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Environmental Research 99 (2005) 369–377
Environmental Research
www.elsevier.com/locate/envres

The effect of extremely low-frequency electromagnetic fields on skin and thyroid amine- and peptide-containing cells in rats: An immunohistochemical and morphometrical study ☆

Vesna Rajkovic[a,b,*], Milica Matavulj[a], Olle Johansson[b]

[a]*Department of Biology, Faculty of Sciences, Trg Dositeja Obradovica 2, Novi Sad 21000, Serbia and Montenegro*
[b]*Experimental Dermatology Unit, Department of Neuroscience, Karolinska Institute, Stockholm, Sweden*

Received 20 August 2004; received in revised form 18 January 2005; accepted 22 February 2005
Available online 6 April 2005

Abstract

The aim of this study was to investigate the influence of extremely low-frequency electromagnetic fields (ELF-EMFs) on mast cells (MCs), parafollicular cells, and nerve fibers in rat skin and thyroid gland. The experiment was performed on 24 2-month-old Wistar male rats exposed for 4 h a day, 7 days a week for 1 month to EMFs (50 Hz, 100–300 μT, 54–160 V/m). After sacrifice, samples of skin and thyroid were processed for indirect immunohistochemistry or toluidine blue staining and then were analyzed using the methods of stereology. The antibody markers to serotonin, substance P, calcitonin gene-related peptide (CGRP), and protein gene product 9.5 (PGP) were applied to skin sections and PGP, CGRP, and neuropeptide Y (NPY) markers to the thyroid. A significantly increased number of serotonin-positive MCs in the skin and NPY-containing nerve fibers in the thyroid of rats exposed to ELF-EMF was found compared to controls, indicating a possible EMF effect on skin and thyroid vasculature.
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Keywords: Electromagnetic field; Skin; Thyroid gland; Rat; Stereology

1. Introduction

Among a broad range of biological effects of extremely low-frequency electromagnetic fields (ELF-EMFs) on almost all organ systems in various mammalian organisms, alterations in the neuroimmune system have also been proposed. EMFs have been found to alter the level of neurotransmitters or their metabolites (Seegal et al., 1989; Prato et al., 1995; Lai and Carino, 1999) and the content of biogenic amines in the spinal ganglion (Merkulova, 1990). The contents of calcitonin gene-related peptide (CGRP), somatostatin, and protein S-100 were found altered in the cutaneous nerve fibers in subjects expressing sensitivity to EMF exposure (Johansson et al., 1996). Based on criteria of morphological appearance, number, and the distribution of mast cells (MCs) in the skin of healthy subjects exposed to EMFs, it has been shown that these cells are susceptible to the EMF influence (Johansson et al., 2001), as are MCs in the intestine, lymph nodes, thyroid, and brain (Iurina et al., 1997; Matavulj et al., 1999; Cook et al., 2000).

Nerve fibers in skin and thyroid gland contain a number of mediators targeting adjacent cells via synaptic as well as paracrine actions that maintain the homeostasis of these organs under physiological conditions. The microanatomical relations of nerve fibers and MCs as well as their functional relationship are

☆ The study was performed on white laboratory rats of the Wistar strain and was conducted with the permission of the Ethical Committee on Animal Experiments of the University of Novi Sad.

*Corresponding author. Department of Biology, Faculty of Sciences, Trg Dositeja Obradovica 2, Novi Sad 21000, Serbia and Montenegro. Fax: +381 21 450 620.
E-mail address: vesna.rajkovic@ib.ns.ac.yu (V. Rajkovic).

0013-9351/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.envres.2005.02.003

recognized in skin (Bauer and Razin, 2000), but these are not confirmed for the thyroid.

The nociceptive nerve fibers of the skin contain, among other peptides, substance P (SP) and CGRP. It is known that CGRP exerts vasodilatation in skin, while SP is responsible for vasodilatation and protein extravasation (Holzer, 1998) and is considered to induce degranulation of MCs (Suzuki et al., 1995). The population of MCs in the skin contains a number of preformed mediators in its granules (histamine, serotonin, etc.), but can de novo synthesize additional biologically active molecules (leukotrienes, prostaglandins, etc.) upon cell activation. Apart from CGRP and SP, MC-derived serotonin has a vasoactive role in the skin as well (Gershon et al., 1975; Askenase, 1979).

