

Electromagnetic field, conduct cross in the open field and neurochemical correlate blood and urine on the background of the supply of oils of grape seeds

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Abstract

The influence of an electromagnetic field (EMF) – network frequency causes minimal CNS dysfunction in Wistar rats in ontogenesis, which is expressed by a decrease of the levels of glutamate and aspartate, methionine and serine, in the blood – lysine, taurine, tyrosine, catecholamine and serotonin, an increase GABA and glycine in the blood, proline and cysteine in the urine. The ratio of excitatory/inhibitory mediator amino acids is significantly reduced. The ratio of essential/non-essential amino acids and the total level of amino acids also decrease in the blood. Against the background of taking the herbal preparation (grape seed oil), the identified changes disappear or smooth out, which is manifested in the behavior of rats in the open field – the animals are characterized by a smaller number of urinations and boluses, an increase in the total time of grooming and a small number of translocations.

KEYWORDS: EMF; amino acids; rat behavior



Introduction

Among the effects associated with exposure to an electromagnetic field (EMF), neurotropic and psychotropic effects occupy a special place, since the nervous system, along with the endocrine, cardiovascular and reproductive systems, is one of the most sensitive to the effects of EMF [1]. In connection with the ecological crisis, special attention is paid to finding ways to protect against the effects of chronic low-frequency EMF exposure in natural conditions [2,3]. As protection against EMF, it is possible to use biologically active substances of natural origin that can activate the body's protective resources, affecting mainly the neurohumoral and immune-hematopoietic systems. The general nonspecific resistance of the body increases, the endogenous background of resistance is stimulated (a complex of endogenous biologically active compounds: amines, thiols and other antioxidants that perform protective functions and suppress the accumulation of a destructive excess of peroxidation products). Due to the absence (or low) toxicity and good tolerance, products of natural origin are recommended as food additives that increase the overall nonspecific resistance of the body, stimulating protective, antioxidant reserves. Among the biologically active substances of natural origin, representatives of bioflavonoids – oligomeric proanthocyanidins – antioxidants that prevent cell degeneration occupy the central place. They also contain vitamin E. It should be noted that many components of plants are included in the list of medicinal herbal raw materials [4]. The pharmaceutical market today advertises many medicinal compositions containing special dietary supplements with antioxidant action. It is generally accepted that natural substances exhibit a much more optimal therapeutic and prophylactic effect than synthetic counterparts do.

In this aspect, grape seed oil (*oleum Vitini Vini Pezae*) deserves special attention. A number of researchers [5,6] convincingly showed that among the majority of known oils recommended as food additives, the highest content of essential fatty acids is found in grape seed oil, the physicochemical constants and biological activity of which depend on climatic and geographical conditions [7,8].

Studies related to the action of network frequency EMF on Wistar rats before and after feeding with a food supplement from grape seeds in Georgia have not been carried out.

The aim of the study is to determine the nature of the action of the dietary supplement from the Saperavi grape seed oil on some biochemical correlates of blood plasma and urine, as well as the behavior of rats in an open field before and after exposure to EMF of the network frequency. Studies of this nature contribute to the search for optimal ways of active prevention of EMF exposure in urban environments.

Materials and Methods

The experiments were carried out on two-month-old Wistar rats weighing 76-85 g. The animals were bred in the nursery of our Center and kept under the same conditions of care and free access to water and stable food (barley, corn, sunflower, cabbage, carrots and bread with milk. They were also given a multivitamin "Undevit").

The animals were divided into three groups of 10 rats each. Control group A ate a standard diet. Experimental group B also had a standard diet and was subjected to a continuous magnetic field for 30 days. Experimental group C received a standard diet and peritoneal 0.5 ml of a 5% solution of grape seed oil (Natural Extra) "Saperavi" for 10 days before exposure to a magnetic field.

The electric field was applied using an original induction coil (coil diameter 750 mm, length 2200 mm). The coil had several sections of turns and a control unit of two types. The first of them made it possible to influence the high voltage of a stationary EMF of the mains frequency, and the second was universal – for the impact of a stationary and simulated frequency. The large dimensions of the coil made it possible to study complex forms of behavior and their ethological correlates even directly during the action of an EMF. Groups C and B were exposed to an electromagnetic field with a frequency of 50 Hz, 1.5 MT.

As behavioral comparison criteria, ethological parameters were used, determined by the method of the so-called "Open Field", which was a round chamber with a diameter of 80 cm, divided into 32 identical sectors. It was illuminated from a height of 1 m with a 200-watt lamp. The duration of each rat in the "Open Field" was 180 seconds. The results were transmitted by a video camera to a computer equipped with a special Rat Watcher program. The program was a significantly improved version of the Pole-91 program previously developed at our Center [9]. It is provided for personal computers such as IBM PC and runs on the Windows operating system.

