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Light and electron microscopic study of the thyroid gland in rats exposed to power-frequency electromagnetic fields

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Summary

The effect of 50 Hz electromagnetic field (EMF) on thyroid gland was studied using light and transmission electron microscopes. Two-month-old male rats were exposed to an EMF (100–300 μT, 54–160 V m^{-1}) for 4 h a day, 5 days a week for 1 month. A predominance of microfollicles with less colloid content and dilated blood capillaries was found in the EMF group. Stereological counting showed a statistically significant increase of the volume density of follicular epithelium, interfollicular tissue and blood capillaries as well as the thyroid activation index, as compared to the controls. The volume density of colloid significantly decreased. Ultrastructural analysis of thyroid follicular cells in the EMF group revealed the frequent finding of several colloid droplets within the same thyrocyte with the occasional presence of large-diameter droplets. Alterations in lysosomes, granular endoplasmic reticulum and cell nuclei compared to the control group were also observed. Taken together, the results of this study show the stimulative effect of power-frequency EMF on thyroid gland at both the light microscope and the ultrastructural level.

Key words: thyroid gland, 50 Hz electromagnetic field, light microscopy, stereology, ultrastructure.

Introduction

Thyroid structural and functional alterations may be caused by various forms of non-ionizing radiation such as radiofrequency (RF) fields, including microwaves (RF fields at high frequencies) and power-frequency fields. In rats, a reduced uptake of iodine by the thyroid and lower levels of plasma thyroid-stimulating hormone (TSH) was found after exposure to 28 MHz continuous-wave radiation (Wright et al., 1984). The inhibition of the functional activity of the thyroid in rats was attributed to a single and double exposure to electromagnetic waves at millimetre range (60 GHz) (Kozhevnikova et al., 1989). Single exposure to microwaves increased plasma TSH levels and resulted in a higher activity of the rat thyroid (Saddiki Traki et al., 1986), and an increased thyroxin (T4) concentration (Lu et al., 1985). However, repeated irradiation of rats with microwaves decreased the functional activity of the thyroid gland (Lu et al., 1985; Navakatikian et al., 1990).

A histophysiological study of the thyroid in 2 month-old rats exposed to a 50 Hz electric field (50 kV m^{-1}) for 8 h a day for 4 weeks showed no effect on plasma TSH, T4 and thyroid structure, but the level of triiodothyronine (T3) was decreased (Portet and Cabanes, 1988). Increased thyroid gland activity was observed after 15 min exposure of rats to 20 mT, 50 Hz electromagnetic field (EMF), as judged from the increased cyclic adenosine monophosphate (cAMP) in the gland, and increased T3 and T4 (reviewed by Zagorskaya, 1989). Exposure to 0.1 mT, 50 Hz EMF for 3 h a day for 20 or 30 days decreased the thyroid activity (reviewed by Zagorskaya et al., 1990).

In our previous studies, we investigated the influence of 50 Hz EMF (50–500 μT) for 2–6 months on male rats exposed from 1-day old. Results of these studies demonstrated the effect of EMF on thyroid follicular epithelium, follicular colloid content, interfollicular connective tissue and mast cells (Matavulj et al., 1999; Matavulj et al., 2000; Rajkovic et al., 2003). The observed changes pointed to a decreased thyroid activity after 3, 5 and 6 months of exposure and enhanced thyroid activity after 2 months.

In the present study, we aimed to investigate the possible harmful effects of power-frequency EMF on thyroid gland structure in 8-week-old male rats exposed to EMF for a period of 4 weeks. In addition to light microscopy and an ultrastructural analysis, stereological point counting was performed in order to support the histological findings with numerical data.

Materials and methods

Animal maintenance

The experiment was performed on 2-month-old male Wistar rats (*Ratus norvegicus albinus* Berkenhaut 1769). Animals were housed in plastic cages under laboratory conditions at 20±2°C and subjected to a controlled photoperiod (14 h:10 h light:dark). Animals (N=15) were exposed to the influence of EMF for 4 h a day, 7 days a week for 1 month. Controls (N=15) were maintained in a same environment, but without the presence of artificially produced EMF.