The thyroid gland harbors three populations of cells from which bioactive molecules originate: sympathetic–adrenergic nerves, MCs, and parafollicular (PF) cells (Melander, 1977). In the intrathyroidal adrenergic nerve fibers that terminate near blood vessels and thyroid follicles, noradrenalin is colocalized with neuropeptide Y (NPY), which is known to increase glandular blood flow (Michalkiewicz et al., 1993). The influence on thyroid microcirculation, and also on thyroid hormone synthesis and secretion, is attributed to amine-containing MCs, which store histamine and serotonin in their granules (Melander and Sundler, 1972; Melander et al., 1975). PF cells contain several peptides, including CGRP, which is also found in nerve fibers around blood vessels, follicles, and PF cells. The coexistence of CGRP with SP has been shown for many nerve fibers, while for PF cells indications of colocalization with calcitonin within the same granules have been found (Grunditz et al., 1986). Under certain experimental conditions, the CGRP from PF cells has been shown to display an inhibitory role in thyroid hormone secretion (Ahren, 1991).

In the present study, we set out to investigate whether power-frequency EMFs can affect skin, which is permanently exposed to a variety of external environmental stimuli, and the thyroid gland, a well-vascularized organ with a superficial anatomical position in mammals. We used selected antibody markers to cutaneous and thyroid gland nerve fiber peptides and for the granular content of MCs and PF cells to demonstrate whether ELF-EMFs could affect them in a manner visible by morphological screening and stereological quantification. The immunohistochemical methods we used enabled us to demonstrate serotonin in cutaneous MCs, SP and CGRP in nerve fibers of the skin, as well as NPY, and protein gene product (PGP) and CGRP in thyroid nerve fibers; the latter two were also used for the demonstration of PF cells in the thyroid gland. For the visualization of intrathyroid MCs, the classical histological staining of toluidine blue was performed.

2. Materials and methods

2.1. Animals

The experiment was performed on 24 white male rats of the Wistar strain. Animals were 1.5 months old when they arrived in the laboratory, and they were kept for 2 weeks, until the beginning of the experiment. Animals were housed under laboratory conditions at $20 \pm 2\,^{\circ}C$ and subjected to a controlled photoperiod (14 h light, 10 h dark). Access to tap water and pelleted food was omitted during exposure hours. Twelve animals were exposed to the influence of ELF-EMF for 4 h a day, 7 days a week for 1 month. Twelve animals served as controls; they were handled in the same manner as the exposed group and maintained in a similar environment, but without the presence of artificially produced ELF-EMF. The investigation was made with the permission of the Ethical Committee on Animal Experiments of the University of Novi Sad.

2.2. Exposure system and the EMF

The exposure system, by which ELF-EMF was produced, was composed of a single coil of solenoid 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 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 regarding the geomagnetic field; it was inhomogeneous and of decaying intensity along the animal cages, with values of 300 μT and 160 V/m on the side of the cage near the coil and 100 μT and 54 V/m on the opposite side, while the value of the electric field at any other point in the room was less than 10 V/m. The residential values of the magnetic (AC Milligaussmeter, Model 42B-1, Monitor Industries, USA) and electric fields (HI-3607 E.L.F. Sensor, Holaday Industries, USA) were measured to be 0.2 μT and 2.9 V/m, while the value of the geomagnetic field (Gauss/Tesla Meter, Model 4048, F.W. Bell, USA) was 40 μT. The estimated value of the magnetic field inclination was 61.2°.

2.3. Specimen preparation

Immediately after the last hour on the last day of exposure, animals were sacrificed via diethyl ether narcosis. Samples of skin from the interscapular region were taken as well as samples of thyroid gland with adjacent parts of the trachea and surrounding connective tissue. All specimens were fixed at 4 °C in a mixture of paraformaldehyde (4%) and saturated picric

acid (14%). Thereafter, the tissue samples were rinsed in 0.1 M Sörensen's buffer containing 10% sucrose, 0.01% NaN_3, and 0.02% bacitracin and cut into 14-μm-thick sections using a cryostat (Microm, Heidelberg, Germany). Sections were further processed for indirect immunohistochemistry or toluidine blue staining.

