NORMALIZING EFFECT OF ELECTROMAGNETIC STIMULATION ON BLOOD QUANTITATIVE INDICES IN DEPRESSED RATS ON THE BACKGROUND OF OXYTOCIN

[1]Bukia N., [1]Butskhrikidze M., [1]Machavariani L., [2]Svanidze M., [3]Jojua N.

[1]LEPL Ivane Beritashvili Center of Experimental Biomedicine, Tbilisi; [2]Iv. Javakhishvili Tbilisi State University; [3]European University, Tbilisi, Georgia

Depression is a common illness that severely limits psychosocial functioning and diminishes the quality of life. In 2008, WHO ranked major depression as the third cause of burden of disease worldwide and projected that the disease will rank first by 2030. Depression is linked to systemic inflammation. There is an association between hematological inflammatory markers and depression symptoms [12].

Oxytocin (OXT) is a nanopeptide hormone, synthesized in magnocellular and parvocellular neurons of the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus. OXT causes uterine contractions during labor and milk ejection during lactation in mammals. The hormone is transported to various areas of the brain in addition to the pituitary [4]. The OXT receptor, a member of the G protein-coupled receptor family, is expressed widely in the central nervous system (CNS), especially in the ventromedial nucleus of the hypothalamus, the central nucleus of the amygdala, the head of the caudate-putamen and the hippocampus [4]. Therefore, OXT acts as a neurotransmitter/neuromodulator to regulate a range of CNS functions in males and females, including emotional, parental, affiliative, and sexual behaviors, as well as spatial and social memories, such as mother-infant bonding [3,4,8-11,14]. Oxytocin mediates an antidepressant-like effect in male mice, which disappears in OTR knockout (KO) mice [5,6]. The administration of intranasal OXT in clinical populations seemed to improve aspects of social cognition, including emotion recognition, and mood [2,3,8].

Oxytocin reduces the activity of brain regions that produce anxiety and might involve in the rehabilitation of depressive-like behavior. The effect of OT on anxiety might be mediated by the de-activation of the hypothalamus-hypophysis-adrenal axis. Depressed patients consistently exhibit hyperactivity of the hypothalamus–pituitary–adrenal (HPA) axis. HPA axis activity is regulated by the secretion of the corticotropic hormone-releasing factor (CRF), vasopressin (AVP) and oxytocin (OXY) from the hypothalamus, which finally stimulates the secretion of the glucocorticoids from the adrenal cortex. Glucocorticoids interact with their receptors (GRs) in multiple target tissues including the HPA axis by feedback inhibition [7,15]. The activity of HPA also can be inhibited by OXY which affects GRs. In depression-like conditions, OXY neurons elevate release of this hormone within the hypothalamus and the amygdala and have a suppressive effect on GR expression in the hippocampus.

The electro-magnetic stimulation (EMS) is a noninvasive treatment method, which is used as a complementary to the drug for the treatment of different neurodegenerative diseases. Repetitive electromagnetic stimulation (EMS), is used in the treatment of moderate depression [1].

The goal of this investigation was to study the quantitative characteristics of blood cells after premedication of oxytocin in depressed rats on the background of EMS.

Material and methods. The experiments were conducted on mongrel, albino male rats, weighing 150-200 g (n=14). Proceeding from the goals set, the experimental group (clomipramine-induced depressed rats) and the control group of rats were involved in the experiments. The experimental group was divided into subgroups. Some rats from the subgroup were given EMS, OXY (4 μg/ per animal for 10 days), or EMS and OXY simultaneously. The Control group of rats received the same amount of saline.

Electro-Magnetic stimulation - The parameters of EMS (stimulus frequency, number, and duration of stimuli,) which partially or fully inhibited behavior manifestation of depression, were established during pilot experiments. For repetitive EMS the following parameters: 10000 -15000 Hz frequency, 1,5 m/Tesla, for 15 min, during 10 consecutive days were used.

