LOW-FREQUENCY LOW INTENSITY MAGNETIC FIELD (50 Hz; 2,7 mT) INFLUENCES ON HEMATOLOGICAL PARAMETERS FOLLOWING CHEMICAL SYMPATHECTOMY IN WISTAR RATS

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Keywords: substantia nigra, low-frequency low intensity magnetic field, 6-OHDA, hematological parameters

Abstract: Involving of central dopaminergic pathways and electromagnetic field (ELF-EMF) (50 Hz; 2.7 mT) in erythrocytes dynamic modulation was assessed in right-unilateral substantia nigra lesioned Wistar rats. Specific lesions of the dopaminergic neurons located in substantia nigra pars reticulata were produced with 6-hydroxydopamine (6-OHDA) (8µg/4µl i.c.v., Sigma), a noradrenergic-selective neurotoxin. The rats were pretreated 30 min before the 6-hydroxydopamine infusion with 25 mg/kg i.p. desipramine (Sigma) to protect noradrenergic projections. 12 days after the operation, the hematological parameters (the total number of erythrocytes, hematocrit and hemoglobin level) were evaluated. The sympathectomy-induced severe reduction in hematological parameters under low-frequency low intensity magnetic field exposure.

INTRODUCTION

A link between the central nervous system and the immune system has been demonstrated via two major routes, the hypothalamic-pituitary-adrenal axis and the autonomic nervous system (Madden and Felten, 1995). Our laboratory has examined the immunomodulatory role of the autonomic nervous system by focusing on sympathetic noradrenergic innervation (Hritcu et al., 2006). 6-OHDA is taken up selectively by noradrenergic (NA) nerve fibers via a high-affinity catecholamine uptake carrier. 6-OHDA rapidly destroys NA nerve fibers in the periphery and depletes norepinephrine (NE) in lymphoid organs by more than 90% (Madden et al., 1994a). Sympathectomy-induced changes in T and B cell responses have been examined most extensively in immune and non-immune mice (Miles et al., 1981; Madden et al., 1989, 1994a,b; Kruszewska et al., 1995). The effects of sympathectomy are dependent on the strain of mouse, the lymphoid organ assessed, and immune status of the mouse. For example, in the spleen of non-immune BALBrc mice, Con A-induced T cell proliferation was transiently reduced 3 days post-sympathectomy, in association with decreased production of the T cell-derived cytokines, interleukin (IL)-2 (a potent inducer of T cell proliferation) and interferon (IFN)-γ (an important regulator of cell-mediated immunity) (Madden et al., 1994b). The lymphoid organ-dependent effects of sympathectomy demonstrate the complexity of the interactions between the SNS and T lymphocytes (Madden et al., 1995). Part of the difficulty in studying SNS interactions with the immune system in vivo is the tendency of the organism to maintain homeostasis. If the activity of the SNS is reduced, the healthy organism can rapidly upregulate the availability of catecholamines and increase sensitivity of target cells to catecholamines.

An array of experimental studies has been conducted in different species to evaluate the effects of low-frequency (50-60 Hz) waves with different field intensities (0.1-1000 mT) on immune system. The available literature on immune function has both strengths and weaknesses. Most of the studies conducted to date have investigated a wide variety of immune function endpoints, including immune system structures, cell- and humoural-mediated immunity, and innate immunity (Mevissen, 1999). In our previous studies we reported that low-frequency low intensity magnetic field exposure is linked with severe abnormalities of the immune reactivity (Maniu et al., 2005; Hritcu et al., 2005; Maniu et al., 2006).

In this study, we have assessed the reactivity of rats following chemical simpathectomy with 6-OHDA under electromagnetic field (50Hz; 2,7 mT) exposure.

MATERIALS AND METHODS

Animals

The experiments were carried out on male Wistar rats weighing 180-200g at the start of the experiment. They were fed and allowed to drink water at libitum. They were housed under natural day/night conditions (22^0C, 50% umidity). Rats were acclimated to the new housing conditions for at least 1 week prior to drug treatment.

Neurosurgery and drug administration

The rats were anesthetized with sodium pentobarbital (45 mg/kg b.w. i.p.). The substantia nigra pars reticulata was lesioned right-unilateral by stereotaxic microinjections of eight migrograms (free base) 6-hydroxydopamine, dissolved in 4 µl physiological saline containing 0.1% ascorbic acid, administered through the Hamilton syringe over 4.50 min. The syringe was left in place for 5 min after injection before being slowly removed. The rats were pretreated 30 min before the 6-hydroxydopamine infusion with 25 mg/kg i.p. desipramine (Sigma) to protect noradrenergic projections. Sham-operated rats received an injection of desipramine, followed by vehicle only in the substantia nigra. The following

coordinates were used: 5.5 mm posterior to bregma; 2.0 mm lateral to the midline; 7.4 mm ventral to the surface of the cortex (Paxinos and Watson, 1998). The hematological tests were began 12 days after the operation.

After 12 days, whole heparinized blood was collected. To determine the total number of erythrocyte, hematocrit and hemoglobin level a COULTER® Ac·T™ 5diff CP were used.