2.4. Immunohistochemistry

In order to demonstrate MCs or nerve fibers in skin and nerve fibers and/or PF cells in thyroid samples, the indirect immunofluorescence technique was used. Sections were kept at 4 °C overnight in a humid atmosphere during incubation with the following primary antibodies: rabbit antibodies to serotonin (5-HT; 1:500; Verhofstad et al., 1983), mouse or rabbit antibodies to protein gene product 9.5 (PGP 9.5; 1:2000; UltraClone), rabbit antibodies to substance P (1:400; a gift from professor A.C. Cuello, Department of Pharmacology & Therapeutics, McGill University, Montreal, Canada), rabbit antibodies to CGRP (1:600; Peninsula Laboratories), and rabbit antibodies to NPY (1:400; Amersham International). Sections were further rinsed in phosphate-buffered saline (PBS), incubated for 30 min at 37 °C in rhodamine (TRITC)-conjugated donkey anti-rabbit IgG (1:160; Jackson), rinsed, and mounted. For PGP and serotonin double-staining, fluorescein (FITC)-conjugated donkey anti-mouse IgG (1:160; Jackson) was also used. All antibodies were diluted in 0.3% Triton X-100. To test for any possible nonspecific binding of the primary antisera, PBS was applied to certain sections instead of each primary antibody. For observation, a Nikon Microphot-FXA fluorescence microscope was used. All sections were blind-coded and analyzed by the same observer.

2.5. Stereological analysis

Sections stained according to the immunohistochemistry protocol were used for quantitative estimation of MCs, nerve fibers, and PF cells, as were toluidine blue-stained thyroid sections. Skin sections were analyzed starting from the epidermal–dermal junction and thyroid sections from the middle of the lobe (facing the trachea) to the periphery.

Serotonin-containing MCs, PGP-, SP-, CGRP-, and NPY-positive nerve fibers, and CGRP- or PGP-labeled PF cells were estimated according to the principles of design-based stereology. The number of immunoreactive cells or nerve fibers per projected square millimeter was counted using a special microscopic frame under the 20 × objective on two skin sections per sample and two test fields per section or two thyroid sections per sample and four test fields per section. Immunoreactive nerve fibers innervating hair follicles and/or blood vessels were not counted.

Analysis on toluidine blue sections was performed using multipurpose stereological grid M42 placed in the ocular of a Reichert light microscope on four thyroid sections per sample and 100 test fields per animal using ocular 10 × and objective 40 × magnification. The numerical and volume density of degranulated (partially and fully) perifollicular and stromal MCs was determined. Further, the ratio of these two cell groups for both stereological parameters was also calculated.

Estimations were made by the same observer on blind-coded sections. A nonparametric Mann–Whitney test was used for statistical analysis.

3. Results

3.1. Analysis of skin samples

3.1.1. Serotonin (MCs)

Serotonin-positive MCs in skin of the control and the exposed animals were mainly situated in the upper and deep dermis (Figs. 1a and b). An increased number of serotonin-positive MCs with a weak fluorescence and a decreased cell volume was observed in samples of the exposed group (Fig. 1b). MCs under the epidermis were characterized by a variable morphology accompanied by

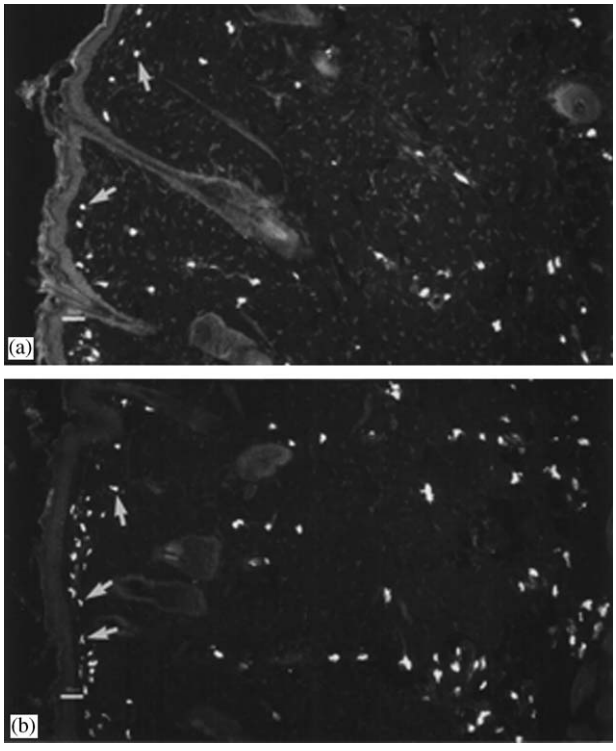


Fig. 1. Serotonin-positive mast cells in the skin of a control animal (a) and an animal exposed to ELF-EMF (b). Brightly fluorescent cells of mainly uniform size and shape (arrows) are seen in (a). The papillary dermis in (b) was found populated with a higher number of cells with diverse shape, size, and fluorescence intensity (arrows). Bars, 50 μm.

a reduced cell size, which suggests degranulation of these cells (Fig. 2b). This was noted in both groups, but most prominently in the exposed group (Figs. 2a and b). In double-stained sections, degranulated serotonin-positive MCs were seen in frequent contact with PGP-positive nerve fibers in the upper dermis of exposed animals. According to the stereological analysis, the increased number of MCs containing serotonin in the exposed group was statistically significant ($P<0.05$) compared to the number in the control group (Fig. 3).

