

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

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Parafollicular (PF) cells in the thyroid gland are known to produce mainly calcitonin, involved in the homeostasis of calcium, but also a number of other regulatory peptides affecting the TSH (thyroid stimulating hormone)-regulated thyrocyte activity (reviewed in Sawicki, 1995). Although PF cells are distinct from follicular cells in their embryologic origin and physiology, recent experimental studies indicate that PF cells are subjected 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, and emanating primarily from power lines and electric appliances, are widespread in human living and working environments. Anthropogenic influences and animal studies are considered and carried out at both extremely low-(50/60Hz) and high-frequency ranges. Literature data regarding the biological relevance of 50/60 Hz EMFs demonstrate a variety of morphophysiological effects on the endocrine system and thyroid. Previous experimental studies demonstrated a decrease or increase in 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 in 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 EMF exposure was estimated as well.

The experiment was performed on 72 male rats of Mill Hill strain. The animals were housed in laboratory conditions at a temperature of $22\pm 2^{\circ}\text{C}$ and subjected to a natural photoperiod. Access to tap water and pelleted food was unlimited. A total of 36 animals were exposed to 50 Hz EMFs (50-500 μT) (AC milligaussmeter, model 42B-1, Monitor Industries, USA) from the second postnatal day (PND), 7 h/day, 5 days/week for 3 months. For the EMF treatments, the previously described exposure system was used (Rajkovic et al., 2003). Thirty-six animals served as controls and were maintained in a separate room free of any generating appliances. The investigation was made with permission of the Ethical Committee on Animal Experiments of the University of Novi Sad.

After the exposure period of 3 months ended, the first group of 14 animals (group I) was killed.

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

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

The rest of the animals were subjected to recovery evaluation of the thyroid PF cells and killed after 1 week (n=14) (group II), 2 weeks (n=14) (group III) and 3 weeks (n=14) (group IV) following the 3-month EMF exposure.

The thyroids and adjacent parts of the 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 the trachea and fixed in 4% glutaraldehyde (Merck, Darmstadt, Germany), postfixed in 1% osmium-tetroxide (Fluka, Basel, Switzerland), embedded in epon resin (Merck, Darmstadt, Germany) and cut on an ultramicrotome (LKB, Bromma, Sweden) into 1 µm thick sections. The histological analysis of the PF cells was performed on the 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 the 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 multipurpose 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 exposed groups. *P* values less than 0.05 were considered significant.

The use of a silver impregnation method yielded strong, dark cytoplasmic granule staining which enabled a clear light microscopic identification, analysis and quantification of PF cells in the thyroid. The results revealed a decrease in the PF cell number after the EMF exposure and after the 1st and 2nd recovery weeks, as well as increases in volumes of these cells, as evidenced by light microscopic analysis of both paraffin and epon sections. The appearance of a number of PF cells with a decreased argyrophil reaction pointing to cell degranulation was a prominent morphological finding in the experimental group subjected to 3 months' exposure to EMFs and a recovery period of 1 week. A detailed stereological analysis of the 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 type of experiment reported in a previous (Rajkovic et al., 2003), pointed to a significant decrease in thyroid activity after a 3 month exposure to EMFs and during recovery periods. With regard to PF cells, our previous studies demonstrated an increased number of PGP (protein-gene product 9.5)-positive cells and a 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 μ T) (Rajkovic et al., 2005). Having these results in mind, it could be presumed that a 3 month exposure to EMFs starting very early in postnatal life (PND 2) caused PF cells to adapt to the treatment. Consequently, following the EMF removal during the first recovery week, the reaction of the PF cells was their activation as demonstrated by cell hypertrophy and degranulation.

Based on the present results and the previous data on thyroid follicles from the same study, it could be concluded that 1) PF cells and follicular cells reacted to EMF exposure similarly by their decreased activity, 2) PF cells are the endocrine cell

population in the thyroid which are less susceptible to EMFs, and 3) the activation of PF cells after the first recovery week following EMF exposure indicate a possible moderate involvement of these cells in overall thyroid reaction to certain environmental influences.

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