Evaluation of rat thyroid gland morphophysiological status after three months exposure to 50 Hz electromagnetic field

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Abstract

Objective of our study was to use morphophysiological criteria in order to determine the sensitivity of male rat thyroid gland to an extremely low frequency electromagnetic field (ELF-EMF) influence and the ability of the gland to repair after period of exposure. Animals were exposed to 50 Hz, 50–500 μT ELF-EMF for 3 months when a part of them (group I) were sacrificed, while the rest of animals were subjected to recovery evaluation of the gland and sacrificed after 1 (group II), 2 (group III) and 3 (group IV) weeks. Histological and stereological analyses were performed on paraffin and semifine thyroid gland sections. Serum T3 and T4 were also determined. Histological and stereological analyses showed that the volume density of follicular epithelium and thyroid activation index decreased, while the volume density of colloid and capillary network increased in group I, II and III. The values of all these parameters in group IV were similar to corresponding controls. Serum T3 and T4 concentrations were significantly lower in all exposed animals, except in group I. Results of this study demonstrate that after significant morphophysiological changes caused by ELF-EMF exposure thyroid gland recovered morphologically, but not physiologically, during the investigated repair period.

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1. Introduction

In the last few decades, various and profound investigations have been carried out in order to investigate the possible biological effects of extremely low frequency electromagnetic fields (ELF-EMF) on mammals, including the human population. Among others, the endocrine system was also an object in a number of these investigations. The endocrine gland that has drawn the most considerable attention was the pineal gland, particularly when the controversial relation between exposure to ELF-EMF and increased risk of certain cancers mediated by decreased melatonin concentration was proposed (Stevens, 1987; Loscher and Mevissen, 1994; Brainard et al., 1999; Graham et al., 2001). Regarding the endocrine system, besides pineal studies, the sensitivity of pituitary gland, adrenal gland and thyroid gland to electromagnetic field (EMF) influence was investigated too, as well as of the endocrine pancreas, testicles and ovaries (Ossenkopp et al., 1972; Zagorskaya, 1989; Picazo et al., 1995; Zagorskaya et al., 1990; Forgas et al., 1998; Burchard et al., 1998; Feria-Velasco et al., 1998; Uscebrka et al., 1999; Matavulj et al., 2000).

Our previous investigations of ELF-EMF influence on thyroid gland have shown that in toto exposure of rats to these fields alters thyroid activity (Matavulj et al., 1996). We have also found that the type of alteration depends on the duration of exposure and it is reflected as either increased or decreased activity of the thyroid. According to our findings, thyroid gland shows increased activity after 2 months of ELF-EMF exposure and decreased activity after 5 and 6 months, as measured by histological and stereological parameters (Matavulj et al., 1996).

In continuation to these experiments, as the aim of the present study we appointed the investigation of morphological and physiological characteristics of rat thyroid gland after 3 months of 50 Hz EMF exposure and during a recovery period of 3 weeks.
2. Materials and methods

Experiment was performed on 89 male rats of Mill Hill strain. Animals were housed in laboratory conditions with 22±2 °C temperature and subjected to a natural photoperiod. Access to tap water and pelleted food was unlimited. A total of 47 animals were exposed to the influence of ELF-EMF from 24 h after birth, 7 h a day (from 07:00 a.m. to 14:00 p.m.), 5 days a week for a period of 3 months. Forty-two animals served as controls and were maintained in a separate room free of any appliances involved in generation. The investigation was made with permission of the Ethical Committee on Animal Experiments of the University of Novi Sad.

The exposure system, which produced ELF-EMF, was made of a single coil of 2.5 mm thick copper wire placed on a wooden frame in 1320 turns. The coil was energized from standard 220 V, 50 Hz and 16 A via an autotransformer. The autotransformer provided 60 V output and was used in order to reduce the electric field which was measured to be 10 V/m. Cages with animals were placed symmetrically on both sides of the coil. The coil produced EMF of decaying intensity along the cages with a 500/H9262 T value on the opposite side.

After the exposure period, first group of 12 animals (group I) was sacrificed starting at 08:00 a.m. the next day following the last day of exposure. The rest of animals were subjected to recovery evaluation of the gland and sacrificed after 1 week (12 animals; group II), 2 weeks (12 animals; group III) and 3 weeks (11 animals; group IV) after 3 months of ELF-EMF exposure. Control animals were sacrificed with their corresponding ELF-EMF exposed animals.

Following sacrifice, removed thyroids were prepared for either standard histological sections or for semifine sections. Thyroid glands with adjacent parts of trachea and surrounding connective tissue were fixed in Bouin’s solution and processed using standard procedure for embedding in paraffin and cut on a rotation microtome in 5 µm thick sections. Thyroid glands with adjacent parts of trachea and surrounding connective tissue were removed from trachea and fixed in 4% glutaraldehyde, postfixed in 1% osmium-tetraoxide, embedded in epon resin (each lobe separately) and cut on a LKB ultramicrotome in 1 µm thick sections. Histological analysis of the gland was performed on paraffin slices stained with haematoxylin–eosin (H&E) and PAS alcian blue as well as on semifine sections stained with toluidine blue-cresyl violet.