For hematological analysis of blood HumaCount 30 TS was used. This analyzer allows us to quantify the number of red blood cells, leukocytes (agranulocytes and granulocytes), platelets, hemoglobin, the hematocrit in 1 ml blood of rats. Determination of these parameters is very important because EMS affected the whole body, including the bone marrow, which is the main place of blood cell genesis. The trunk blood was collected from each rat. The hematological analysis was performed 2 weeks later after OXY injection and EMS.

The obtained results were processed using an adequate statistical program. Data reliability was assessed using parametric and non-parametric techniques, with the use of one- and two-way layout of factorial analysis.

Results and discussion. *Impact of EMS and OXY on the Red blood cells count in depressed rats*

Depression caused decreasing number of red blood cells (RBC), increasing of Mean corpuscular volume (MCV) and Red Cell Distribution Width (RDW) compared to the control group of rats. EMS, as well as oxytocin injection, caused an increase in RBC content. MCV and RDW returned to normal limits. Against the background of simultaneous exposure of oxytocin and EMS, the rates returned to data obtained in intact rats. On the background of oxytocin injection, the amount of hemoglobin was increased also. Additional EMS did not change these data significantly, but in this case, obtained results did not differ from the results of control, non-depressed rats.

The chronic increases of the RDW (%) content during a long time, develop anemia, and leads to deterioration of the functional state of the organism. Since depression causes abrupt changes in RDW (%), we think that one of the markers of depression can be used to determine RDW (%) in the blood. In our opinion, this should be related to nutritional deficiency, which leads to a decrease in iron and vitamin B12 in the body. At this time, due to the violation of erythropoiesis, not enough erythrocytes are produced, and the already existing RBCs increase in size. As a result, RBC sizes become highly variable (Fig.1 a, b, c, d, e).

Since depression is associated with anemia, it is possible to assume that the symptoms of depression can be reduced by modulating the blood system. Thus, in the treatment of depression, it would be appropriate to use medications that activate erythropoiesis. Iron therapy, as well as vitamin B12 therapy, can be considered here.

The weight of clomipramine induced depressed rats was significantly lower than the weight of non-depressed rats (Fig. 2).

