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CORRECTION OF DISTURBANCES OF FUNCTIONAL ACTIVITY OF THE CENTRAL NERVOUS SYSTEM IN RATS WITH THE POST-TRAUMATIC STRESS DISORDER MODEL USING REMEDY WITH A HIGH MAGNESIUM CONTENT

[1]Gushcha, S. G.; [1]Oleshko, A. Ya.; [1]Bakholdina, E. I.; [2]Badiuk, N. S.*; [1]Zabolotna I. B.
[1]State Institution "Ukrainian Research Institute of Medical Rehabilitation Therapy of Ministry of Health of Ukraine", Odessa, Ukraine
[2]International European University, Kyiv, Ukraine

*badiuk_ns@ukr.net

Abstract

The problem of post-traumatic stress disorder (PTSD) is becoming increasingly important, which requires research using models of PTSD in animals, which cause pathogenetically sound development of methods and means of correction of PTSD in humans. Purpose: to establish the effectiveness of the use of magnesium-containing agent in the search for a model of PTSD.

Methods: Wistar rats on the background of the development of PTSD models receive intragastric 5% aqueous solution of balneological product with magnesium content - 0.95 g / l The functional state of the central nervous system (CNS) and emotional activity of rats, biochemical and immunological marketing pathological processes were evaluated.

Results. Rats have been shown to improve their psycho-emotional state - signs of fear and inhibition disappear. The detoxifying and biliary function of the liver undergoes positive changes. The activity of reamination enzymes (ALT, AST) is restored, the content of total protein and the balance of protein fractions are normalized. The indicators of the leukocyte formula are restored, the content of circulating immune complexes of heterophilic antibodies is normalized. In general, the signs of systemic disorders that occur during the reproduction of the PTSD model disappear.

Conclusions. The effectiveness of using a tool with a high magnesium content to adjust the development of the PTSD model has been experimentally shown.

Key words: *post-traumatic stress disorder, psychophysiological disorders, functional activity of the central nervous system, metabolism, magnesium*

Introduction

Existing methods, means of correction and treatment of PTSD do not always meet the expected results, which requires the development of new pathogenetically sound approaches to treatment [1, 2, 3]. But this requires a deeper understanding of the pathogenesis of PTSD, which will serve as an effective basis for the development of drugs and medicines based on products of natural origin. In this aspect, animal models allow us to study the processes of pathogenesis of such a serious condition as PTSD, and to obtain the results of the treatment quite quickly [4, 5, 6].

Mammals demonstrate biologically preserved behavioral and neurobiological responses to valence stimuli that underlie PTSD models in rodents [7, 8].

Since one of the most important macronutrients in the body is magnesium, and many pathological conditions, including chronic distress are accompanied by its loss, we considered it possible to investigate the use of compounds with high magnesium to correct PTSD in the experiment [9, 10, 11, 12, 13].

The aim of the work is to establish the effectiveness of magnesium-containing agent in rats with the PTSD model.

Methods

Experimental studies were performed on 40 clinically healthy male rats (Wistar line), aged 6-8 months, weighing 180 - 210 in compliance with the requirements of humane treatment of experimental animals, regulated by the relevant documents [14, 15, 16]. During the experiment, the animals were in the experimental-biological clinic (vivarium) of the State Institution "Ukrainian Research Institute of Medical Rehabilitation Therapy of Ministry of Health of Ukraine", Odessa Research in the vivarium (Protocol № 12 dated 23.12.2020 from the Bioethics Commission). The physiological, morphological, immunological and biochemical methods used in the study are given in the methodological recommendations and approved by the Ministry of Health of Ukraine [17].

Reproduction of the PTSD model was performed by applying a rat that was under light etheric anesthesia of moderate traumatic brain injury (TBI). To do this, the rat was placed in a pencil case, where its head was fixed, and on top, from a height of 65 on the tube was dropped in a free fall semicircular cylinder weighing 85 g.

After trauma, the rats reproduced immobilization and cold stress, for which the animal was placed in a pencil case 15 cm long and 5 cm in diameter and placed in a refrigerator at a temperature of + 4 - 6 °C for 4 hours [18].

Rats were divided into 3 groups. The first group - 16 intact animals (which were not exposed to any influences), their data served as a control. The second group - 12 animals, which reproduced the model of PTSD. 3 group of rats (12 animals), which against the background of the development of the PTSD model received 5.0 g / l aqueous solution of preformed balneological agent "Magnesium Oil".

Magnesium oil solution was injected into the esophagus of rats with a soft probe with an olive, once a day, at a dose of 1% of the animal's body weight, in the evening (approximately at 17.00), taking into account the peculiarities of the daily biorhythm of rats. The obtained indicators were compared with the corresponding indicators of 1 group of intact animals.