Results of histological analysis were quantified by stereological analysis performed on up to 4 sections per sample and 60 test fields per animal. The volume densities of thyroid follicles, follicular epithelium, colloid, interfollicular tissue and capillary network were determined, as well as the activation index of the thyroid gland. The activation index, which represents the ratio of the volume density of follicular epithelium to the volume density of colloid was introduced by Kalsnik (1981). All histological and stereological analyses were made by the same researcher.

Blood samples were collected from each animal immediately after decapitation for the determination of triiodothyronine (T3) and thyroxine (T4) concentrations. After centrifugation serum was frozen at −20 °C until used. Kits produced by INEP Zemun (Yugoslavia) were used for radioimmunological analysis (RIA).

Results obtained from stereological and hormonal analyses were expressed as mean ± S.E. Statistical significance of differences between control animals and ELF-EMF exposed animals was determined using Student’s t-test.

3. Results

The most prominent morphological characteristics of the thyroid gland in animals sacrificed after 3 months exposure to ELF-EMF compared to controls, were the absence of apical protrusions on thyrocytes, rare presence of large colloid droplets in these cells and occasional appearance of follicles with extremely low epithelium (Fig. 1a). The same characteristics were detected in animals subjected to the recovery evaluation of the gland and sacrificed at the end of the first and second week after exposure (Fig. 1b and c). Thickness of thyroid follicular epithelium in animals sacrificed at the end of the third week following the exposure, was almost the same as in the corresponding control group (Fig. 1a and d). Rough epithelium–colloid border in thyroid follicles of these animals was noted too (Fig. 1d). Colloid in the follicles of all ELF-EMF exposed animals was amass, mainly homogenous and explicitly PAS positive.

Characteristic histological appearance of the gland in ELF-EMF exposed animals was substantiated with the results of stereological analysis.

In animals sacrificed immediately at the end of the exposure, stereological analysis of thyroid glands showed significant increase \( p < 0.05 \) of the volume density of thyroid follicles (Vvf) compared to the control group (Fig. 2). Volume density of follicular epithelium (Vve) was insignificantly lower in exposed animals, while the volume density of colloid (Vvk) was significantly increased \( p < 0.01 \); Fig. 2). As a consequence, activation index of thyroid gland \( 1a; \text{ratio of } Vve \text{ to } Vvk \) decreased significantly \( p < 0.01 \); Fig. 3). Volume density of interfollicular tissue (Vvi) decreased as well \( p < 0.01 \); Fig. 4). An increase of the volume density of capillary network (Vvc) in the gland of exposed animals was also noted, although it was not significant (Fig. 4). Serum T4 concentration was slightly, insignificantly higher than in the control group, while the level of T3 was reduced and as well insignificantly (Fig. 5a and b).

In animals subjected to recovery evaluation of the thyroid gland and maintained during 1 and 2 weeks after ELF-EMF exposure, values of the volume density of thyroid follicles were similar to corresponding controls (Fig. 6a and b).
Fig. 1. (a–d) Follicular epithelium of thyroid gland. (a) Control animal. Normal histological appearance of thyroid follicular epithelium with cylindrical thyrocytes of convex apical membranes. (b and c) Animal sacrificed after three months exposure to ELF-EMF and first recovery week and animal sacrificed after three months exposure to ELF-EMF and second recovery week, respectively. Absence of apical protrusions on follicular cells, plane epithelium–colloid border and amassed colloid. (d) Animal sacrificed after three months exposure to ELF-EMF and third recovery week. Rough epithelium–colloid border in thyroid follicles. Note the similar thickness of follicular epithelium with the control. H&E; 385x.
Volume density of follicular epithelium and activation index of thyroid gland were significantly decreased ($p < 0.01$) in both groups (Figs. 3 and 6a and b, respectively). Volume density of colloid significantly increased ($p < 0.01$; Fig. 6a and b) in each group of exposed animals and the capillary network as well ($p < 0.01$ after the first and $p < 0.05$ after the second recovery week; Fig. 4). Increased volume density of interfollicular tissue after the first recovery week was not statistically significant compared to control group, while it significantly decreased ($p < 0.05$) after the second week (Fig. 4).

Concentration of T4 decreased in exposed animals: significantly after first ($p < 0.01$) and insignificantly after second week of recovery period (Fig. 5a). Decreased concentration of T3 was significant after both first ($p < 0.05$) and second ($p < 0.01$) recovery week (Fig. 5b).

Thyroid gland of animals sacrificed at the end of the third recovery week, was generally characterized by restoration of stereological parameters to control values. This tendency refers to the volume densities of follicular epithelium, colloid, interfollicular tissue and capillary network as well as activation index of the thyroid gland (Figs. 3 and 6c).
In spite of morphological signs of thyroid recovery, determined serum levels of both thyroid hormones, T4 and T3, still remained at significantly lower concentrations ($p < 0.01$) compared to the corresponding control group (Fig. 5a and b).

