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CHRONIC EXPOSURE TO ELECTROMAGNETIC FIELDS: EFFECTS ON THYROID PARAFOLLICULAR CELLS IN RATS

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Abstract: Based on the presented results and the previous data on thyroid follicles, it could be concluded that (i) PF cells and follicular cells reacted to EMF exposure similarly by their decreased activity, (ii) PF cells are the endocrine cell „population" in the thyroid less susceptible to EMFs, and (iii) the activation of PF cells after the first recovery week following EMF exposure indicates a possible moderate involvement of these cells in overall thyroid reaction to certain environmental influences.

Key words: Parafollicular cell, thyroid gland, electromagnetic field, stereology

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INTRODUCTION

Parafollicular (PF) cells in the thyroid gland are known to produce mainly calcitonin which is involved in the homeostasis of calcium, but also a number of other regulatory peptides affecting TSH (thyroid stimulating hormone)-regulated thyrocyte activity (reviewed in Sawicki, 1995). Although PF cells are distinct from follicular cells by their embryologic origin and physiology, recent experimental studies indicate that PF cells are subject to the regulatory control of pituitary TSH and indicate a probable interaction between PF and follicular cells in a paracrine manner (Morillo-Bernal et al., 2009).

Electromagnetic fields (EMFs) originating from artificial sources, primarily power lines and electric appliances, are widespread in the human living and working environment. Antropogenic influences and animal studies are considered and carried out at both an extremely low- (50/60Hz) and high-frequency range. Literature data regarding the biological relevance of 50/60 Hz EMFs demonstrate a variety of morphophysiological effects including the endocrine system and the thyroid. Previous experimental studies demonstrated a decrease or increase in the thyroid activity in rats after different durations of exposure to EMFs as measured by serum levels of thyroid hormones or judged by the thyroid morphological features (Udintsev et al., 1978; Zagorskaya and Rodina, 1990; Rajkovic et al., 2006). However, studies on EMF effects on PF cells are very scarce. Boorman et al. (1999) demonstrated the significant increase in the combined incidence of PF cell adenomas and carcinomas of the thyroid in male rats, while Oglodek and Mos (2006) found a significant increase of PF cells in female rats after exposure to EMFs.

In the present study, the potential of 50 Hz EMFs to affect PF cells in male rats using morphological and morphometrical criteria was evaluated. The status of these cells in three recovery time lags after the EMF exposure was estimated as well.

MATERIALS AND METHODS

The experiment was performed on 72 male rats of Mill Hill strain. Animals were housed in laboratory conditions at 22±2°C and subjected to a natural photoperiod. Access to tap water and pelleted food

was unlimited. A total of 36 animals were exposed to the influence of 50 Hz EMFs (50-500 μT) (AC milligauss meter, model 42B-1, Monitor Industries, USA) from the second post-natal day (PND), 7 h/day, 5 days/week during 3 months. For the EMF treatments, the exposure system was used as previously described (Rajkovic et al., 2003). Thirty-six animals served as controls and were maintained in a separate room free of any appliances involved in generation. The investigation was made with the permission of the Ethical Committee on Animal Experiments of the University of Novi Sad.

After the exposure period of 3 months, the first group of 14 animals (group I) was sacrificed. The rest of the animals were subjected to recovery evaluation of the thyroid PF cells and sacrificed after 1 week (n=14) (group II), 2 weeks (n=14) (group III) and 3 weeks (n=14) (group IV) following the 3-month exposure to EMFs.

Thyroids with adjacent parts of trachea and surrounding connective tissue were fixed in Bouin's solution (picric acid: Merk, Darmstadt, Germany), embedded in paraffin and cut on a rotation microtome in 5 μm thick sections. Thyroids designated for semi-fine sections were removed from trachea and fixed in 4% glutaraldehyde (Merck, Darmstadt, Germany), post-fixed in 1% osmium-tetroxide (Fluka, Basel, Switzerland), embedded in epon resin (Merck, Darmstadt, Germany) and cut on a ultra-microtome (LKB, Bromma, Sweden) in 1 μm thick sections. Histological analysis of PF cells was performed on paraffin sections stained with a silver impregnation method (silver nitrate: Merk, Darmstadt, Germany, hydroquinone: Sigma, Deisenhofen, Germany) according to Fernandez Pascual as well as on semi-fine sections stained with toluidine blue-cresyl violet (Carlo Erba, Milano, Italy; Edward Gurr Ltd., London, UK, respectively).