The investigation was carried out with the permission of the Ethical Committee on Animal Experiments of the University of Novi Sad, Serbia and Montenegro.

Exposure system and the EMF

The exposure system, by which EMF was produced, was made of a single coil of solenoid type (Electronic Equipment Factory 'Novkabel', Novi Sad, Serbia and Montenegro) equipped with a cooling system and energized from 50 Hz, 220 V and 10 A *via* an autotransformer, which provided a 100 V output. Cages with animals in were placed on both sides of the coil, perpendicular to the coil axis, at a 12 cm distance, and were covered with a plastic lid. The coil axis was parallel to the lines of force of the geomagnetic field (north–south direction). The EMF produced by the coil was in the horizontal direction with regard to the geomagnetic field; it was inhomogeneous and of decaying intensity along the animal cages, with values of 300 μT and 160 V m^{-1} on the side of the cage near the coil and 100 μT and 54 V m^{-1} on the opposite side. The value of the electric field at any other point in the room was less than 10 V m^{-1}. The residential values of the magnetic (AC milligaussmeter, model 42B-1, Monitor Industries, Boulder, USA) and electric fields (HI-3607 ELF sensor, Holaday Industries, Eden Prairie, USA) were measured to be 0.2 μT and 2.9 V m^{-1}, and the value of the geomagnetic field (Gauss/Tesla meter, model 4048, F. W. Bell, Orlando, USA) was 40 μT.

Light microscopy

Immediately after the last hour on the last day of exposure, 10 exposed and 10 control animals were killed in diethyl ether narcosis. Samples of thyroid gland were taken in a tissue block, composed of trachea, oesophagus and surrounding connective tissue, and fixed at 4°C in a mixture of paraformaldehyde (4%; Merck, Darmstadt, Germany) and saturated picric acid (14%; Merck, Darmstadt, Germany). Thereafter, the tissue samples were rinsed in 0.1 mol l^{-1} Sörensen's buffer containing 10% sucrose (Merck, Darmstadt, Germany), 0.01% NaN_3 (Merck, Darmstadt, Germany) and 0.02% Bacitracin (Sigma Chemicals Co., St Louis, USA), cut into 14 μm thick sections using a cryostat (Microm, Heidelberg, Germany) and stained with Haematoxylin–Eosin (both stains from Merck, Darmstadt, Germany) and analyzed.

Electron microscopy

After death, thyroids taken from five exposed and five control animals were fixed in 2.5% glutaraldehyde (Merck, Darmstadt, Germany) in 0.2 mol l^{-1} sodium cacodylate buffer (pH 7.4) (Fluka, Basel, Switzerland) at 4°C and postfixed in 1% osmium tetroxide (Fluka, Basel, Switzerland) for 1 h. Specimens were dehydrated through a graded series of acetone (J. T. Baker, Deventer, Holland) and then in propylene oxide (Merck, Darmstadt, Germany), and embedded in Epon resin (Merck, Darmstadt, Germany). Sections of 0.5 μm and 1 μm thickness were obtained using an LKB ultramicrotome (8800) (LKB, Bromma, Sweden), and they were stained with Toluidine Blue–Cresyl Violet (Carlo Erba, Milano, Italy; Edward Gurr Ltd., London, UK, respectively) to select areas for further sectioning. These areas were cut with a diamond knife into ultrathin sections, collected on 0.3% Formvar (Agar Scientific Ltd., Cambridge, UK)-coated copper grids (100 mesh) (Agar Scientific Ltd., Cambridge, UK), contrasted with uranyl acetate (Merck, Darmstadt, Germany) and lead citrate (Merck, Darmstadt, Germany), and examined and photographed using a JEOL JEM-1230 (JEOL-USA Inc., Peabody, USA) transmission electron microscope linked to a MEGA VIEW camera.