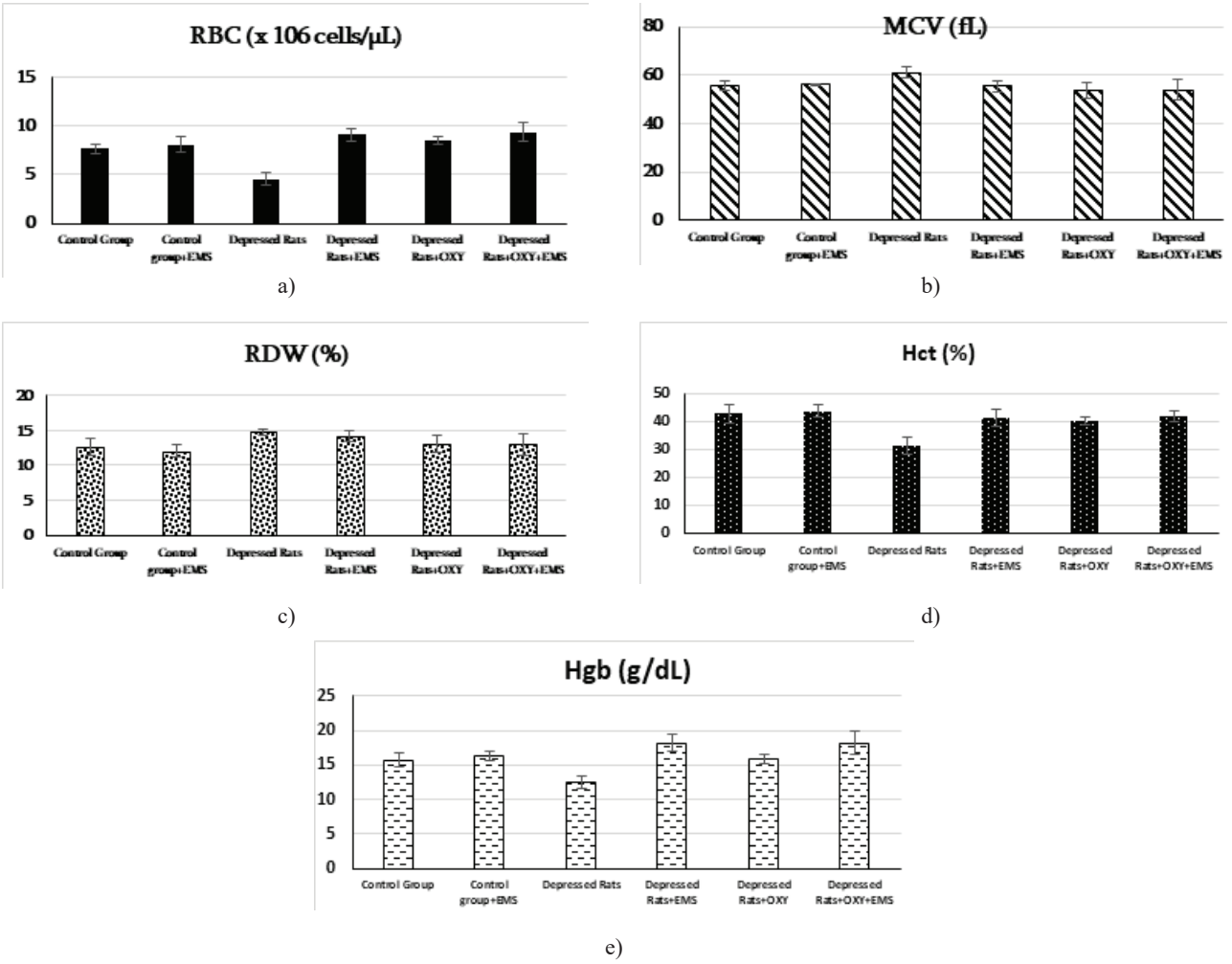

Fig. 1 (a.b.c.d.e) - The effects of EMS and OXY on the RBC (x 106 cells/µL), MCV (fL), RDW (%),Hct (%), Hgb (g/dL) in various experiment conditions

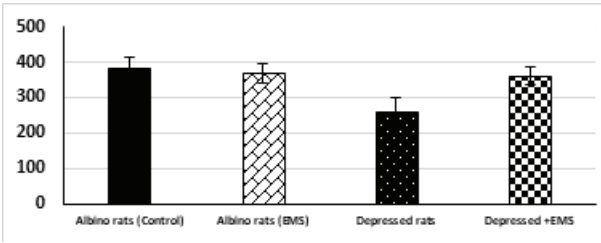

Fig. 2. Body weight (g) in experimental (depressed) and control group of rats after EMS

Impact of EMS and OXY White blood cell count in depressed rats. Increased white blood cell (WBC) count and red cell distribution width (RDW) is associated with negative clinical outcomes in a wide variety of pathological conditions. WBC is a non-specific inflammatory marker and RDW is also strongly related to other inflammatory markers.

Increased level of WBC in depressed rats was decreased after oxytocin injection and EMS. Despite the growth of white blood cells, depression decreased lymphocyte content. Injected oxytocin did not change the content of lymphocytes, but in light of the 10-day of EMS, the content of lymphocytes ($P \leq 0.05$) increased. It is possible to think that the decrease in lymphocytes in depressed rats may explain the immunological dysfunction characteristic of a depressive condition, which can be corrected by EMS.

Depression increases the levels of cytokines (particularly interleukin-1) in the blood, which activate the hypothalamic-pituitary axis and by activation of glucocorticoid receptors, lead to the development of depressive behavior [2]. Therefore, depression of cytokines or glucocorticoid receptors might lead to a reduction of depressive-like behavioral manifestations. On the other hand, an increased level of cytokines causes effects on the hippocampus, hypothalamus, and brain stem. In particular, the working memory gets worse as a result of the inhibition of hippocampal long-term potentiation, which is the basis for memory consolidation. Eventually, the cognitive function deteriorates.

The number of granulocytes did not change in depressed rats. This parameter does not change as a result of exposure to oxytocin and EMS.