Preformed balneological agent "Magnesium Oil" (Doctor "Magnesium" Chernivtsi, Ukraine), is characterized as boron, iodine-bromine chloride magnesium very strong brine.

The formula of the chemical composition is as follows:

$$\begin{matrix} I\ 0{,}068 \\ Br\ 3{,}04 \\ H_3BO_3\ 0{,}065 \end{matrix} \quad M_{339{,}88} \quad \frac{Cl\ 97\ SO_4\ 3}{Mg\ 95\ (Na+K)\ 5}$$

In a dilution of 5.0 g / l preformed balneological agent "Magnesium oil" is characterized as bromine chloride magnesium of low mineralization and corresponds to the following formula:

$$M_{5,11} \quad \frac{Cl\ 95 SO_4\ 4 HCO_3\ 1}{Mg\ 95\ (Na+K)\ 3\ Ca\ 2}$$

The use of the solution began the day after exposure to stressors and continued until the end of the experiment - 8 days. On the 9th day, the animals underwent a set of studies, which began with the assessment of the reactions of the higher parts of the CNS, namely, changes in the behavior of experimental rats and their emotional state.

These studies were conducted according to the generally accepted method of "open field" [19, 20]. The rat was placed in the center of the site and for 6

minutes visually recorded behavioral indicators. During the experiment, the sequence and duration of each act were recorded in seconds using a stopwatch and data were recorded. In the study of locomotor activity recorded the number of exits to the center of the device "open field", the number and duration of stops - indicators of motor activity; the number of crossed squares, the number of vertical struts and the number of glances at the minks performed by rats were indicative research behavior.

Features of the emotional state of animals were determined by the number and duration of grooming, the number of boluses and urination.

Metabolic studies were performed by biochemical methods involving serum. To assess the functional state of the liver, the activity of enzymes - alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was determined, pigment metabolism was examined by the content of total bilirubin and its fraction. To assess inflammatory and destructive processes, the content of total protein and its fraction (albumin, α1-, α2-, β-, γ-globulins) was determined, and the content of urea and creatinine was determined to assess the functional state of the kidneys.

Laboratory tests determined the number of leukocytes, the value of ESR and leukocyte formula (percentage of neutrophils, acidophils, monocytes, lymphocytes). Assessment of the state of the humoral immune system was performed by determining the content of circulating immune complexes (CIC) and the content of heterophilic antibodies (HA), namely natural antibodies to hemolysins and heterophilic agglutinins.

Statistical processing of the data obtained was carried out using the program for biomedical research Statistica. Significant shifts were considered those that were within the confidence limits according to the Student's tables $p < 0.05$.

Results

The development of PTSD in rats of group 2 was characterized by a decrease in motor activity: the number of exits to the center of the site decreases by 38%, and the duration of stops on the contrary - increases by 137% (Table 1). Approximate research behavior of animals is also declining, as evidenced by a significant decrease (by an average of 48%) in the number of crossed squares, the number of vertical struts and the number of peeks into the mink. The emotional state of rats with the PTSD model was characterized by manifestations of fear - ($p < 0.01$) a significant decrease in the number of defecations ($p < 0.01$) and an increase in the number of acts of urination ($p < 0.01$). Moreover, the urination of the animal was carried out by placing them at the beginning of the experiment in the center of the device "open field".

The negative fact of the emotional state of rats was a significant ($p < 0.05$) increase in the number of grooming acts by 36%, while maintaining the duration of grooming acts at the level of the control group ($p > 0.5$). Grooming of animals was carried out not only short-term (its duration was 1-4 seconds), but convulsive and chaotic in nature. In addition, half of the period of the experiment, the animals moved embarrassed around the perimeter of the device "open field". Sometimes they got stuck in one of the corners of the "open field" device. The animals showed signs of fright and embarrassment. That is, it can be argued that modeling PTSD causes disorders in rats of behavior, emotional status and cognitive functions.

In addition to disorders of the psychophysiological sphere, rats with a model of PTSD found changes in metabolism. The results of these studies are shown in table 2. When simulated in rats, PTSD revealed a significant decrease in the activity of ALT ($p < 0.01$) and AST ($p < 0.05$), which indicates inhibition of liver function. At the same time, the Ritis index increased significantly ($p < 0.01$), which indicates the presence of hypoxic processes in the liver tissues of rats. Violation of biliary function of the liver was found - an increase in total ($p < 0.05$) and indirect bilirubin ($p < 0.001$).

Another event that attracts attention is the increase in total protein content ($p < 0.01$) due to a significant increase in the content of globulins (α-1, α-2 and γ), which against the background of a decrease in albumin content ($p < 0.01$), indicates a violation of protein metabolism and activation of the inflammatory process in the body of rats. At the same time, the concentration of creatinine and urea increases significantly, which is evidence of impaired renal function.

