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STEREOLOGICAL ANALYSIS OF THE ADRENAL GLAND OF FEMALE RATS AFTER EXPOSURE TO AN ELECTROMAGNETIC FIELD

GORDANA UŠĆEBRKA*, D. ŽIKIĆ*, MILICA MATAVULJ**, VERICA MILOŠEVIĆ*** and B. LAŽETIĆ****

*Faculty of Agriculture, Dositeja Obradovića 8, Novi Sad, Yugoslavia, **Institute of Biology, Faculty of Science,, Novi Sad, Yugoslavia, ***Institute for Biological Research "Siniša Stanković", Beograd, Yugoslavia, ****Medical Faculty, Novi Sad, Yugoslavia

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Some results about the long-term effect of a low frequency electromagnetic field (LF-EMF; 50Hz) on the functional activity of the adrenal gland of female rats, strain Mill Hill, are presented in the paper. Stereological analysis of the cortex and medulla of the adrenal gland was performed using the M42 multipurpose test system (Weibel, 1979). Every tenth section of serially cut gland was measured, at the enlargement 100X and the thickness of the sections was 5 μm.

Exposure to LF-EMF caused an increase of body weight (13.59%; $p<0.05$) and a decrease of absolute (18.03%; $p<0.05$) and relative weight of the adrenal gland (13.03%; $p<0.01$). Stereological analysis indicated a smaller relative volume of the adrenal cortex ($p<0.05$) and increased the relative volume of the adrenal gland medulla ($p<0.05$).

Clear differentiation of all zones was seen: zona glomerulosa, zona fasciculata, zona reticularis in both treated and control groups of animals. Changes in structure were clearly visible in the adrenocorticocytes of zona fasciculata and zona reticularis in treated animals. Prominent hyperemia was seen also, in the cortex as well as in the medulla of adrenal glands of the treated animals. In the medulla adrenalocytes and noradrenalocytes could be seen and between them there were exceptionally widened blood vessels in the treated animals.

These results suggest that long term exposure to LF-EMF has a biological effect on the structure of the adrenal gland of female rats within six months.

Key words: adrenal gland, electromagnetic field, stereology

INTRODUCTION

The effect of magnetic fields on biological mechanisms is still poorly understood. It was considered for a long time that the effect of magnetic fields was identical to the effects produced by high and low temperatures, ultraviolet rays and noise (Nakagawa, 1984). However, magnetic fields in the organism, besides a thermoregulative response may cause neuroendocrine and neurochemical modulations as well as reactions concerning behavior (Michaelson, 1982). Magnetic fields have been shown to influence the release of a number of neurotransmitters (Kavaliers and Ossenkopp, 1987), with these actions possibly involving alterations in neuronal calcium levels and fluxes (and possibly other divalent ions) and in the stability of calcium binding to neuronal membranes (Blackman et al., 1985, Kavaliers and Ossenkopp, 1986).

The aim of this work was to simulate LF-EMF, of intensities that can be met in the environment and to find out how the EMF affects the adrenal gland, which is one of the indicators of neuroendocrine balance.

MATERIAL AND METHODS

The effect of LF-EMF was investigated on five treated and five control laboratory female rats of the Mill Hill strain.

The treated animals were exposed to alternating EMF beginning from 24h after birth to six months of age, 7 hours a day, 5 days a week. At the beginning of the experiment the animals were housed together. After 6 weeks, when the sexes should be separated, the females were separated from the males.

The exposure system, by which the LF-EMF was produced, consisted of a single coil of 4 mm thick wire placed in 1320 turns. The coil was energized from standard 220 V, 50 Hz and 16 A outlets via an autotransformer. The autotransformer provided a 60 V output and was used in order to reduce the electric field which was measured as less than 10V/m everywhere in the room. One half of the coil had a south-east/north-west orientation of axes, and the other half was orthogonal to that direction. Cages with animals were placed symmetrically on both sides of the coil. The coil produced a magnetic field of decaying intensity along the cages from 500 μT to 50 μT.