The following parameters were recorded: animal motor activity (by which the time spent to leave the central circle was noted), the number of sector lines crossed, the number of translocation cycles and time spent, and the average line crossing speed. Orientation-exploratory activity was assessed by the number of vertical standing up, according to the average time spent on one standing up. The idea of emotional activity was assessed by the number of boluses and urinations. Stereotypical activity was judged by the number of grooming cycles, the amount of time spent on them, etc. Attention was paid to the number of cycles of immobility, the average time spent on one cycle.

The level of free amino acids in plasma and urine was determined by high-performance liquid chromatography on an RTS-1 chromatograph (Waters, USA) according to



the method [10], and catecholamines on the same device according to the method [3]. Blood was taken from the distal part of the tail after appropriate treatment.

The results of the study were subjected to statistical processing using Student's criteria for the computer program SPSS version 13 ANOVA.

Saperavi grape seed oil was obtained by pressing at low temperatures [11]. This method is the most gentle, as it is carried out without additional heating of the oil. An increase in pressure in the mixture is accompanied by a natural increase in temperature up to 35°C, which does not adversely affect the quality of the oil. The oil obtained in this way has a pronounced aroma and taste, and biologically active substances (vitamins, polyunsaturated fatty acids, pigments, etc.) are almost completely preserved. The oil is not subjected to refining, which significantly reduces its quality. The considered method does not allow to obtain oil in a large volume, it is called "Natural Extra". After the end of the first phase of the process, the pressings are separated, the residue is mixed and pressed again. Obtained after the secondary extraction is filtered, it is also of high quality and is not subjected to refining. It's called Natural Delicate Oil.

Results

After exposure to EMF group B, a distinct change in the level of amino acids is observed – namely, compared with the control, there was a decrease in the blood plasma of proline, methionine, tyrosine, glutamic acid, and aspartic acid. The content of asparagine, glutamine, valine, tryptophan and threonine also decreased. The content of leucine and cysteine did not change. Amino acids in the urine are increased. Compared to the control, the level of glycine, asparagine, aspartic acid and tryptophan decreased. The level of valine, glutamine, threonine also moderately decreased, the content of tyrosine and phenylalanine did not change, the level of cysteine, proline, leucine and alanine.

The effect of EMF on rats treated with a food supplement (group C) turned out to be significantly different.

Out of the plasma monoamino monocarboxylic amino acids, the levels of alanine and leucine did not change, valine and threonine increased and glycine decreased, while in the urine the level of valine and glycine increased, leucine, threonine decreased, and alanine did not change. Out of the monoamino dicarboxylic acid amino acids in plasma and urine, the levels of aspartic and glutamic acids, as well as asparagine and glutamine, increased significantly. Among sulfur-containing amino acids, plasma and urinary methionine levels increased, while plasma cysteine levels

increased and remained unchanged in plasma. In the group of aromatic amino acids, plasma tyrosine levels increased, while plasma and urine phenylalanine levels did not change. The content of heterocyclic amino acids (proline and histidine) in plasma remained unchanged, while in urine it increased and decreased respectively. Tryptophan levels in plasma and urine increased.

As a result of exposure to EMF, significant changes were observed in relation to biogenic amines. In plasma, there was a decrease in the level of adrenaline, norepinephrine and dopamine, while in the urine only a decrease in dopamine.

And the levels of norepinephrine and adrenaline significantly increased (A-B). The content of serotonin in plasma decreased, in urine it did not change, but the level of 5-indoacetic acid increased significantly.

The effect of the magnetic field on rats treated with a food supplement (group C) turned out to be significantly different.

The levels of dopamine, norepinephrine, adrenaline and serotonin in plasma increased significantly in group C rats compared to group B, while the content of dopamine, adrenaline and 5-indolacetic acid in the urine increased. Serotonin levels have not changed.

The following observations were made in the "open field". Comparative analysis of the behavior of control (A) and grape-seed oil-fed rats (B) groups in the "open field" showed a difference in motor activity. The latent period of exit from the central circle changed, which turned out to be shorter in the rats of the control group (1.9 ± 0.8 instead of 3.2 ± 0.6 seconds). For group B, the number of crossed lines is much higher compared to the rats of the control group A (48.6 ± 2.0 and 42.8 ± 5.0 seconds). The difference is significant both in the time spent on translocation and in the percentage of time spent on translocation in relation to the total time spent on the experiment. From this it follows that the rats of group B move faster compared to the control ones and spend on average less time to overcome the cells – $30.0 \pm 4.2\%$ instead of $35.6 \pm 4.0\%$. This conclusion is confirmed by the number of cycles of immobility. The table shows that in rats of the control group these indicators are higher (13.2 ± 0.2 sec.) than in animals of group B (4.1 ± 0.5 sec). In addition, the rats of groups A and B also differ in their orientational activity in the open field. The number of vertical rises was 17.0 ± 1.01 (group A), and in group B rats it was 22.1 ± 0.5 . The average time of vertical risings for control animals was 0.8 ± 0.2 sec., and for group B it was 1.6 ± 0.3 sec. An increase in the number of vertical standing and a significant increase in the time spent on orientation while in one or another compartment means that the rats are examining the compartment in which they are located. Comparison of the stereotypical activity of rats of groups A and B in the open field showed that, although the number of grooming cycles in animals differs little, the rats of group B spend 9.7 ± 0.3 seconds per cycle, and the control rats – 4.9 ± 0.1 seconds.