Reproduction of the PTSD model caused changes in the state of peripheral blood and the humoral part of the immune system (table 3).

This is evidenced by a significant increase in the number of leukocytes and ESR. The redistribution of formed blood elements was established: the percentage of neutrophils increased ($p < 0.05$), and the percentage of monocytes and lymphocytes decreased significantly, which indicates the presence of signs of inflammation in rats. The content of CEC increased by 12% (which is a leading marker of the development of pathological processes in the body), and the content of heterophilic antibodies (HA) decreased by 54%, which also indicates the development of inflammatory processes and weakening of the immune system.

Thus, the data on changes in metabolic parameters indicate the development of systemic changes in rats during the development of PTSD.

In rats of group 3 after completion of the solution of "Magnesium oil" found a significant decrease in motor activity and orientation-research behavior, even in comparison with 2 with a group of rats with unadjusted PTSD (table 1). In comparison with the 1st control group, the number of rats in the 3rd group decreases the number of exits to the center of the site by 52%, and the duration of stops increases by 220%. Indicators that characterize the orienting-research behavior are reduced by an average of 63%.

The emotional state of rats in this group was characterized by the disappearance of fear. When placing rats in the center of the device "open field", they did not urinate large in size. The increase in the number of urination and bolus by 63% and 158% compared to 1 control group, we do not consider the manifestations of fear, and the inherent magnesium diuretic and laxative effects. In addition, the disappearance of signs of fear and overexcitement by restoring the amount of grooming, and a significant increase in the duration of grooming by 36%. That is, 2/3 of the experimental period the animals calmly and with pleasure engaged in grooming. At the same time, they did not look retarded.

When analyzing the metabolism of rats of group 3 (table 2), found higher than in rats of group 2 activity of enzymes (ALT and AST), which indicates the restoration of detoxification function of the liver while increasing the evacuation of toxic metabolic products - normalizes the concentration of total bilirubin restoring the ratio of its fractions. The content of creatine and urea is reduced to the level of control, which indicates the stimulation of the excretory function of the kidneys.

The content of total protein is restored and the ratio of its fractions is normalized, which indicates the normalization of protein-synthesizing activity in the body of rats, which should have a positive effect on the neural population of the CNS.

In rats of group 3, a decrease in the number of leukocytes and the level of ESR to the level of intact rats was found (table 3). The leukocyte formula showed a restoration of the percentage of monocytes, a significant decrease in the percentage of acidophiles and a slowdown in the redistribution of neutrophils / lymphocytes - neutrophils decreased significantly, and lymphocytes increased relative to group 2, but did not reach level 1 (control).

Positive changes were found in the indicators of the humoral part of the immune system: the content of CIC and the content of HA was restored to level 1 of the group. Thus, the use of magnesium-containing agent has a positive effect on the state of peripheral blood and immune system in rats against the background of the development of PTSD - limits the intensity of inflammatory processes.

Conclusions

The administration of exogenous Mg^{2+} to rats with a PTSD model promotes the normalization of the detoxification function of the liver, restoration of its biliary system and activation of the urinary system, which provides enhanced excretion of toxic metabolic products formed during metabolic restructuring during the development of PTSD. By reducing the toxic load, this leads to the normalization of the functional activity of the central nervous system and the emotional behavior of animals.

In addition, Mg^{2+}, as a Ca^{2+} antagonist, entering in significant quantities with the use of a solution of "Magnesium oil" also has a protective effect on the state of the brain pulp, contributing to a decrease in the functional activity of the central nervous system,

which has a beneficial effect on the psycho-emotional state of rats.

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Table 1. Indicators of the functional state of the CNS and emotional activity of rats with a model of PTSD and rats that received a solution of "Magnesium oil" against the background of the development of PTSD

Indicators	Group 1 control (intactrats)	Group 2 withthe PTSD model	Group 3 with the PTSD model and the "Magnesium Oil" course
	($M_1 \pm m_1$)	($M_2 \pm m_2$)	($M_3 \pm m_3$)
Number of exits to the center, n	1,37 ± 0,26	0,86 ± 0,06*	0,66 ± 0,032*
Stops, n	1,64 ± 0,12	2,17 ± 0,15*	1,44 ± 0,09
Stops, c	38,00 ± 8,96	90,14± 12,6*	122,18 ± 1,78*
Number of crossed squares, n	63,14 ± 3,98	33,29 ± 2,80*	29,47 ± 0,44*
Number of vertical stance, n	8,20 ± 0,15	6,00 ± 0,13*	3,25 ± 0,23*
The number of peeks in the caves, n	9,60 ± 0,74	6,29 ± 0,93*	6,59 ± 0,93*
Grooming, n	1,74 ± 0,27	2,50 ± 0,05*	1,90 ± 0,21
Grooming, c	21,67 ± 1,23	25,44 ± 1,29	29,44 ± 1,73*
Number of acts of defecation (bolus), n	1,87 ± 0,15	1,23 ± 0,11*	3,06 ± 0,22*
Number of acts of urination, n	4,28 ± 0,22	5,14 ± 0,12*	11,08 ± 0,35*