Stereology

Cryostat sections stained with Haematoxylin–Eosin were analyzed using a multipurpose stereological M42 grid placed in the ocular of a Reichert light microscope. Every fourth serial section was analysed (in total three thyroid sections per gland sample) and 60 test fields per thyroid sample, using 10× ocular and 40× objective. Counting was performed starting from the middle of the thyroid lobe (facing the trachea) to the periphery. The volume density [in mm^3 mm^{-3} (after Weibel, 1979)] of follicular epithelium (V_{ve}) and colloid (V_{vk}) were determined and further used to calculate the volume density of follicles (V_{vf}) ($V_{vf}=V_{ve}+V_{vk}$) and the thyroid activation index (Ia) (Ia$=V_{ve}/V_{vk}$). The volume density of interfollicular tissue (V_{vi}) was also determined.

Semithin sections were analyzed in order to determine the numerical [N_{vc}, in mm^{-3} (after Weibel, 1979)] and volume (V_{vc}) density of the capillary network in the thyroid interfollicular space. Counting was performed on 60 test fields per thyroid sample on one randomly selected section using the ocular magnification of 10× and an immersion objective.

Estimations were made by the same observer on blind-coded sections. A non-parametric Mann–Whitney U-test was used for statistical analysis of differences between the control and the exposed group. P values less than 0.05 were considered significant.

Results

The thyroid gland of control animals was characterized by the predominance of macrofollicles rich in a colloid material, whereas the lobes in the exposed group showed numerous microfollicles with less colloid content (Fig. 1a,b, Fig. 2a–c). The thyroid stroma in exposed rats consisted of wider connective tissue septa (Fig. 2b,c) and more dilated blood

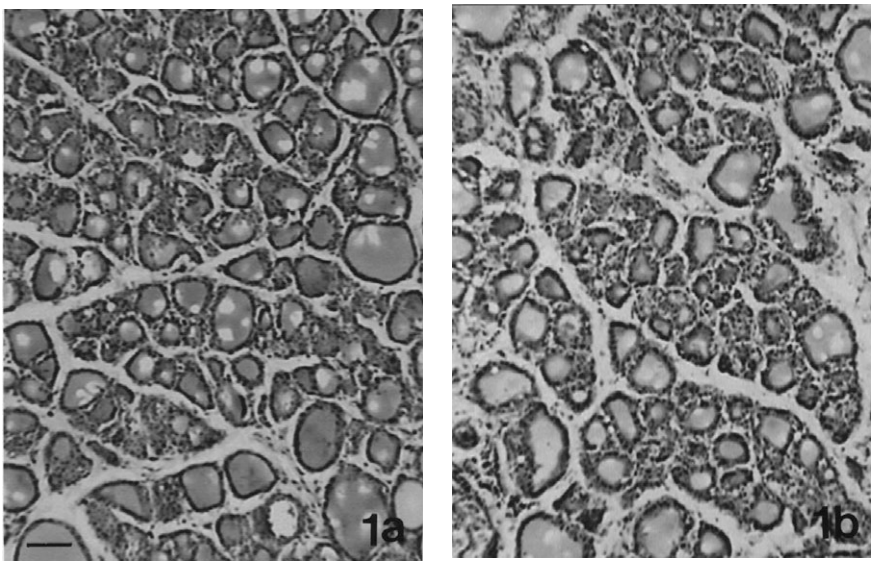

Fig. 1. Photomicrographs of frozen sections of the thyroid gland stained with Haematoxylin–Eosin in a control animal (a) and an animal exposed to 50 Hz EMF (b). Thyroid parenchyma is composed mainly of macrofollicles rich in colloid content in a. Follicles of different diameter are present in b with a preponderance of smaller (microfollicles) over larger follicles. Microfollicles have low to very low colloid content in b. The lobular structure is more prominent in b with each lobule completely divided by connective tissue (compared with a). Both photomicrographs are of the same magnification. Scale bar, 200 μm.

capillaries (Fig. 3b) compared with those of the controls (Fig. 2a, Fig. 3a).