At the age of six months the animals were weighed and slaughtered. Adrenal glands were isolated, weighed and fixed in Bouin's fixative and embedded in paraffin.

For light microscopy stereological investigations, the whole left adrenal gland was serialy cut at the thickness of 5 μm. Stereological measurements were made on each section by means of the M42 multipurpose testing system (Weibel, 1979) at a magnification 10x10. Of the stereological parameters the volume of cortex, medulla and zones was estimated. Statistical analysis was done on a personal computer, using the Microstat program and the significance of differences was tested by the Student's "t" test.

RESULTS

In Table 1., the results are presented for the weight of the animals and their adrenal glands.

Table 1. Body weight and absolute and relative weight of the adrenal glands in control and treated female rats

Group	Body weight (g)	Adrenal gland weight	
		Absolute (mg)	Relative (mg%)
Control	206 ± 15.2	71.2 ± 8.5	34.6 ± 3.4
Treated	234 ± 11.4*	58.2 ± 6.6*	29.4 ± 3.2**

Mean ± S.D
*p<0.05, **p<0.01

Significant differences were found in the body weight, absolute weight of the adrenal glands ($p<0.05$) and relative weight of the adrenal glands ($p<0.01$), between the control and treated animals.

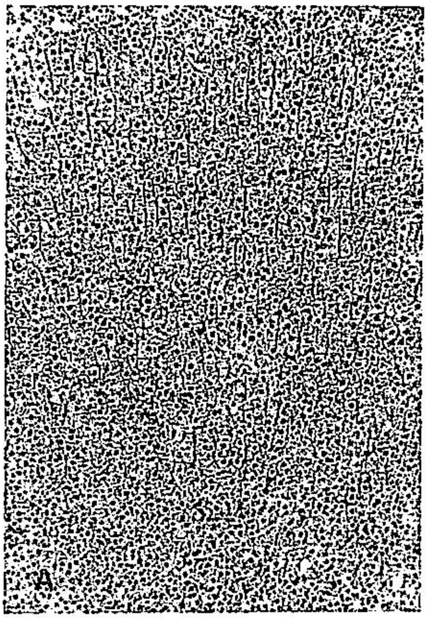

Figure 1. Relative volume (R.V.) of cortex and medulla of adrenal glands in the control and treated animals

The values for the relative volumes of cortex and medulla in the control and treated animals are presented in Figure 1. A statistically significant difference in the relative volumes of cortex and medulla was found between the control and treated animals ($p<0.05$).

In Figure 2., where the values for relative volumes of individual zones are given, it may be seen that there was no statistically significant difference between the zones of control and experimental animals.

Analysis under the light-microscope indicated a clear differentiation of all zones of cortex. It was found that the cortex of treated animals was narrower.

The outer zona fasciculata in control animals was made up of numerous large light spongiocytes regularly distributed, while in treated rats both light and dark individual spongiocytes were observed in the zona fasciculata and a change of fasciculate structure was seen (Figure 3).

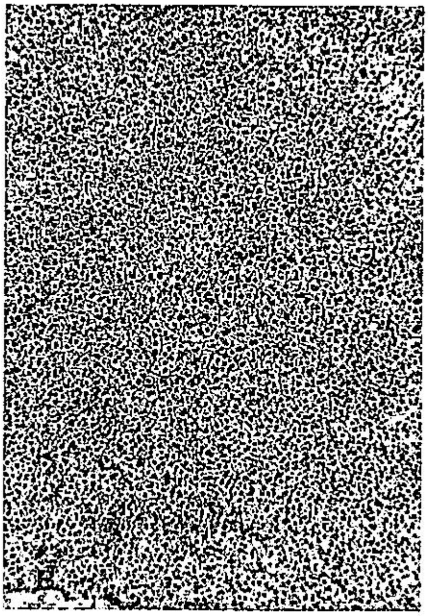

Figure 2. Relative volume (R.V.) of cortical zones of adrenal glands in the control and treated animals

Zona reticularis of the control animals was characterized by adrenocorticocytes of smaller diameter than zona fasciculata, distributed in the form of a net. Zona reticularis of animals exposed to LF-EMF was characterized by dilatation of the blood vessels, great differences in size and color between adrenocorticocytes (Figure 4), pyknosis of nuclei and necrosis of cells.