Thus, based on the data obtained, it can be concluded that rats treated with grape seed oil are characterized by lower anxiety, which is manifested in fewer urinations and boluses, a high number of total grooming times, and a small number of translocations. As a result of exposure to a magnetic field (group C), the behavior of rats in the open field changed significantly compared to the indicators of the control group A.

The exit time from the central circle significantly decreased (1.2 ± 0.5 sec and 1.9 ± 0.8 sec, respectively), the number of crossed cells significantly decreased (34.4 ± 2.9 and 42.8 ± 5.0 sec, respectively), and the number of immobility cycles also decreased (10.1 ± 1.8 and 13.2 ± 0.2 sec), increased the specific time of immobility during the testing period (50.2 ± 4.0 and $30.3 \pm 4.7\%$) and translocation during the testing period (38.0 ± 3.5 and $35.6 \pm 4.0\%$), respectively. Thus, they move more slowly, as a result of which the number of crossed cells decreases. Also in group C, there is a very low number of rises to the paws, which is especially reduced in the middle circle and adjacent cells (9.8 ± 1.0 and 17.0 ± 1.01 , respectively). This may be due to a decrease in the activity of the central nervous system, as a result of which the number of orientation reflexes decreases, and the number of urination and boluses increases sharply (2.5 ± 0.1 and 8.0 ± 0.2). the average number of grooming cycles (4.5 ± 0.7) compared with group A (11.1 ± 0.2) drops sharply against the background of an increase in its total time (6.8 ± 0.1 sec). Behavior of group D rats in a magnetic field and those fed with grape seed oil significantly differs from the behavior of group C animals. The data indicate low anxiety. The specific time spent on translocation for group C animals is 38.0 ± 3.5 , and for group D animals it is $45.8 \pm 0.6\%$. The specific time of immobility in rats of group C was $50.2 \pm 4.0\%$ sec, and for animals of group D it was $35.9 \pm 0.7\%$, while the number of immobility cycles increased significantly (16.8 ± 0.3), the time to exit from the central circle (3.8 ± 0.4 sec) and the number of crossed lines (40.9 ± 0.5). The rats of group D outperformed group C with a significant increase in the number of vertical standings (14.8 ± 0.6) and their duration (1.9 ± 0.5 sec), as well as in the average number of grooming cycles (7.4 ± 0.5) and their total time (9.6 ± 0.3 sec). The mean number of boluses (1.2 ± 0.4) and frequency (1.5 ± 0.4) appeared to be reduced.

Thus, as a result of exposure to EMF in blood plasma and urine, a certain change in the level of individual amino acids is observed, which themselves or through derivatives affect the functional state of the central nervous system. Increased levels of GABA and glycine. It is accompanied by a decrease in serotonin levels. These shifts not only affect the processes of activation of the brain, but also contribute to the emergence of dysfunction of behavioral processes, which manifests itself in a change in the activity of animals and a decrease in plasma glutamate and aspartate with a parallel decrease in GABA and glycine. Of great importance is a significant change in the qualitative ratio of the amino acid composition of the

plasma, followed by a change in the balance of functionally opposite mediators, the pathogenetic significance of which is the regulation of the functional activity of the central nervous system. In plasma, there is a decrease in the level of serotonin, in the urine only 5-hydroxyindoleacetic acid. The ratio of the levels of dopamine and norepinephrine to serotonin revealed a significant increase in this indicator, which convincingly indicates a decrease in the relative amount of serotonin, that is, there is a pronounced activation of the kynurenine pathway of tryptophan metabolism, which leads to an imbalance of functional processes. Deviation from the optimal level and ratio of biogenic amines and free amino acids, caused by the influence of a magnetic field, changes the biochemical and physiological processes directly or indirectly associated with the implementation of behavioral acts. The change in the level of amino acids and their derivatives in biological fluids and tissues is one of the most important causes of multiple pathology, which manifests itself not only in the dysfunction of the nervous system, but also contributes to the development of a number of nervous and mental disorders as a result of the action of EMF, especially in ontogenesis [12,13].