3.1.2. SP (nerve fibers)

Immunoreactivity to SP was found in thin nerve fibers of the skin epidermis and upper dermis in both investigated groups (Figs. 4a and b). Fibers were rarely observed in other parts of the dermis. In the control, SP-containing fibers were mostly brightly fluorescent (Fig. 4a), while weakly stained in the exposed group, and, therefore, occasionally hardly visible (Fig. 4b). Statistical analysis showed a lowered number of SP fibers in exposed animals compared to controls, but it was not significant ($P = 0.95$).

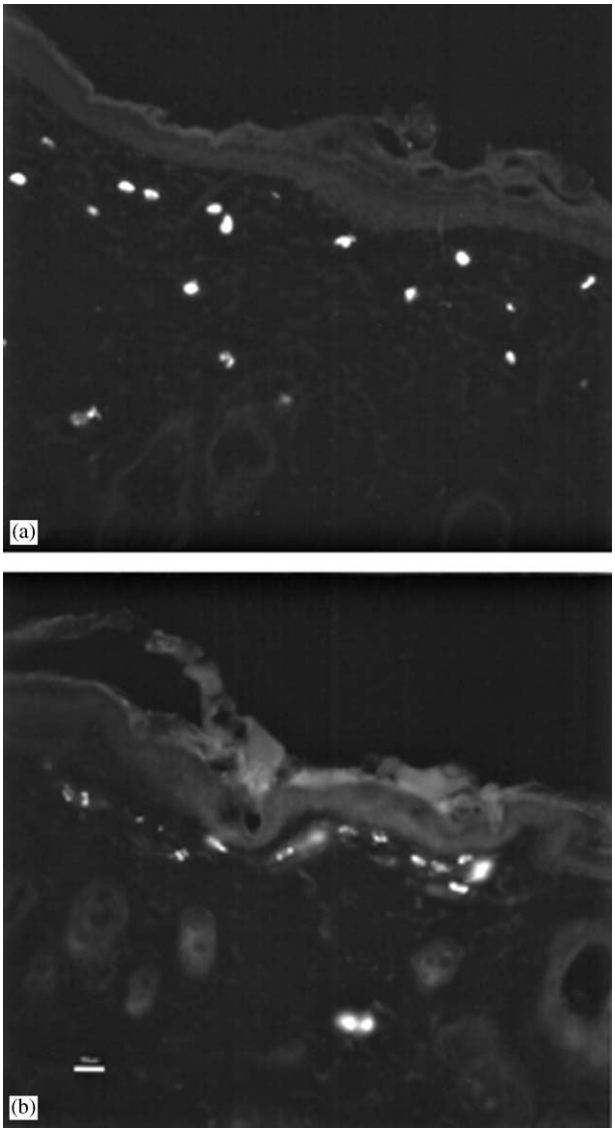


Fig. 2. Serotonin-positive mast cells in the skin of a control animal (a) and an animal exposed to ELF-EMF (b). Degranulated cells apposed to the epidermis and serotonin-containing granules released into the cutaneous connective tissue are observed in (b) and predominantly intact mast cells in (a). Both photomicrographs are of the same magnification. (b) Bar, 50 μm.

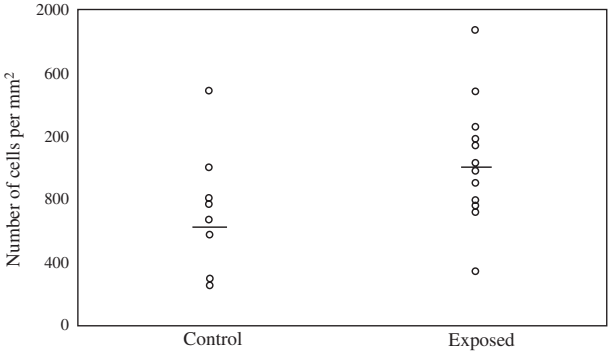


Fig. 3. Number of serotonin-positive mast cells per projected square millimeter of skin in control animals and animals exposed to ELF-EMF. According to the Mann–Whitney U test the difference between the groups was significant at $P<0.05$.