The medulla of the adrenal gland was made up of medullocytes, connective tissue, nerves and blood vessels. Adrenalocytes were numerous in control and treated animals, but the treated animals had more noradrenalocytes, cells with dark cytoplasm, that control animals. Marked hyperemia was characteristic for the medulla in animals exposed to alternating LF-EMF (Figure 4).

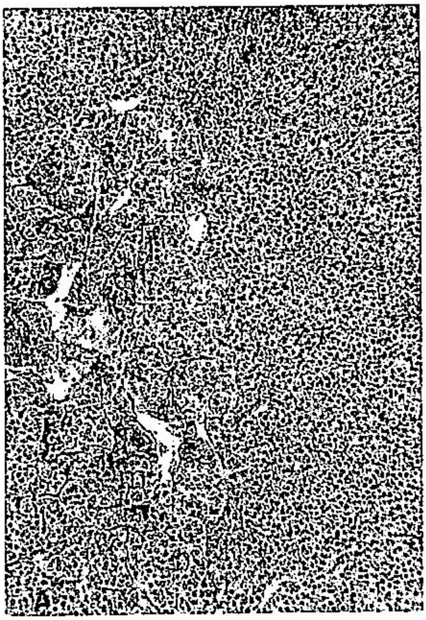

Figure 3. Adrenal cortex of control (A) and treated (B) animals (H&E; 63X1.6)

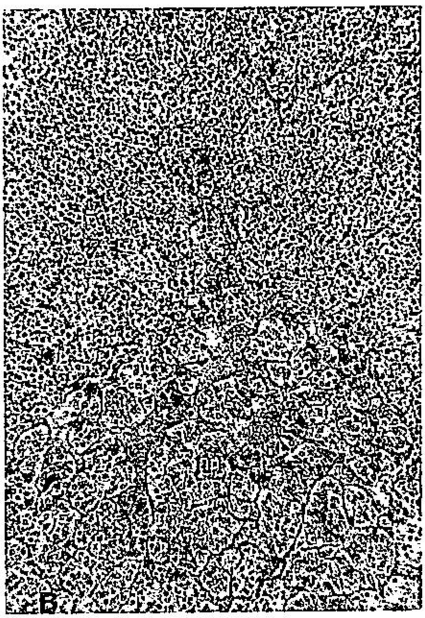

Figure 4. Zona reticularis (zr) and medulla (m) of control (A) and treated (B) animals; dilatated blood vessels (∧) (H&E; 63X1.6)

DISCUSSION

The results obtained show a decrease of the relative volume of cortex by 4.21%($p<0.05$) and an increase of the relative volume of medulla by 4.22% ($p<0.05$) in the adrenal glands of treated female rats associated with long term alternating LF-EMF treatment.

It is clear that LF-EMF as a factor in the environment represents an important ecological influence, which is able to cause a complex adaptive reaction of the organism. The effects of LF-EMF on these structures depend on the frequency, intensity and time of exposure (Tenford and Kaune, 1987, Glaser, 1992). LF-EMF represents a stressogenic factor which increases the activity of all parts of the sympathoadrenal system (Saharov et al. 1981). LF-EMF elicits a nonspecific adaptive reaction (Zagorskaya, 1989, Zagorskaya et al. 1990). The latent period for reaction of an organism to EMF is longer than in other reactions and therefore some researchers overlooked the effect of EMF on the organism (Bogoljubov, 1981). Changes in growth and weight of the endocrine glands suggest possible disturbance of the endocrine system and biological reaction caused by LF-EMF (Mikolajczyk et al. 1993.)

Our results show that LF-EMF of the strength tested, expresses a depressive action on the adrenal cortex, which is in agreement with the data of Kuzmina (1984) who found that chronic exposure to constant MF at first increases the activity, to be replaced by depression of the adrenal cortex after 15 days of exposure.