Thyroid follicular cells of a control animal with characteristic ultrastructural features are shown in Fig. 4a,b. In rats exposed to EMF, several colloid droplets within the same thyrocyte were frequently observed in the apical part of the cell (Fig. 4c,d). A number of large diameter droplets were seen (Fig. 4c,d, Fig. 5a–c), in contrast to control thyroids where they were a rare finding. In most follicular cells of the exposed group, the number of lysosomes in the apical cytoplasm was decreased compared with their abundance in the thyrocytes of control animals (Fig. 4a–c). Thyrocytes with hypertrophic granular endoplasmic reticulum and dilated cisternae with amorphous electron-lucent contents were also observed in the thyroid of exposed animals (Fig. 5a–c). In some cells, nuclei were often irregular in shape with incisions and a higher density of chromatin material than in controls (Fig. 4a–d).

Results of the stereological analysis showed a significantly increased volume density of follicular epithelium (V_{ve}) ($P<0.05$), a decreased in volume density of colloid (V_{vk}) ($P<0.01$) and an increased thyroid activation index (Ia) ($P<0.01$) in the exposed animals as compared to the controls (Table 1). The differences between the groups of the volume density of thyroid follicles (V_{vf}) was not statistically significant ($P>0.05$; Table 1). In the exposed rats, the volume density of the interfollicular tissue (V_{vi}) and the volume density of capillaries (V_{vc}) were significantly increased (both at $P<0.05$) compared to controls (Table 1). The increased numerical density of capillaries (N_{vc}) was not statistically significant between the control and the exposed group at the $P=0.05$ level according to the Mann–Whitney test (Table 1). Although the statistical analysis showed a non-significantly altered volume density of the follicles and a significantly altered volume density of the interfollicular tissue, the P values for these parameters are on the border of non-significance/significance,

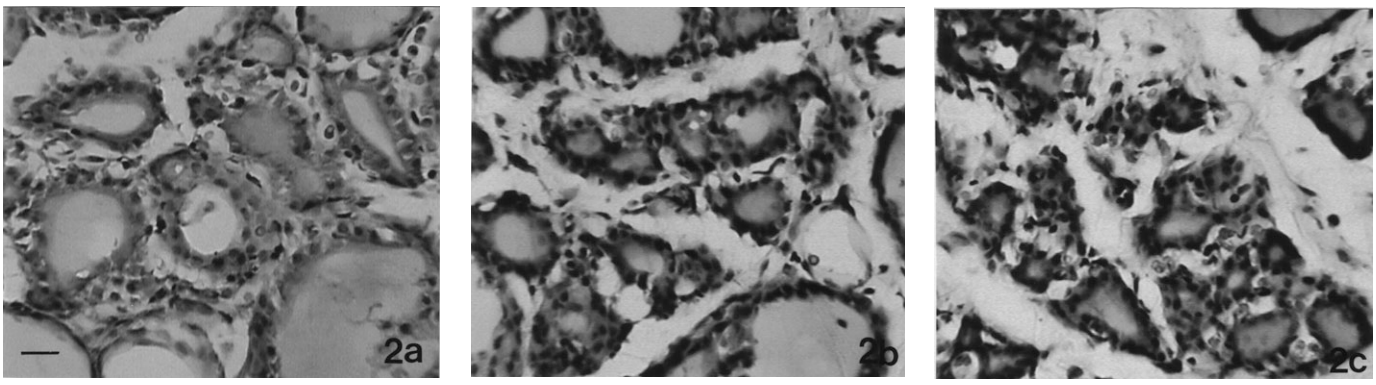

Fig. 2. Photomicrographs of frozen sections of the thyroid gland stained with Haematoxylin–Eosin from a control animal (a) and animals exposed to 50 Hz EMF (b,c). Note the difference in follicle size in a compared to b and c. Occasional follicles with a squamous cell lining, probably due to a low activity state can be seen in a–c. There is a prominent increase in connective tissue content in b and c compared to a. All photomicrographs are of the same magnification. Scale bar, 50 μm.

