergic, Gluergic, serotonergic and DAergicpathways[33]. In addition to affecting the activity of the GABA-A receptor, toluene also blocks currents transmitted by Gluergic NMDA receptors. After exposure to toluene, selective changes occur in the subunit and brain area in GABA receptors during brain growth. Changes in GABA receptors during the use of toluene may result in neurocirculatory disorders[34].

GABA receptors ligand Cl-channels and mediate rapid

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inhibitory transmission in mammalian CNS[35]. GABA-A receptors are involved in controlling the plasticity and circulation of the developing brain. In rats exposed to toluene, α1 and α2 GABA-A receptor RNA levels were significantly increased in the cortex, striped body, and cerebellum[34].

Toluene can cause adaptation to low-level regulation of synaptic GABA-A receptor, while enhancing the effects of extrasynaptic receptors[36]. After exposure to toluene, the level of α5 subunit in the hippocampus rises, which may contribute to an increase in extrasynaptic activation in response to GABA[34]".

In our study, changes in GABA exchange in the brain after exposure to toluene were at a higher level in the age-related developing brain. The developing brain is characterized by greater sensitivity to neurotoxins, including toluene, than in adult animals[31]. Rats are more susceptible to toluene intoxication in the first month of postnatal ontogenesis[37].

After 15 minutes of repeated exposure of toluene at concentrations of 8000 and 12000 ppm, the levels of GABA and Glu changed depending on the studied brain structure[13]. Neurochemical changes after exposure to toluene depend on concentration, brain structure, and age[11].

The change in the amount of GABA due to the effect of toluene was associated with presynaptic effects of toluene to increase the release of toluene from nerve terminals and independent

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changes in inositol 3-phosphate in intracellular calcium stores[38].

GABAergic reactions develop over time during exposure to toluene. This is supported by gene expression researchers who show that gene expression is more inclusive for signal pathways involving GABA receptors 18 hours after exposure to toluene compared to 6 hours after exposure[39].

Toluene facilitates neuronal inhibition through GABA-A receptor[40]. It can directly (through repeated collateral inhibition) and indirectly (through inhibition of excitatory afferents) change the level of GABA at the phosphorylation levels of glutamic acid decarboxylase (GAD2) by protein kinase Cε[41]. Finally, either Glu or GABA requires energy, and a deficiency in toluene-induced energy homeostasis leads to disruption of the cycle and neurochemical levels[42].

By altering and inhibiting the NMDA receptors, possibly subunit components, toluene[43] causes oxidative stress in several brain sections[15]. Decreased Ca^{2+} signals due to the effect of toluene associated with NMDA receptors can impair many aspects of CNS function, thereby leading to CNS damage.

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Toluene causes tissue damage by increasing oxygen radicals[44]. As a powerful cleanser of free radicals, melatonin reduces oxidation-based neurotoxicity[45]. The brains of animals exposed to toluene vapors and treated with melatonin reduce free radical production, lipid peroxidation and gliosis[46]. Melatonin has a neuroprotective or neuro-rescue effect by neutralizing the overproduction of free radicals or stimulating gene expression of antioxidant enzymes[47]. Melatonin probably protects the nervous system from toluene toxicity through antioxidant mechanisms[48]. Melatonin affects both the activity of antioxidant enzymes and the level of cell mRNA for these enzymes[45].

Melatonin, a powerful endogenous antioxidant, protects neurons from oxidative stress in many disease models. A possible mechanism can be attributed to its ability to reduce oxidative stress and maintain a balance between GABAergic and Gluergic transmission.

Melatonin increases the synthesis of GABA[49]. Melatonin may interact with other subtypes of benzodiazepine receptors to influence

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neurosteroidogenesis and cyclic adenosine monophosphate production, which can modulate GABAergic activity in the CNS[50]. GABA can protect the brain from oxidative stress[51]. Central effects of melatonin include modulation of GABAergic function[52].