Morphological investigations point to reduction of cortical layer as well as to disturbance of architectonics, especially in zona fasciculata and reticularis. Navakatikyan and Marzeev, (1992), showed increased activity of zona glomerulosa of the adrenal gland after 2 months of irradiation by constant microwaves and decreased activity of zona reticularis and increased activity of zona fasciculata after 2 months pulsed microwaves. The cytological changes in the adrenocorticocytes of animals exposed to LF-EMF are in agreement with the results of Jager et al. (1992).

Since chronic stress may cause an increase of lipid droplets in all three zones of the adrenal cortex (Nussdorfer, 1986) changes in the adrenal cortex of animals exposed to EMF have the character of a stress effect, although the mechanisms of the effect are still insufficiently clear. Cain et al., (1992), pointed out that LF-EMF does not have enough energy to damage DNA covalent bonds directly and that its effect on biological systems is achieved through the cell membrane. EMF affects the cell membrane, and intracellular enzymes adenylcyclase and phosphatylethylesterkinase (Blank et al. 1986, Dacha et al. 1993), and could influence a transport protein in the membrane, leading to modification of the flux and subsequently to changes of cellular ion concentrations (Glaser, 1992). In vitro experiments, (Cain et al. 1985, Ferndale and Murray 1986), showed that LF-EMF modifies migration of receptors in the cell membrane, shortens the time of residence of hormones on the membrane surface, which hinders mobilization of Ca^{++} ions ad reduces the activity of adenylcyclase.

Thus, data of other authors (Kuzmina, 1984, Zagorskaya and Rodina, 1990, Jager et al., 1992, Navakatikyan and Marzeev, 1992) and our previous (Ušćebrka et al., 1995) and present results indicate that the adrenal gland, like the whole endocrine system is sensitive to LF-EMF. We suggest that our experimental model may be useful for further verifyng the mechanism of interaction between LF-EMF and the adrenal gland.

Acknowledgements

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STEREOLOŠKA ANALIZA NADBUBREŽNE ŽLEZDE ŽENKI PACOVA POD UTICAJEM ELEKTROMAGNETNOG POLJA

UŠĆEBRKA GORDANA, D. ŽIKIĆ, MILICA MATAVULJ, VERICA MILOŠEVIĆ i B. LAŽETIĆ

SADRŽAJ

U radu su izneti rezultati o dugotrajnom delovanju promenljivog elektromagnetnog polja (EMP) niske frekvencije (50 Hz) na funkcionalnu aktivnost nadbubrežne žlezde ženki pacova soja Mil Hill.

Stereološka analiza kore i srži nadbubrežne žlezde rađena je višenamenskim test sistemom M42 (Weibel, 1979). Meren je svaki deseti rez serijski isečene žlezde, na uveličanju 100x, a debljina reza je bila 5 μm.

Delovanje niskofrekventnog EMP je dovelo do povećanja mase tela (13.59%; $p<0.05$), smanjenja apsolutne (18.03%; $p<0.05$) i relativne mase nadbubrežne žlezde (13.03; $p<0.01$). Stereološkom analizom je konstatovan manji relativni volumen kore nadbubrežne žlezde ($p<0.05$) i povećan relativni volumen srži nadbubrežne žlezde ($p<0.05$).

U obe grupe životinja uočava se jasna diferencijacija svih zona: zona glomerulosa, zona fasciculata, zona reticularis. Promene u strukturi se uočavaju u adrenokortikocitima zone fasciculate i zone reticularis u tretiranih životinja. Takođe je uočena izrazita hiperemija kako u kori tako i u srži nadbubrežne žlezde tretiranih životinja.

U srži nadbubrežne žlezde mogu se uočiti adrenalociti i noradrenalociti, a između njih se uočavaju izrazito prošireni krvni sudovi u oglednih životinja.

Ovi rezultati ukazuju da dugotrajno delovanje promenljivog EMP ima biološki efekat na funkciju nadbubrežne žlezde.