Note 1. ($M_1 \pm m_1$), ($M_2 \pm m_2$) and($M_3 \pm m_3$) are arithmetic means with errors;
* - significant changes ($p < 0,05$) were calculated in comparison between groups 1 and 2;
** - significant changes ($p < 0,05$) were calculated in comparison between groups 1 and 3;
the data of the control group of animals were accepted as 100%.

Table 2. Biochemical and immunological parameters of rats with the model of PTSD and rats that received a solution of "Magnesium oil" against the background of the development of PTSD

Indicators	Group 1 control (intactrats)	Group 2 withthe PTSD model	Group 3 with the PTSD model and the "Magnesium Oil" course
	($M_1 \pm m_1$)	($M_2 \pm m_2$)	($M_3 \pm m_3$)
ALT, U/l	133,09±4,68	108,53±3,77*	125,91±3,12
AST, U/l	278,84±6,57	248,74±4,77*	261,34±879*
Ritis Index	2,10 ± 0,07	3,16 ± 0,12*	2,27 ± 0,15
Total bilirubin, mkmol / l	5,79 ± 0,81	8,50 ± 0,97*	6,21 ± 0,26
Direct bilirubin, mkmol / l	1,98 ± 0,32	2,40 ± 0,25	2,19 ± 0,15
Bilirubin indirect, mkmol / l	3,81 ± 0,51	6,10 ± 0,72*	4,02 ± 0,63
Total protein, g / l	68,70 ± 2,74	60,35 ± 1,21*	67,59 ± 1,21
Albumin, g / l	25,80 ± 1,08	9,31 ± 1,02*	23,02 ± 1,02
α-1 Globulin, g / l	8,28 ± 0,51	13,16 ± 1,19*	10,56 ± 0,44*
α-2 Globulin, g / l	10,70 ± 0,62	15,37 ± 0,65*	11,47 ± 1,38
β- Globulin, g / l	11,80 ± 1,27	6,21 ± 0,28*	10,21 ± 1,28
γ- Globulin, g / l	11,10 ± 0,73	16,30 ± 1,14*	12,33 ± 0,95
Creatinine, mkmol / l	47,80 ± 0,63	63,16 ±0,48*	53,18 ± 1,36
Urea, mmol / l	2,80 ± 0,27	5,23 ± 0,42*	2,29 ± 0,64

Note 1. ($M_1 \pm m_1$), ($M_2 \pm m_2$) and($M_3 \pm m_3$) are arithmetic means with errors;
* - significant changes ($p < 0,05$) were calculated in comparison between groups 1 and 2;
** - significant changes ($p < 0,05$) were calculated in comparison between groups 1 and 3;
the data of the control group of animals were accepted as 100%.

Table 3. Peripheral blood parameters and immunological parameters of rats with a model of PTSD and rats that received a solution of "Magnesium oil" against the background of the development of PTSD

Indicators	Group 1 control (intactrats)	Group 2 withthe PTSD model	Group 3 withthe PTSD model and the "Magnesium Oil" course
	($M_1 \pm m_1$)	($M_2 \pm m_2$)	($M_3 \pm m_3$)
Leukocytes, 10^9/l	5,50 ± 0,88	9,29 ± 0,21*	6,01 ± 0,21
Erythrocyte sedimentation rate, mm/h	1,54 ± 0,09	1,85 ± 0,10*	1,63 ± 0,13
Neutrophils, %	12,79 ± 0,68	29,80 ± 1,40*	25,30 ± 1,32*
Acidophiles, %	2,25 ± 0,14	2,74 ± 0,19	1,50 ± 0,29*
Monocytes, %	3,72 ± 0,15	2,50 ± 0,17*	3,25 ± 0,25
Lymphocytes, %	81,20 ± 0,87	65,00 ± 1,40*	69,95 ± 1,70*
circulating immune complexes, mg/ml	5,70 ± 0,20	6,41 ± 0,17*	6,15 ± 0,22
HA, cu	6,00 ± 0,80	2,82 ± 0,49*	5,86 ± 0,15

Note 1. ($M_1 \pm m_1$), ($M_2 \pm m_2$) and($M_3 \pm m_3$) are arithmetic means with errors;
* - significant changes ($p < 0,05$) were calculated in comparison between groups 1 and 2;
** - significant changes ($p < 0,05$) were calculated in comparison between groups 1 and 3;
the data of the control group of animals were accepted as 100%.