Impact of EMS and OXY on Platelets (PLt) count in depressed rats. In depressed rats the amount of PLt and PDW were decreased, MPV was increased. Thrombocytopenia can be associated with leukocytosis, as well as with a weakened immune system. On the background of oxytocin injection, there was a tendency to an increase in platelet number, although the results were not statistically significant. The platelet count returned to the normal level after Additional EMS in rats with oxytocin premedication.

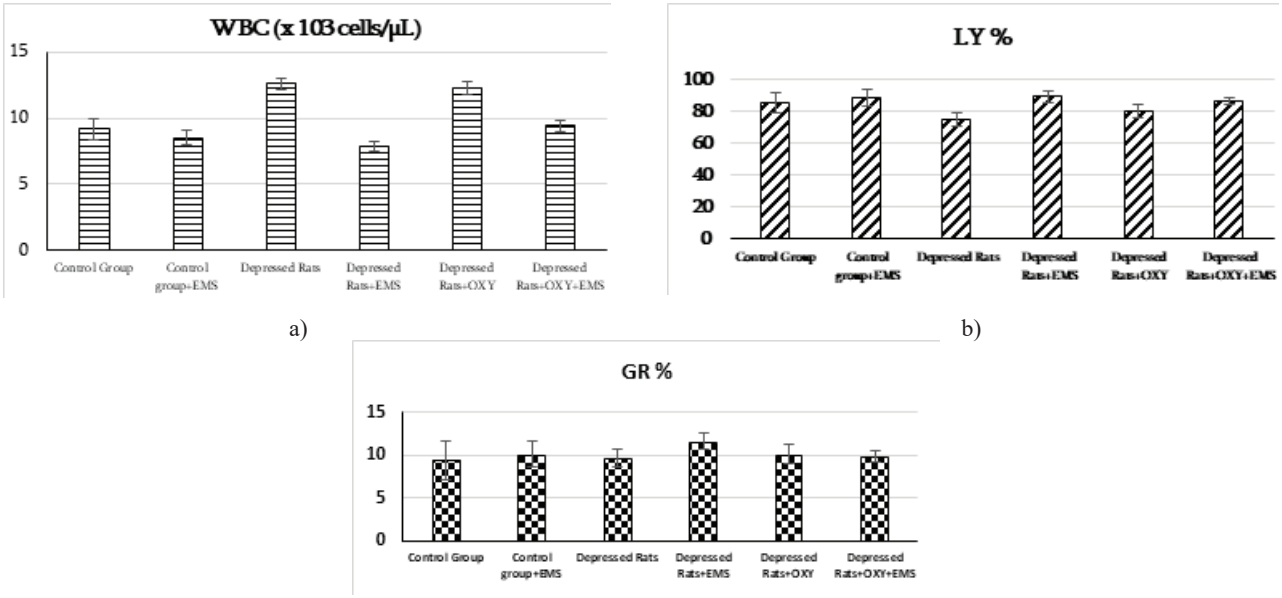

Fig. 3 (a, b, c). The effects of EMS and OXY on the WBC (x 103 cells/μL), LY %, GR % in various experiment conditions