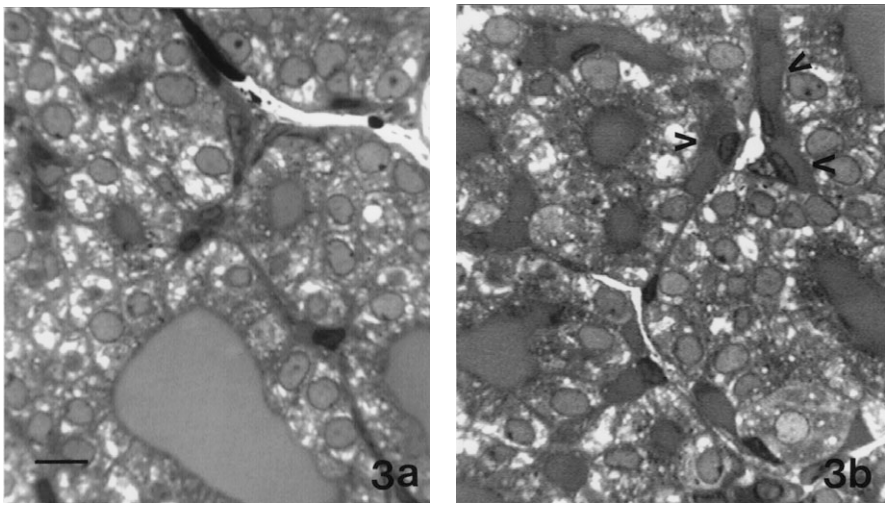

Fig. 3. Photomicrographs of semithin sections of the thyroid gland stained with Toluidine Blue–Cresyl Violet in a control animal (a) and an animal exposed to 50 Hz EMF (b). There is a more extensive capillary bed (carets) in b compared with a. Both photomicrographs are of the same magnification. Scale bar, 25 μm.

where the *P* value for the first parameter is 0.053 and for the second is 0.048 (Table 1).

Discussion

Light and electron microscopic investigations of thyroid glands in 2-month-old rats after exposure to 50 Hz EMF for one month demonstrate hypertrophy and hyperplasia of the thyroid follicular cells and the connective tissue, microfollicular rearrangement of thyroid lobes and lowered volume of follicular colloid content. Ultrastructural analysis of thyrocytes pointed to alterations in number and morphology of lysosomes and in the process of colloid engulfment by thyrocytes in the EMF exposed rats.

TSH is a major regulator of the thyroid gland morphology and physiology, as it affects a wide variety of aspects of thyroid function. TSH is responsible for the morphological appearance of thyroid follicles and the synthesis and secretion of thyroid hormones leading to hypertrophy and hyperplasia of the follicular cell (McNabb, 1992). One of the early response of TSH-stimulated thyroid follicular cells is engulfment of colloid material from the follicular lumen into the apical cytoplasm of thyrocytes, in the form of membrane-bound colloid droplets. Administration of TSH to rats pretreated with thyroxin resulted in formation of numerous pseudopods on the apical surface of thyrocytes observed with both transmission and scanning electron microscopes (Ekholm et al., 1975; Kawada and Naito, 1978).