3.1.3. CGRP (nerve fibers)

Nerve fibers containing CGRP were found primarily in the epidermis and upper dermis of the skin, but they were also present in other parts of the dermis (Figs. 4c–f). The area of upper dermis was usually populated with thicker fibers (Figs. 4c–e) and the epidermis with thinner ones (Fig. 4e). In the exposed group, thin fibers were found in a noticeably higher number, especially in the epidermal region. These fibers were often characterized by weaker fluorescence. The calculated value of the CGRP-positive nerve fiber number in the skin of the exposed group was higher than in the control group, but it was not statistically significant ($P = 0.64$).

3.2. Analysis of thyroid gland samples

3.2.1. PGP 9.5 (nerve fibers)

The neuronal marker PGP 9.5 enabled a general overview of the thyroid gland nerve fibers found around thyroid follicles and blood vessels (Figs. 5a and b). There were no apparent differences between the exposed and the control groups of animals except in the counted number of fibers. However, the lowered number of fibers in the exposed group was not significant compared to the controls ($P = 0.50$).

3.2.2. CGRP (nerve fibers)

Nerve fibers containing CGRP were visible around thyroid follicles and blood vessels in both groups, but they were abundantly observed in the gland connective

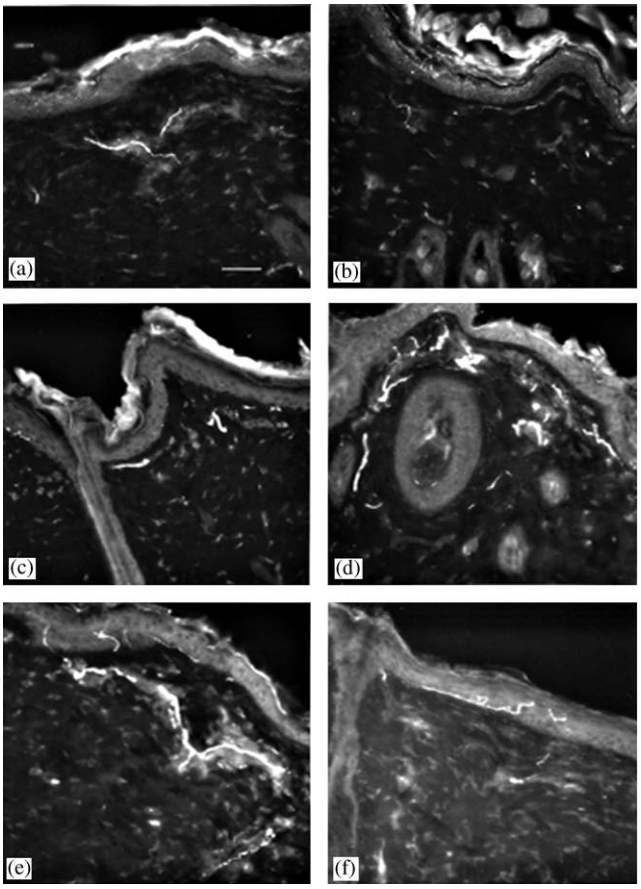


Fig. 4. SP- and CGRP-positive nerve fibers in the skin of control animals (a and c) and animals exposed to ELF-EMF (b, d, e, and f). Brightly fluorescent SP-containing nerve fibers are found in (a) while very thin and weakly fluorescent fibers are seen in (b). A few CGRP-positive nerve fibers are observed in (c) and numerous in (d) and (e). Note the CGRP-containing nerve fibers of different thickness and fluorescence intensity in the epidermis in (e) and (f). All photomicrographs are of the same magnification. (a) Bar, 100 μm.

tissue of the exposed group. In these animals, nerve fibers showed a decreased fluorescence compared to controls, especially those in contact with PF cells. The prominent increase in the number of CGRP-positive nerve fibers in exposed animals was, however, not significant in comparison to the controls ($P = 0.07$).

3.2.3. NPY (nerve fibers)

Thyroid sections stained using the NPY antiserum revealed nerve fibers situated in close proximity to follicles and blood vessels. The NPY-positive nerve fibers related to thyroid follicles appeared as thin fibers with pale fluorescence in both the control and the exposed groups (Figs. 6a and b). The increased number of these fibers in the exposed animals was statistically significant compared to controls ($P<0.01$) (Fig. 7).