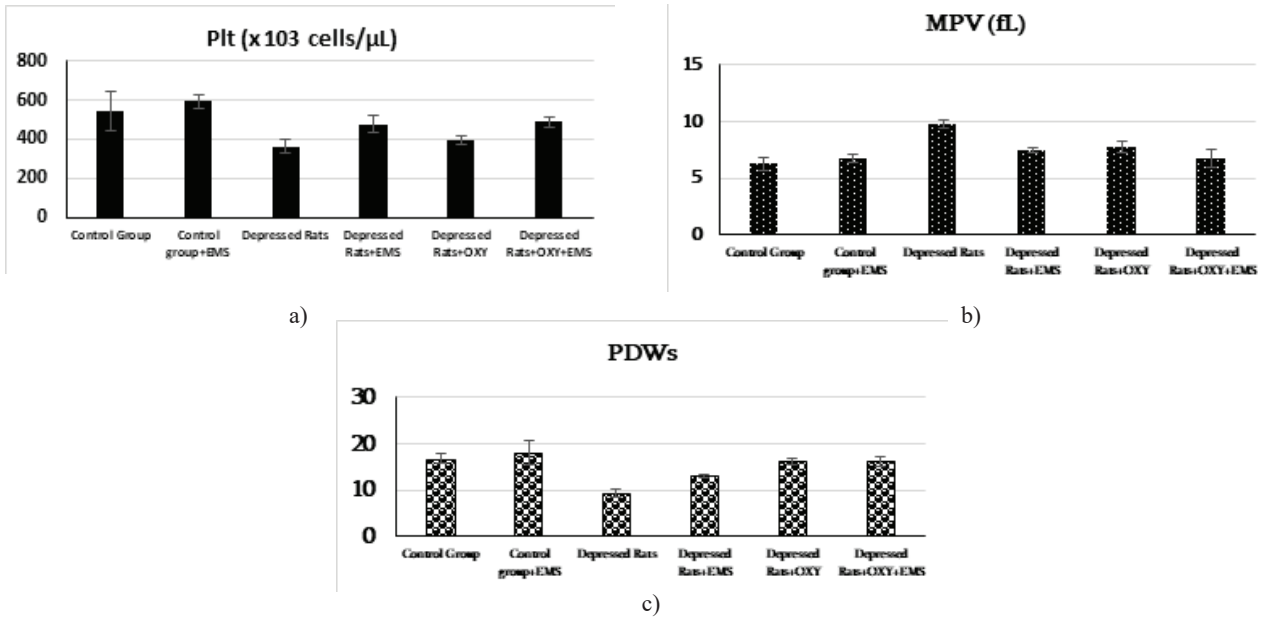

Fig. 4 (a, b, c.). The effects of EMS and OXY on the Plt (x 103 cells/μL), MPV (fL), PDWs in various experiment conditions

MPV is a key characteristic of platelet size and is a potential marker of platelet reactivity. Variation in platelet size is indicative of a change in platelet function. Therefore, platelet parameters are markers that are thought to be changed in response to systemic inflammation, internal bleeding, bone marrow pathology or different neurological diseases [12]. Under the influence of oxytocin and EMS, the mean platelet volume MPV (fL) was normalized.

The PDW -indicator of volume variability in platelets size. It changes with platelet activation and reflects the heterogeneity in platelet morphology. The decreased level of PDW was increased to a normal level after OXY and EMS. It was suggested that EMS effects thrombopoietic agents which induce the growth and maturation of megakaryocytes.

Therefore, Oxytocin injection did not effect on PLt amount but normalized amount of PDW and MCV. Additional EMS normalized all three parameters.

This study demonstrate that PLT counts and PLT parameters can probably be used as adjunct to clinical evaluation of depression. Oxytocin, as well as EMS, stabilized the blood cells quantitative characteristics. The data obtained from the blood tests might be used as a marker for determination the degree of depression and the general condition of the body. The analysis of these data is of great importance for medical practice.

Conclusion. In clomipramine-induced depressed rats, the oxytocin alone, or together with EMS can restore the blood cell imbalance. Oxytocin with simultaneous action of EMS have antidepressant effects, which would strengthen the notion of its therapeutic potential treatment strategy for depression.

The research was supported by the Shota Rustaveli National Foundation FR 17_333.