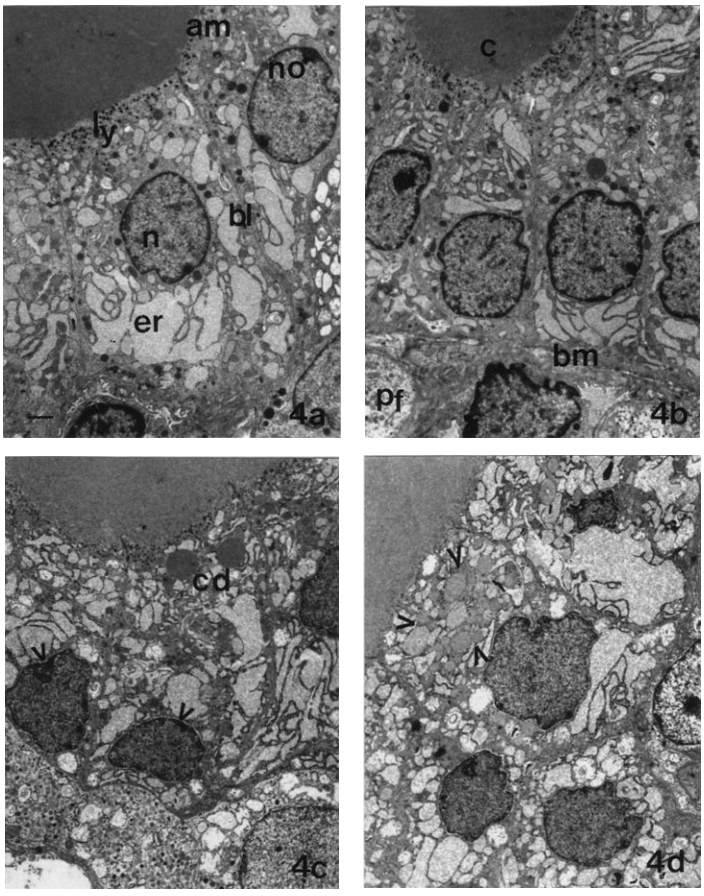

Fig. 4. Electron micrographs of a part of the follicle wall showing the ultrastructure of follicular cells in control animals (a,b) and animals exposed to 50 Hz EMF (c,d). In a and b the follicular epithelial cells have euchromatic nuclei (n) and prominent excentrically positioned nucleoli (no). In the treated cells the nuclei are placed basally in the cell (carets in c) and the euchromatin is darker in appearance. The rough endoplasmic reticulum (er) is relatively well developed in both cisternal and vesicular form in a–c. The baso-lateral compartments (bl) of some cells in a, c and d have distended cisternae (er). There are more pleomorphic electron-dense lysosomes (ly) under the apical membrane (am) of thyrocytes in a and b compared with c. Lysosomes of different electron density are seen in c. Note two large colloid droplets (cd) in the apical cytoplasm of the same thyrocyte in c and a large group of droplets of various diameter occupying nearly the entire apical region of one follicular cell (carets in d). c, colloid; bm, basement membrane; pf, parafollicular cell. All photomicrographs are of the same magnification. Scale bar, 1 μm.

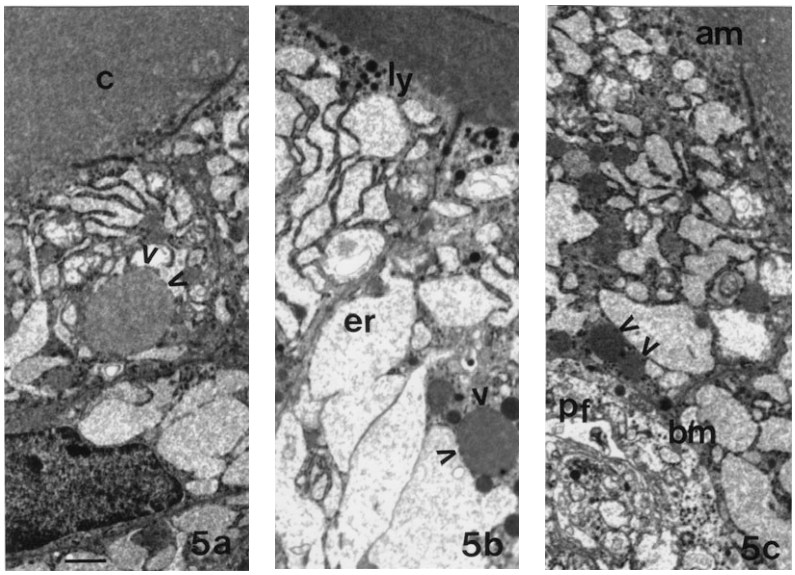

Fig. 5. (a–c) Electron micrographs of follicular cells in animals exposed to 50 Hz EMF. The significant features are: a giant colloid droplet (carets) in a; a large colloid droplet (carets) surrounded by lysosomes in the central part of a thyrocyte in b and near the basal membrane (carets) in c; pleomorphic lysosomes (ly) in the apical cytoplasm in b; rough endoplasmic reticulum (er) with amorphous contents of different density and dilated cisternae in a, b and c. c, colloid; am, apical membrane; bm, basement membrane; pf, parafollicular cell. All photomicrographs are of the same magnification. Scale bar, 1 μm.