ELF-EMF exposure

The rats were exposed to ELF-EMF (50Hz; 2.7 mT), 20 minutes daily, over a 5 days period, 1 week after the operation.

Histological control

The rats were killed with an overdose of sodium pentobarbital (100 mg/kg i.p.) followed by a transcardial infusion of 0.9% saline and a 10% formalin solution. The brains were removed and placed in a 30% sucrose/formalin solution. The brains were frozen and cut into coronal sections (50 μm) using a freezing microtome and stained with cresyl violet for verification of the point of the syringe needle. Only experimental data from lesions correctly located in the substantia nigra were used for statistical analysis.

Statistical analysis

Results were expressed as mean ± S.E.M. The results were analyzed statistically by means of the Student's "t" test. $p<0.05$ was taken as the criterion for significance.

RESULTS AND DISSCUSIONS

1. Effect of the chemical simpathectomy and ELF-EMF (50Hz; 2.7 mT) exposure on hematological parameters

Experimental data were registered 12 days after the 6-OHDA administration. 6-OHDA and ELF-EMF exposure impaired significantly the total number of erythrocyte ($p<0.03$) (Figure 1.), hematocrit value ($p<0.01$) (Figure 2.) and more significantly hemoglobin level ($p<0.0001$) (Figure 3.) compared with sham-operated groups.

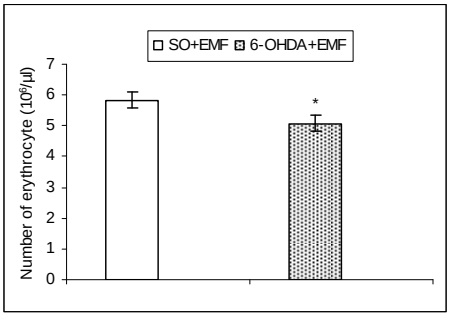

Figure 1. Changes of the total number of erythrocyte tested 12 days after chemical sympathectomy under ELF-EMF (50Hz; 2.7 mT) exposure. Values are means ± SEM (n=4 per group). *$p<0.03$ vs. control group.

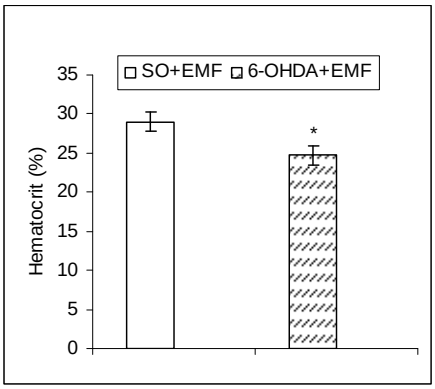

Figure 2. The effect of the chemical sympathectomy under ELF-EMF (50Hz; 2.7 mT) exposure on hematocrit value tested 12 days after the neurosurgery. Values are means ± SEM (n=4 per group). *p<0.01 vs. control group.

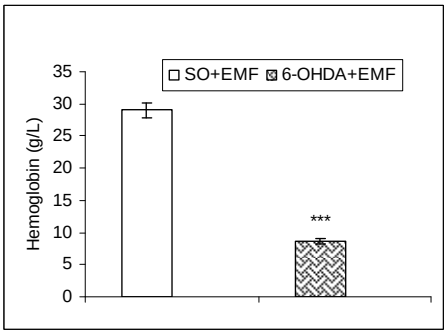

Figure 3. The effect of the chemical sympathectomy under ELF-EMF (50Hz; 2.7 mT) exposure on hemoglobin level tested 12 days after the neurosurgery. Values are means ± SEM (n=4 per group). *p<0.0001 vs. control group.

We have presented the evidence that substantia nigra pars reticulata have a crucial role in hematopoesis regulation in combination with ELF-EMF exposure). In our experiments we used procedure of ELF-EMF exposure following chemical simpathectomy by held rats orizontally in the tubes for a continuous 20 minutes, daily, during 5 days period, without food and water, which is particularly stressful. By means of this particularly stressful we observed considerably decrease of hematological parameters registered on 12th day after 6-OHDA administration, tested by the total number of erythrocyte, hematocrit value and hemoglobin level.

The presence of adrenergic receptors on erythrocyte demonstrates a modulatory role of noradrenergic system in hematopoiesis.

An array of experimental studies has been conducted in different species to evaluate the effects of EMF (50 Hz) waves with different field intensities. The influence on different immune response after EMF exposure depends of the time and period of exposure as we concluded by literature. The available literature reveals different data about EMF exposure effects. In our study EMF exposure present suppressive after 5 days period of exposure.

In view to describe the mechanisms involved in substantia nigra and EMF actions on the hematological parameters of rats, future research is required.

CONCLUSIONS

On the basis of our results obtained by 6-OHDA administration, we can conclude that in the rats, the central dopaminergic neurons from substantia nigra have a crucial role in hematological processes under magnetic field exposure (50 Hz; 2, 7 mT).

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Acknowledgements. This research was supported by the National Council of Scientific Research and University Education (CNCSIS), Romania.

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