REFERENCES

1. Bergmann T.O., Karabanov A., Hartwigsen G., Thielscher A., Siebner H.R. Combining non-invasive transcranial brain stimulation with neuroimaging and electrophysiology: current approaches and future perspectives. // Neuroimage. 2016, 140, 4–19.
2. De Cagna F., Fusar-Poli L., Damiani S, Rocchetti M., Giovanna G., Mori A., Politi1 P, Brondino N. The Role of Intranasal Oxytocin in Anxiety and Depressive Disorders: A Systematic Review of Randomized Controlled Trials // Clinical Psychopharmacology and Neuroscience 2019;17(1):1-11.
3. Fang A, Treadway MT, Hofmann SG. Working hard for oneself or others: Effects of oxytocin on reward motivation in social anxiety disorder. // Biol Psychol 2017;127:157-162.
4. Feeser M, Fan Y, Weigand A, Hahn A, Gärtner M, Böker H, et al. Oxytocin improves mentalizing-pronounced effects for individuals with attenuated ability to empathize. // Psychoneuroendocrinology 2015; 53:223-232.
5. Katoh A, Fujihara H, Ohbuchi T, Onaka T, Hashimoto T, Kawata M et al. Highly visible expression of an oxytocin-monomeric red fluorescent protein 1 fusion gene in the hypothalamus and posterior pituitary of transgenic rats.// Endocrinology 2011; 152:2768–2774.
6. Kroll-Desrosiers AR, Nephew BC, Babb JA, Guilarte-Walker Y, Moore Simas TA, Deligiannidis KM. Association of peripartum synthetic oxytocin administration and depressive and anxiety disorders within the first postpartum year. // Depress Anxiety 2017; 34:137-146.
7. Martins, C.S.; Elias, D.; Colli, L.M.; Couri, C.E.; Souza, M.C.; Moreira, A.C.; Foss, M.C.; Elias, L.L.; de Castro, M. HPA axis dysregulation, NR3C1 polymorphisms and glucocorticoid receptor isoforms imbalance in metabolic syndrome. // Diabetes Metab. Res. Rev. 2017, 33.
8. Matsushita H, Tomizawa K, Okimoto N, Nishiki T, Ohmori I, Matsui H Oxytocin mediates the antidepressant effects of mating behavior in male mice. // Neurosci Res 2010; 68:151–153.
9. Neumann ID, Slattery DA. Oxytocin in general anxiety and social fear: a translational approach. // Biol psychiatry 2016;79: 213-221.
10. Rocchetti M, Radua J, Paloyelis Y, Xenaki LA, Frascarelli M, Caverzasi E, et al. Neurofunctional maps of the ‚maternal brain' and the effects of oxytocin: a multimodal voxel-based meta-analysis. // Psychiatry Clin Neurosci 2014; 68:733-751.
11. Rutigliano G, Rocchetti M, Paloyelis Y, Gilleen J, Sardella A, Cappucciati M, et al. Peripheral oxytocin and vasopressin: Biomarkers of psychiatric disorders? A comprehensive systematic review and preliminary meta-analysis. // Psychiatry Res 2016; 241:207-220.
12. Shafiee M., Maryam Tayefi M., Seyed Mahdi Hassanian, Zahra Ghaneifar, Mohammad Reza Parizadeh, Amir Avan, Farzad Rahmani, Zahra Khorasanchi, and all. Depression and anxiety symptoms are associated with white blood cell count and red cell distribution width: A sex-stratified analysis in a population-based study. // Psychoneuroendocrinology 2017; 84:101-108.
13. Stasi R., Bosworth J., Rhodes E., Willis F., Gordon-Smith E. Thrombopoietic agents. // Blood Rev. 2010;24(4-5):179-90.
14. Tomizawa K, Iga N, Moriwaki A, Matsushita M, Li ST et al. Oxytocin improves long-lasting spatial memory during motherhood through MAP kinase cascade. // Nat Neurosci 2003; 6:384–390.
15. Wulsin, A.C.; Herman, J.P.; Solomon, M.B. Mifepristone decreases depression-like behavior and modulates neuroendocrine and central hypothalamic-pituitary-adrenocortical axis responsiveness to stress. // Psychoneuroendocrinology 2010, 35, 1100–1112.

SUMMARY

NORMALIZING EFFECT OF ELECTROMAGNETIC STIMULATION ON BLOOD QUANTITATIVE INDICES IN DEPRESSED RATS ON THE BACKGROUND OF OXYTOCIN

[1]Bukia N., [1]Butskhrikidze M., [1]Machavariani L., [2]Svanidze M., [3]Jojua N.

[1]LEPL Ivane Beritashvili Center of Experimental Biomedicine, Tbilisi; [2]Iv. Javakhishvili Tbilisi State University; [3]European University, Tbilisi, Georgia

Depression is linked to systemic inflammation. There is an association between blood cell count and/or hematological inflammatory markers with depression symptoms. Oxytocin reduces the activity of brain regions that produce anxiety and might involve in the rehabilitation of depressive-like behavior. Repetitive EMS is used in the treatment of moderate depression. So, the goal of this investigation was to study the quantitative characteristics of blood cells after EMS on the background of oxytocin premedication of in depressed rats.