The increase in the amount of GABA due to the effect of toluene protects brain cells from destruction by increasing the delay processes in the brain. Continuous exposure to high concentrations of toluene indicates that the recovery of impaired GABA exchange process is difficult and even irreversible and the organism exposed to the effect of this ecotoxicantis susceptible to diseases related to impaired GABA exchange.

As a result of intra-abdominal injection of melatonin after exposure to toluene, changes in the levels of all components involved in GABA exchange were significantly reduced, became closer to control and restored either in full or part. The results show that the recovery processes that occur under conditions of toluene intoxication due to the action of melatonin depend on the stage of development of postnatal ontogenesis and the brain structure under study. In case the impairment of GABA exchange is at a higher level under the condition of toluene intoxication, its recovery process is much slower.

Due to the antioxidant and neuroprotective properties of melatonin, melatonin reduces the neurotoxicity of toluene and ensures partial recovery of impaired GABA exchange in the CNS.

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CONCLUSION

1. Chronic effects of toluene at different stages of postnatal ontogenesis have led to an increase in the amount of GABA, a decrease in the amount of Glu and Asp, an increase in the activity of GDC enzyme, and a decrease in the activity of GABA-T enzyme in the tissues and mitochondria of various structures of the brain of rats compared to the control. These results suggest that the chronic effects of toluene have resulted in significant changes in GABA exchange in the brain.

2. The changes in the amount of GABA, Glu, Asp, GAD and GABA-T in the brain structures of rats at different stages of postnatal ontogenesis as a result of chronic exposure to toluene are directly proportional to the dose-dependent effect of this ecotoxicant. The chronic effects of toluene at a dose of 1000 mg/kg caused more significant changes in GABA exchange compared to its effects at doses of 500 and 200 mg/kg.

3. As the chronic effect of toluene is prolonged, there was an increase in the amount of GABA, an increase in the activity of GAD, a decrease in the amount of Glu and Asp, and a decrease in the activity of GABA-T compared to the control. Changes in GABA exchange due to the effect of toluene at a dose of 25 mg/kg for 3 months were higher than the effects of 1 and 2 months.

4. As a result of the chronic effects of toluene, the levels of all components involved in GABA exchange have changed depending on the age of the animals. In the later period of postnatal ontogenesis, the changes in the structure of the brain in different structures of the brain compared to the previous periods were slightly lower than in the control. Due to the chronic effects of toluene, the changes in the indicators of GABA, Glu, Asp, GAD and GABA-T in the cerebellum are more pronounced than in the other structures studied.

5. Melatonin partially restores GABA exchange in the brain of rats under the conditions of toluene intoxication. The level of recovery in the exchange of GABA under the conditions of toluene intoxication of melatonin is inversely proportional to the level of

impairment due to toluene.

6. Based on the evidences of the research, we can conclude, that due to the effect of toluene the increase in the amount of GABA, which is an inhibitory mediator, and the decrease in the amount of Glu and Asp, which are excitatory mediators, lead to the predominance of inhibitory processes over excitatory processes and neuron delay in the CNS.

PRACTICAL RECOMMENDATIONS

1. In the event of toluene intoxication, GABA medicines may be prescribed after exposure to toluene, due to the high content of GABA in the brain and low levels of Glu and Asp.

2. Melatonin can be used to eliminate intoxication under appropriate conditions due to its property to partially restore GABA metabolism in brain structures and its antioxidant properties in case of toluene intoxication.

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LIST OF ABBREVIATIONS

AFO - active form of oxygen
Asp – aspartate (asparagine acid)
CNS – central nervous system
GABA – gamma-aminobutyric acid
GABA-T – GABA-aminotransferase (EC 2.6.1.19)
GAD – glutamate acid decarboxylase (EC 4.1.1.15)
Glu – glutamate (glutamine acid)
MAC –maximum allowable concentration
NMDA - N-metyl-D-aspartate

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