A food supplement (grape seed oil) contributed to a certain regulation of the changes caused by exposure to EMF. In the group of rats irradiated with EMF and treated with a food supplement, there is an increase in plasma glutamate and aspartate, which is accompanied by a decrease in GABA and glycine, which helps to optimize the ratio of activating/inhibiting amino acids. Obviously, a change in the balance of functionally opposite mediators contributes to the process of regulating metabolic shifts. The above problems of the effect of EMF and food additives on the level of individual biochemical constants of the organism and the holistic behavior of animals require further study. Meanwhile, multiorgan lesions characteristic of EMT, according to modern data are largely associated with the primary violation of the functional processes of the CNS, including primary cerebrovascular disorders [14,15]. In this regard, the study of the impact of the electromagnetic field on various forms of behavior against the background of food additives can be of practical importance.

Discussion

The data obtained indicate that the decrease in anxiety after a certain time after the use of the herbal preparation is due not only to the residual effects of organic changes in the brain structures associated with the organization of behavior, in particular, spe-



cific glutaminergic neurons of the “attack center” of the hypothalamus but also with the activation the entire adaptive-compensatory system in the process of restitution from exposure to EMF. The clarification of these issues will deepen the current understanding of the chronic effects of industrial EMF (frequency 50 Hz, inductance 1.5 MT) and the effect of grape seed oil on neurochemical correlates and dysfunction of the nervous system of animals.

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References:

1. Grigoriev YuG. Electromagnetic fields and public health. *Hygiene and Sanitation*. 2003;3:14-16.
2. Kikalishvili B, Zurabashvili D, Nikolaishvili M, Zurabashvili Z, Giorgobiani I. Fatty acid composition of grape seed oil and its action in the form of a dietary supplement. *Georgian Medical News*, 2011;191:73-77.
3. Kikalishvili B, Zurabashvili Z, Turabelidze D, Shanidze L. Fatty acids of grape seed oils, velvet tree bark and amaranth seeds and their biological activity. *Georgian Medical News*, 2012;11(212):73-76.
4. Medicinal plants: Pharmacological and phytochemical studies. Kazakhstan. Proceedings of the international conference, “Pharmacy Kazakhstan” 2009; :87-91.
5. Nadareishvili K, Meskhishvili I, Ormotsadze G, Nadareishvili D, Iordanishvili G, Nikolaishvili M. // Proceedings of the Academy of Sciences of Georgia (biological series). 2003;29(5-6):667-672.
6. Nadareishvili K, Meskhishvili I, Nadareishvili D, Ormotsadze G, Nikolaishvili M, Iordanishvili G. // Radiation biology. Radioecology. 2005;45(2):133-145.
7. Nikolaishvili M, Tamasidze A. Influence of the electromagnetic field

- of the mains frequency on rats during intermittent or continuous exposure. *Georgian Medical News*, Tbilisi, 2006;12: 91-94.
8. Fetisova A, Pinkov V, Pikhodkiy A. Features of modern methods of obtaining fatty oil from vegetable raw materials and promising technologies. *Proceedings of the VII International Congress "Actual problems of creating new drugs of natural origin"*, SPB: 2008; :93-99.
 9. Borjanovic SS, Jankovic S, Pejovic Z. Related Articles, Links ECG changes in humans exposed to 50 Hz magnetic fields. *J Occup Health*. 2005;47(5):391-396.
 10. Catecholamine on supelcosilcolumns, catecholamines and metabolites typically found in blood plasma and urine. Supelco, chromatography supplies. Macherey-Nagl-Duten M.V. HPLC programme. U84/03. Getmany. Separation of catecholamines in urine and plasma. U.S. EPA-600 Nethocls. 2014; – 67.
 11. Dasdag S, Sert C, Akdag Z, Batun S. Effects of extremely low frequency electromagnetic fields on hematologic and immunologic parameters in welders. *Arch Med Res*. 2002;33(1):29-32.
 12. Jeong JH, Choi KB, Yi BC, et al. Effects of extremely low frequency magnetic fields on pain thresholds in mice: roles of melatonin and opioids. 2000;20:259-264.
 13. Mchedlishvili N, Nikolaishvili M, Omiadze N, Abutidze M, Gulua L. Natural food red colorant with radioprotective properties. *Proceedings of Georgian Academy of Sciences Biological Series*. 2009;7(3-4).
 14. Nikolaishvili M, Iordanisvili G, Jariashvili T, Petriashvili E, Museliani T. Effect of power-frequency electromagnetic field (EMF) on aggressive behavior at stages of ontogenesis. *Bulletin of the Georgian Academy of Series*. 2009;35(1-2):49-53.
 15. Waters chromatography system and chemistries and biological application. Division of Bohm and Haas 1196. Switzerland. *Amino Acids by Reversed Phase HPLG*. 2014:6.