3.2.4. Toluidine blue (MCs)

Partially and fully degranulated MCs in the thyroid gland were divided into cells situated perifollicularly and

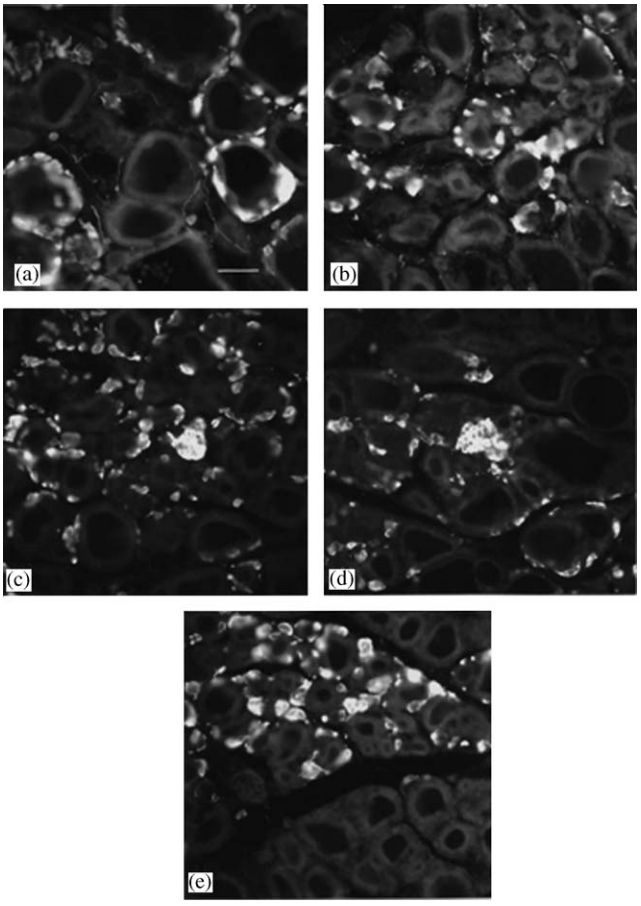


Fig. 5. PGP- and CGRP-positive nerve fibers and parafollicular cells in the thyroid gland of control animals (a and c) and animals exposed to ELF-EMF (b, d, and e). A pattern of distribution of nerve fibers and parafollicular cells labeled with antibodies to PGP 9.5 is observed (a and b). A higher population density of CGRP-containing parafollicular cells is found in (c) compared to (d). Large groups of cells in the stroma of the gland are seen in (c) and (d). Parafollicular cells populate certain areas of the thyroid lobe, with the adjacent parenchyma lacking such positive cells (e). All photomicrographs are of the same magnification. (a) Bar, 100 μm.

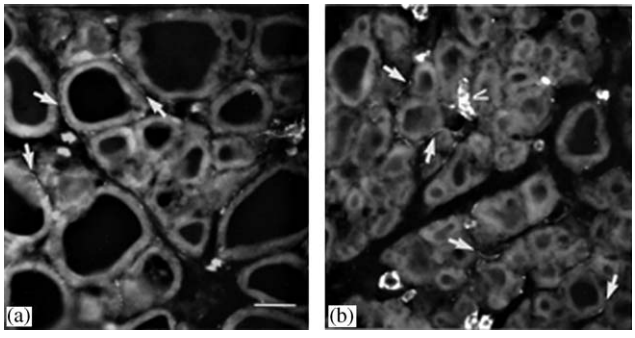


Fig. 6. NPY-positive nerve fibers in the thyroid gland of a control animal (a) and an animal exposed to ELF-EMF (b). Fibers terminating in the vicinity of thyroid follicles are thin and express a pale fluorescence (arrows) in (a) and (b). Note the higher number of fibers and the richly innervated blood vessels with bright fluorescent fibers (arrowhead) in (b). Both photomicrographs are of the same magnification. (a) Bar, 100 μm.