This is followed by the appearance of colloid droplets, first in the apical and later in the deeper parts of the cytoplasm (Ekholm et al., 1975). The deeper cytoplasmic droplets were closely related to small lysosomes. Experiments in *in vitro* conditions demonstrated the same effects. Thyroid cells incubated with TSH were characterized by apical pseudopods and intracytoplasmic colloid droplets (Kawada and Naito, 1978; Rocmans et al., 1978). In mice and rats injected with TSH after pretreatment with thyroxin, the percentage of follicular cells containing colloid droplets and the number of droplets in cells gradually increased with increase of the TSH dose (Gerber et al., 1987).

Our finding of large-diameter colloid droplets in the cytoplasm of thyrocytes, several droplets within the same cell and droplets in close proximity to small lysosomes in rats exposed to power-frequency EMF points to increased stimulation of follicular cells with TSH. The clear sign of this stimulation at the light microscopic level was an increase in the follicular epithelium height and a reduction in the colloid mass, which was supported by the stereological quantification results. Activation index of the thyroid gland, derived from the calculated values of the volume density of follicular epithelium and the volume density of colloid, is a stereological parameter used for correlation to the level of plasma TSH. Based on experimental studies on rats involving TSH measurements in peripheral blood and stereological analysis of thyroids, Kalisnik (Kalisnik, 1981) was able to show a positive correlation between the thyroid activation index and the plasma TSH. In this respect, we found that the thyroid activation index increased in 2-month-old rats exposed to EMF for 1 month. This suggests an increased level of TSH, and subsequently, the influence of TSH on the follicular epithelium.

The TSH stimulative effect on thyroid follicular cell function is modulated by the action of various molecules such as neuropeptides, peptides derived from parafollicular cells and growth factors (reviewed by Ahren, 1991). Norepinephrine (NE) released from intrathyroid nerve endings inhibits the TSH-induced secretion of thyroid hormones and the

Table 1. *The number of observations, median values with the lower and upper quartiles of all investigated stereological parameters in the thyroid gland of control and EMF-exposed animals*

		Control group			Exposed group			
Stereological parameter	*N*	Median	Lower quartile	Upper quartile	Median	Lower quartile	Upper quartile	*P* value*
V_{ve} (mm^3 mm^{-3})	10	0.52	0.49	0.57	0.58	0.54	0.59	0.020
V_{vk} (mm^3 mm^{-3})	10	0.24	0.21	0.32	0.16	0.15	0.18	0.005
V_{vf} (mm^3 mm^{-3})	10	0.80	0.75	0.82	0.74	0.71	0.76	0.053
Ia	10	2.26	1.53	2.90	3.53	3.28	3.87	0.002
V_{vi} (mm^3 mm^{-3})	10	0.19	0.17	0.25	0.25	0.23	0.29	0.048
N_{vc} (mm^{-3})	5	101914	101106	103339	138442	106958	148525	0.117
V_{vc} (mm^3 mm^{-3})	5	0.028	0.025	0.032	0.038	0.038	0.047	0.037

V_{ve}, V_{vk}, V_{vf}, V_{vi} and V_{vc} represent, respectively, the volume density of: follicular epithelium, colloid, follicles, interfollicular connective tissue and capillaries. *I*A, thyroid activation index; N_{vc}, numerical density of capillaries; *N*, number of observations.