Stereological analysis was performed on every fourth serial paraffin section using a multi-purpose stereological grid M42. A total of 60 fields of vision per animal were analyzed under 1000x magnification. The numerical and volume densities of PF cells were determined. A non-parametric Mann-Whitney U test was used for statistical analysis of the differences between each of the control and of the exposed groups. *P* values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

The use of the silver impregnation method yielded strong, dark cytoplasmic granule staining which

Table 1. Median values with the lower and upper quartiles (in brackets) are given of investigated stereological parameters for parafollicular cells in the thyroid gland of control and exposed animals. Nvp represent the numerical density and Vvp the volume density of parafollicular cells.

Experimental group	GROUP 1		GROUP 2		GROUP 3		GROUP 4	
Stereological parameter	Control	EMF	Control	Recovery week 1	Control	Recovery week 2	Control	Recovery week 3
Nvp (mm^{-3})	72536 (53182) (82054)	61919 (47882) (93997)	102104 (79719) (122423)	82093 (51947) (101578)	95853 (65876) (100883)	78989 (55839) (83342)	79178 (56974) (83991)	80730 (69115) (94067)
Vvp (mm^{0})	0.029 (0.025) (0.038)	0.03 (0.02) (0.039)	0.034 (0.029) (0.04)	0.03 (0.021) (0.037)	0.029 (0.025) (0.033)	0.028 (0.026) (0.033)	0.034 (0.026) (0.034)	0.034 (0.029) (0.037)

enabled a clear light microscopic identification, analysis and quantification of PF cells in the thyroid. The results revealed a decrease in PF cell number after the EMF exposure and after the 1st and 2nd recovery week as well as an increase in the volume of these cells, as evidenced by the light microscopic analysis of both paraffin and epon sections. Appearance of a number of PF cells with decreased argyrophil reaction pointing to cell degranulation was a prominent morphological finding in the experimental group subjected to 3 months' exposure to EMFs and the recovery period of 1 week. A detailed stereological analysis of PF cells supported the major histological findings, however the outcomes of the statistical analysis demonstrated no significant differences between the exposed groups and the corresponding controls (Table 1).

Findings on thyroid follicles and thyroid hormones (free serum T3 and T4) from the same experiment as the present study pointed to a significant decrease in thyroid activity after a 3-month exposure to EMFs and during recovery periods (Rajkovic et al., 2003). Regarding PF cells, our previous studies demonstrated an increased number of PGP (protein-gene product 9.5)-positive cells and decreased number of CGRP (calcitonin gene related peptide)-positive cells in male Wistar rats aged two months at the beginning of a 4 week exposure to EMFs (50 Hz, 100-300 μT) (Rajkovic et al., 2005). Taking into consideration these results, it could be assumed that a 3-month exposure to EMFs starting very early in post-natal life (PND 2) caused PF cells to adapt to the treatment. Consequently, following the EMF removal during the first recovery week, the reaction of PF cells was their activation as demonstrated by cell hypertrophy and degranulation.

Based on the presented results and the previous data on thyroid follicles, it could be concluded that: i) PF cells and follicular cells reacted to EMF exposure similarly by their decreased activity, ii) PF cells are the endocrine cell population in the thyroid less susceptible to EMFs, and iii) the activation of PF cells after the first recovery week following EMF exposure indicates a possible moderate involvement of these cells in overall thyroid reaction to certain environmental influences.

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REFERENCES

Boorman, G.A., McCormick, D.L., Findlay, J.C., Hailey, J.R., Gauger, J.R., Johnson, T.R., Kovatch, R.M., Sills, R.C, and *J.K. Haseman* (1999). *Toxicol. Pathol.* **27**, 267-278.

Morillo-Bernal, J., Fernandez-Santos, J.M., Utrilla, J.C., de Miguel, M., Garcia-Marin, R., and *I. Martin-Lacave* (2009). J. Anat. 10.1111/j.1469-7580.2009.01095.

Oglodek, E., and *D. Mos* (2006). *Fizjoterapia.* **14**, 64-69.

Rajkovic, V., Matavulj, M., Gledic, D., and *B. Lazetic* (2003). *Tissue Cell* **35**, 223-231.

Rajkovic, V., Matavulj, M., and *O. Johansson* (2005). *Environ. Res.* **99**, 369-377.

Rajkovic, V., Matavulj, M., and *O. Johansson* (2006). *J. Exp. Biol.* **209**, 3322-3328.

Sawicki, B (1995). *Acta Histochem.* **97**, 389-399.

Udintsev, N.A., Serebrov, V.Y., and *G.I. Tsyrov* (1978). *Bull. Exp. Biol. Med.* **86**, 544-546.

Zagorskaya, E.A., and *G.P. Rodina* (1990). *Kosm. Biol. Aviokosm. Med.* **24**, 65-60