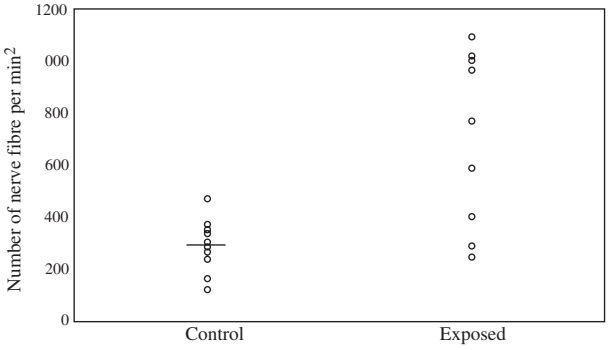


Fig. 7. Number of NPY-immunoreactive nerve fibers per projected square millimeter in the thyroid gland of control animals and animals exposed to ELF-EMF. According to the Mann–Whitney U test, the difference between the groups was significant at $P<0.01$.

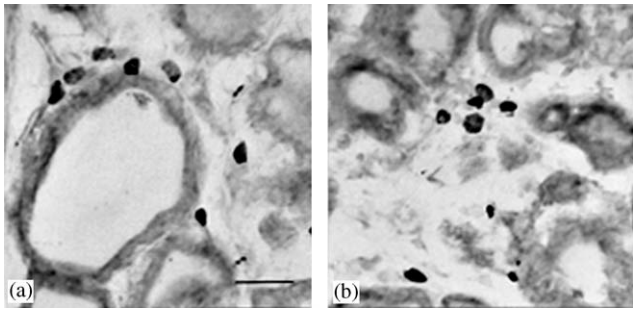


Fig. 8. Mast cells stained with toluidine blue in the thyroid gland of a control animal (a) and an animal exposed to ELF-EMF (b). Degranulated perifollicular mast cells are found in (a) and stromal cells in (b). Both photomicrographs are of the same magnification. (a) Bar, 100 μm.

in the connective tissue stroma of the gland (Figs. 8a and b). Stereological analysis of their numerical and volume density in control and exposed animals showed differences, but they were not statistically significant. The calculated ratio between the two MC groups for both investigated stereological parameters indicated that the stromal MCs exceeded the perifollicular MCs in both groups. However, this was much more prominent in the exposed group than in the control group.

3.2.5. PGP 9.5 (PF cells)

The PGP-positive PF cells were, as is common, found in the connective tissue stroma and in thyroid follicles. PF cells in both control and exposed animals showed a similar pattern of distribution in the thyroid lobes and in their contacts with PGP-positive nerve fibers (Figs. 5a and b). The difference between the groups was the observation of larger groups of stromal PF cells in the exposed specimens and some PF cells with weak fluorescence compared to the controls. The increased number of PF cells in exposed animals showed no statistical significance when compared to controls ($P = 0.54$).

3.2.6. CGRP (PF cells)

The PF cells expressed a strong fluorescence after staining with CGRP in both investigated groups, and a number of cells with weaker fluorescence were also noticed in both groups (Figs. 5c–e). The CGRP-positive cells were found in larger groups in the connective tissue of each group (Figs. 5c and d) and solitary cells in contact with CGRP-containing nerve fibers. The number of cells stained with CGRP was lower in the exposed group compared to the number in the control group (Figs. 5c–e), but it was not significant ($P = 0.45$).

4. Discussion

The results presented above show significantly increased numbers of serotonin-containing MCs and NPY-immunoreactive nerve fibers in skin and thyroid, respectively, of rats exposed to ELF-EMF with the characteristics defined by our exposure protocol.

In rodents, serotonin (or 5-hydroxytryptamine, 5-HT) is synthesized by MCs and released into the surrounding connective tissue after cellular degranulation. The physiological role of this biogenic amine in the skin is known to be vasoactive. Gershon et al. (1975) found that serotonin released from MCs caused the development of gaps between neighboring endothelial cells of postcapillary venules in the skin, leading to increased vascular permeability. Intradermal injections of serotonin were shown to have the same effect (Keahey et al., 1991). Serotonin-positive MCs in samples of skin taken from animals exposed to ELF-EMF in our study were predominantly situated in the upper dermis of the skin. Many of these MCs were characterized by a decreased cell volume and a weak fluorescence, both indications of cell degranulation. Therefore, it could be presumed that the 5-HT released from MCs, which richly populated the papillary dermis, diffused through the connective tissue in the skin and affected cutaneous microvasculature.