*Mann–Whitney *U*-test.

accumulation of cAMP (Ahren et al., 1986). Neuropeptide Y (NPY), co-stored in adrenergic nerve fibres with NE and released concomitantly with this catecholamine upon sympathetic stimulation, potentiate the NE inhibitory action (Ahren, 1986). Our recent finding of an increased number of NPY-positive nerve fibers in the thyroids of rats exposed to EMF (Rajkovic et al., 2005a) raise the possibility of an involvement of neurotransmitters from adrenergic fibers in regulating the thyroid follicle cell function during exposure to EMF. It appears probable that the result of this interfering inhibitory action is related to the present observation of a decreased number of lysosomes in the apical region of thyrocytes and, consequently, to impaired thyroid hormone release in EMF-exposed rats.

Investigation on rats exposed to EMF (50 Hz, 50 μT–500 μT) for 7 h a day, 5 days a week, from the second postnatal day for 2 months yielded equivocal results of increased thyroid activity, as studied by a combination of classical histology and stereology (Matavulj et al., 1999). By contrast, in the thyroids of rats exposed to EMF for a longer period of 3, 5 and 6 months under the same experimental conditions there was morphological and stereological evidence for a decreased activity (Matavulj et al., 1999; Rajkovic et al., 2003). The degree of these changes correlated well with the duration of exposure.

Studies on EMF effects on thyroid gland demonstrated an increased weight and activity of the gland in mice exposed prenatally to 0.5 Hz, 0.15–9 mT EMF (Ossenkopp et al., 1972). In rats exposed to EMF (0.5 Hz; 10^{-6}, 10^{-7} and 10^{-8} T) perinatally, or as adults aged 180–200 days at the beginning of the experiment, no alterations were seen in thyroid follicle morphology or thyroid hormone concentrations (Lafreniere and Persinger, 1979). An increased level of circulating TSH and T4 was found in rats exposed to 50 Hz EMF of 20 mT for 18 h, but a decreased concentration of circulating thyroid hormones after a single exposure to EMF (Udintsev et al., 1978). Levels of TSH were not changed in rats after exposure to 60 Hz and 100 kV m^{-1} electric field for 1 or 3 h (Quinlan et al., 1985). Single exposure of rats to 20 mT EMF demonstrated an affected thyroid activity and a concentration of thyroid hormones that remained lower for 2 months after the exposure (Zagorskaya and Rodina, 1990).

The present investigation demonstrated an increased volume density of blood capillaries in the thyroids of exposed rats. This is consistent with reports of the same finding in rats exposed to EMF (50 Hz, 50 μT–500 μT) from 24 h after birth for 2, 3 and 5 months (Matavulj et al., 1999; Rajkovic et al., 2001). This vascular effect of EMF might be exerted by the action of mediators from mast cells situated around blood vessels and/or nerve fibers terminating nearby, as indicated by our recent results. In 2-month-old rats exposed to EMF for 1 month we observed numerous degranulated mast cells labelled with antibodies to histamine (Rajkovic et al., 2005b) and an increased number of NPY nerve fibers (Rajkovic et al., 2005a). Both mediators are known to increase thyroid blood flow, and histamine additionally increases the capillary permeability (Melander et al., 1975; Michalkiewicz et al., 1993). From a theoretical standpoint, we might argue that the effects of NPY and histamine are expressed during exposure to EMF, which enable the enhancement of blood flow through capillaries and concomitantly enable different molecules, primarily TSH, to be driven to the thyroid by the bloodstream.

The results of the presented study demonstrate the stimulative effect of a power-frequency EMF on the thyroid gland, detected using both light and electron microscopy and substantiated with stereological data. Although the structural alterations in the thyroid gland were not so severe as to point to a hazardous effect of the EMF, they are important in the light of a possible thyroid sensitivity to 50 Hz EMF. Further studies involving EMFs at higher frequencies could hopefully reveal the nature of such alterations in the thyroid tissue under the influence of these fields.

Abbreviations

TSH	thyroid-stimulating hormone
T4	thyroxin
T3	triiodothyronine
cAMP	cyclic adenosine monophosphate
RF	radiofrequency fields
EMF	electromagnetic field

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