4. Discussion

Results of the present study have shown that 3 months exposure of rats to ELF-EMF led to decreased activity of the thyroid gland. This is indicated by increased volume density of thyroid follicles and reduction of connective tissue in interfollicular space, also by amassed intrafollicular colloid, low follicular epithelium, which was extremely flattened in some areas of the gland, and rare presence of intracellular droplets in thyrocytes. Moreover, we also tried to determine the morphological recovery point of the gland, noted at the end of the third week following the exposure. But, as thyroid follicles attained control values in investigated groups of exposed animals, serum T3 and T4 remained at lower concentrations until the end of the investigated recovery period. It seems that the thyroid gland recovery is actually only partial.

According to Kalisnik (1981), thyroid activation index (ratio of the volume density of follicular epithelium to the volume density of colloid) is in positive correlation to plasma TSH, a major factor influencing the thyroid structure and function. This stereological parameter is found to be lowered in animals exposed to ELF-EMF, compared to corresponding controls. Therefore, histological alterations of thyroid follicles in these animals could be explained by the absence of stimulatory effects of TSH on these cells.

In animals subjected to recovery evaluation of the gland, value of activation index was slowly rising through the first and second recovery week and to the end of the third week, when it almost reached the value of corresponding control. This was reflected through the histological appearance of the gland showing normal thickness of follicular epithelium and rough epithelium–colloid border. These findings are the first morphological indications for thyroid reversement to normal histological state.

However, this morphological recovery cannot be supported with physiological status of exposed animals. At this point, it is difficult to predict the possible cause for decreased T3 and T4 concentrations observed from the beginning to the end of the investigated recovery period.

It is well known that, under normal physiological conditions, TSH stimulates thyroglobulin exocytosis, endocytosis and proteolysis of colloid droplets and cytoskeletal protein phosphorylation, important for the pseudopod formation that leads to colloid endocytosis and, ultimately, thyroid hormone release (McNabb, 1992). Possible reasons for low T3 and T4 levels after third recovery week are the impaired exocytotic and endocytotic processes at the apical membranes of follicular cells. This is supported by the presence of unusually large colloid droplets in the subapical region of thyrocytes observed in some exposed animals, which is correlated with the state of disbalance between exocytosis and endocytosis in follicular cells (Pantic, 1974). However, absence of PAS positive colloid droplets was predominant in most animals and, also, reflects impairment of the thyrocyte apical membrane physiology. Possible alterations in enzyme actions involved in cytotic processes in thyrocytes and hormone release should also be taken into consideration. Results of experimental investigations that
demonstrated the EMF effect on enzymes (Blank et al., 1995; Holian et al., 1996; Loschinger et al., 1998; Ding et al., 2001) and alterations in the structure and function of cellular membranes (Lisi et al., 2000; Bordushkov et al., 2000) which are appointed as primary targets of EMF action on biological systems (Teiforode and Kaune, 1987; Goodman et al., 1995), implicate the possible aspect of a direct EMF influence on thyroid gland (Matavulj et al., 1998).

Mediators of direct ELF-EMF influence on thyroid could also comprehend neurotransmitters from intrathyroid nerve endings or active peptides from parafollicular cells or mast cells, as we discussed earlier (Matavulj et al., 1999b). Action of various mediators (e.g. norepinephrine, serotonin, histamine etc.) upon thyrocytes and capillary endothelium would have, as a consequence, altered levels of serum T3 and T4 and dilatation of blood capillaries in interfollicular

Fig. 5. (a–b) Serum concentration of T4 (a) and T3 (b) in control animals and animals exposed to ELF-EMF for 3 months (group I) and during recovery period of 1 (group II), 2 (group III) and 3 weeks (group IV). Mean ± S.E. are given.
Fig. 6. (a–c) Volume density of thyroid follicles (Vvf), epithelium (Vve) and colloid (Vvk). (a) Control animals and animals sacrificed 1 week after the 3 months exposure to ELF-EMF. (b) Control animals and animals sacrificed 2 weeks after the 3 months exposure to ELF-EMF. (c) Control animals and animals sacrificed 3 weeks after the 3 months exposure to ELF-EMF. Mean ± S.E. are given.
space of the thyroid gland. The link between release of different mediators and exposure to EMFs was previously suggested by some authors (Zecchi et al., 1998; Lai and Carino, 1999; Gangi and Johansson, 2000).

According to our results, measured serum T3 and T4 concentrations after first and second recovery week are lower than in corresponding controls. It appears, the circulating TSH is also reduced as indicated by decreased thyroid activation index. In the pituitary–thyroid feedback system, lowered thyroid hormones stimulate release of TSH from pituitary, the effect that probably failed to occur in animals from these two groups. The impairment of hypothalamic–pituitary–thyroid axis as a cause of noted alterations remains questionable since there were no TSH measurements.

Results of our previous investigations on same animal strain and gender, under the same exposure conditions, revealed a different reaction of thyroid follicles to EMF influence in immature rats, showing increased activity after exposure to EMF. However, differences in exposure facilities and experimental protocols among these experiments, make comparisons during estrous cycle of dairy cows exposed to electric and magnetic fields. Bioelectromagnetics 19, 438–443.


References