In the implementation of the project the depressed rats (250-450 g) were used (n=20). For each task two groups of the animal were conducted: experimental group (with EMS) and control group (without EMS). For repetitive (10-days) EMF exposure, the following parameters were used: 10000 -15000 Hz frequency, 1,5 m/Tesla, for 15 min. An animal model of depression was received by subcutaneous injection of Clomipramine from 8 to 21 days of neonatal development. The blood counts were performed 2 months later after clomipramine injection using blood HumaCount 30 TS. This analyzer allows quantifying the number of red blood cells, white blood cells (agranulocytes and granulocytes), platelets, hemoglobin, the hematocrit in 1 ml blood of rats. The hematological analysis was performed 2 weeks later after oxytocin (4 mcg/per animal during 10 days) injection and EMS. The obtained results were processed using an adequate statistical program. In clomipramine –induced depressed rats, EMS independently or with simultaneous injection of Oxytocin caused normalization of blood cells count (Red blood cells, white blood cells and Platelets). More important was the simultaneous impact of EMS and oxytocin.

In depressed rats, the oxytocin alone, or together with EMS can restore the blood cell imbalance.

Keywords: electric-magnetic stimulation, Oxytocin, depression.

РЕЗЮМЕ

ЭЛЕКТРОМАГНИТНАЯ СТИМУЛЯЦИЯ СПОСОБСТВУЕТ НОРМАЛИЗАЦИИ КОЛИЧЕСТВЕННЫХ ПОКАЗАТЕЛЕЙ КРОВИ У ДЕПРЕССИВНЫХ КРЫС НА ФОНЕ ОКСИТОЦИНА

[1]Букия Н.Г., [1]Буцхрикидзе М.П., [1]Мачавариани Л.И., [2]Сванидзе М.Дж., [3]Джоджуа Н.В.

[1]LEPL Центр экспериментальной биомедицины им. И. Бериташвили, Тбилиси; [2]Тбилисский государственный университет им. И. Джавахишвили; [3]Европейский университет, Тбилиси, Грузия

Депрессия связана с системным воспалением. Существует связь между количеством клеток крови и/или гемато-

логическим маркером воспаления с симптомами депрессии. Окситоцин снижает активность областей мозга, вызывающих беспокойство, и может участвовать в реабилитации депрессивно-подобного поведения. Повторяющаяся электромагнитная стимуляция (ЭМС) используется при лечении умеренной депрессии. Целью настоящего исследования явилось изучение количественных характеристик клеток крови после электромагнитной стимуляции на фоне премедикации окситоцином у депрессивных крыс.

Опыты проводились на депрессивных и интактных крысах (n=14) массой 150-200 г. Обе группы подразделены на две подгруппы: экспериментальная (с ЭМС) и контрольная группа (без ЭМС). ЭМС проводили 10 дней (частота 10000-15000 Гц, 1,5 м/тесла, в течение 15 мин). Животную модель депрессии получали путем подкожной инъекции кломипрамина с 8 до 21 дня неонатального развития. Анализ крови выполнен с использованием прибора HumaCount 30 TS. Этот анализатор позволяет определить количество эритроцитов, лейкоцитов (агранулоцитов и гранулоцитов), тромбоцитов, гемоглобина, гематокрита в 1 мл крови крыс. Гематологический анализ проводили спустя 2 недели после инъекции окситоцина (4 мкг/животное в течение 10 дней) и ЭМС. Полученные результаты обрабатывались с помощью статистической программы (ANOVA).

У крыс с депрессией, вызванной кломипрамином, ЭМС самостоятельно или с одновременным введением окситоцина вызвала нормализацию количества клеток крови (эритроциты, лейкоциты и тромбоциты). Более эффективным было одновременное воздействие ЭМС и окситоцина.

Таким образом, результаты проведенных исследований выявили, что у депрессивных крыс ЭМС и окситоцин может восстановить дисбаланс клеток крови.

რეზიუმე

ოქსიტოცინის ფონზე ელექტრომაგნიტური სტიმულაცია ხელს უწყობს დეპრესიულ ვირთაგვებში სისხლის რაოდენობრივი პარამეტრების ნორმალიზებას

[1]ნ.ბუკია, [1]მ.ბუცხრიკიძე, [1]ლ.მაჭავარიანი, [2]მ.სვანიძე,[3]ნ.ჯოჯუა

[1]ივ. ბერიტაშვილის ექსპერიმენტული ბიომედიცინის ცენტრი, თბილისი;
[2]ივ. ჯავახიშვილის თბილისის სახელმწიფო უნივერსიტეტი; [3]ევროპული უნივერსიტეტი, თბილისი, საქართველო

დეპრესია ასოცირდება სისტემურ ანთებასთან. არსებობს ასოციაცია, ერთის მხრივ, სისხლის უჯრედების რაოდენობას და/ან ანთების ჰემატოლოგიურ მარკერებსა და, მეორეს მხრივ, დეპრესიის სიმპტომებს შორის. ოქსიტოცინი ამცირებს განგაშის რეაქციაში ჩართული თავის ტვინის უბნების აქტივობას და, ამდენად, შესაძლოა ჩაერთოს დეპრესიისთვის დამახასიათებელი ქცევის რეაბილიტაციაში. განმეორებითი ელექტრომაგნიტური სტიმულაცია (EMS) გამოიყენება ზომიერი დეპრესიის სამკურნალოდ.

კვლევის მიზანს წარმოადგენს სისხლის უჯრედების რაოდენობრივი მახასიათებლების შესწავლა ელექტრომაგნიტური სტიმულაციის, ოქსიტოცინის და ასევე, ოქსიტოცინისა და ელექტრომაგნიტური სტიმულაციის ერთდროული ზემოქმედების ფონზე დეპრესირებულ და ინტაქტურ ვირთაგვებში.

პროექტის განხორციელებისას გამოყენებული იყო დეპრესირებული და ინტაქტური ვირთაგვები 150-200 გ (n=14). ორივე ჯგუფის ვირთაგვებში შეიქმნა ქვეჯგუფები: ექსპერიმენტული (EMS-ით) და საკონტროლო ჯგუფი (EMS–ს გარეშე). დეპრესიის ცხოველური მოდელის მისაღებად ნეონატალური განვითარების მე-8 დღიდან 21 დღემდე ვირთაგვებისთვის შეყავდათ კლომიპრამინის კანქვეშა ინიექცია. EMS ტარდებოდა 10-დღის განმავლობაში (სიხშირე 10000-15000 ჰც, 1.5 მ/ტესლა, 15 წუთის განმავლობაში). სისხლის რაოდენობრივი ანალიზი ჩატარდა HumaCount 30 TS-ის გამო-ყენებით. აღნიშნული ანალიზატორის საშუალებით შესაძლებელია 1 მლ სისხლში განვსაზღვროთ ერითროციტები, ლეიკოციტები (აგრანულოციტები და გრანულოციტები), თრომბოციტები, ჰემოგლობინი, ჰემატოკრიტი. ჰემატოლოგიური ანალიზი ჩატარდა ოქსიტოცინის (4 მკგ/ცხოველის 10 დღის განმავლობაში) ინიექციისა და EMS-დან 2 კვირის შემდეგ. მიღებული შედეგები დამუშავდა სტატისტიკური პროგრამის (ANOVA) გამოყენებით.

ვირთაგვებში, კლომიპრამინით გამოწვეული დეპრესიით EMS ან EMS-ის და ოქსიტოცინის ერთდროული ზემოქმედება იწვევდა სისხლის უჯრედების (ერითროციტები, ლეიკოციტები და თრომბოციტები) რაოდენობის ნორმალიზებას. განსაკუთრებით ეფექტური იყო EMS-ის და ოქსიტოცინის ერთდროული ზემოქმედების შედეგი. ოქსიტოცინი და EMS აღადგენს ვირთაგვების დეპრესიული მდგომარეობით გამოწვეულ სისხლის უჯრედების დისბალანსს.