MCs containing serotonin were found apposed to nerve fibers positive to SP and CGRP, positioned closely to or being in direct contact with MCs, in the rat mesentery (Crivellato et al., 1991). Substance P has an ability to provoke the release of granules from rat peritoneal and human cutaneous MCs when challenged with this vasoactive transmitter (Ebertz et al., 1987; Amano et al., 1997). CGRP showed the same effect, but was several-fold less potent in augmenting this effect than SP (Piotrowski and Foreman, 1986). Acting upon MCs, SP elicited vasodilatation and vascular permeability via histamine and serotonin (Lam and Ferrell, 1990). However, the microanatomical relations and functional interactions of MCs and nerve fibers are bidirectional: histamine and serotonin released from MCs can affect afferent nerve endings, causing SP and CGRP release from peripheral varicosities, leading to

hyperemia and protein leakage (reviewed in Holzer, 1998).

Considering our results of small alterations in the number of nerve fibers containing SP and CGRP, but also the decreased intensity of SP and CGRP immunoreactivity within the fibers, the interaction of these two neuropeptides with MCs under our experimental conditions may be questionable. This particularly refers to SP because of the very low distribution density of peptidergic fibers harboring this peptide in the skin samples taken from our experimental animals. On the other hand, the number of CGRP-containing fibers was much higher per analyzed field of vision of the skin compared to the number of SP-containing fibers. This could indicate a possible role of CGRP in the skin physiology of rats exposed to EMFs. Under these conditions, MCs would, probably, express their overall effect on the cutaneous vascular bed directly, via serotonin release into the connective tissue of the skin dermis. In our study, serotonin-positive MCs were observed in frequent contact with PGP-positive nerve fibers, indicating that the interaction between them probably occurred under EMF exposure, but whether this is true only for MCs with nerve fibers storing CGRP or for other mediator(s) remains to be elucidated. In addition, one of our previous findings showed a small increase in the number of eosinophil cationic protein-immunoreactive cells in rats exposed to ELF-EMF (data not published). This observation favors a proposed mediator effect on skin vasculature accompanied by moderate eosinophil recruitment, pointing to the involvement of certain nerve fiber-derived mediators.

A number of studies point to neuroimmune responses to the influence of various types of electromagnetic fields. MCs in rats and humans were found to be susceptible to constant magnetic fields, microwaves, and static fields by means of alteration in their number, morphology, and/or mediator content (Doeva et al., 1990; Kalabekov et al., 1995; Donnellan et al., 1997; Johansson et al., 2001). Estimation of the number of CGRP-positive nerve fibers in humans expressing subjective and/or objective skin symptoms during exposure to EMFs showed an increase of these fibers in the papillary dermis (Johansson et al., 1996). Both MCs and sensory nerve fibers in the skin are known to be involved in the response to ultraviolet (UV) B irradiation, playing an important role in the development of UVB-induced immunosuppression (reviewed in Aubin, 2003). Upon UVB irradiation, CGRP is released from nerve endings in the skin, triggering MCs to deplete tumor necrosis factor-alpha (Niizeki et al., 1997). Although it was revealed that UVB exposure increases CGRP, but also SP content, in cutaneous nerve fibers, CGRP was proven to be a dominant neuropeptide in mediating UV effects on skin (Legat et al., 2002; Seiffert and Granstein, 2002). Our results of an increased number of CGRP nerve fibers and degranulated MCs in the skin of exposed animals resemble the cutaneous effects of UVB irradiation, except that the released MC mediator is different. Considering the data in the literature and our findings, we hypothesize that ELF-EMF could indirectly activate MCs to release serotonin by inducing cutaneous nerve terminals to free CGRP.

NPY, another known vasoactive peptide, is considered to induce vascular effects through mediator secretion from MCs (Shen et al., 1991; Mousli and Landry, 1994). The spatial association between MCs and nerve fibers containing neuropeptides has been demonstrated in a number of organs and tissues (Williams et al., 1995), but was not substantiated for the thyroid gland. However, both NPY and MC-derived histamine independently influence thyroid microcirculation. NPY is involved in the regulation of thyroid function by acting as a vasoconstrictor agent and, thereby, increasing thyroid blood flow after binding to NPY Y1 receptors on capillaries (Michalkiewicz et al., 1993; Matsuda et al., 2002). Histamine released from MCs is also known to increase thyroid blood flow and capillary permeability (Melander et al., 1975). Our current results show an increased number of intrathyroid NPY-immunoreactive nerve fibers and a predominance of degranulated stromal MCs over perifollicular ones in rats exposed to ELF-EMF. Additionally, we have previously found a considerable increase of histamine-containing MCs in the thyroid of exposed rats (data not published). Altogether, these observations indicate that thyroid vascular tissue in rats is probably affected during EMF exposure. As a consequence, the microenvironment of increased blood flow and capillary permeability in the gland may enhance the uptake of substrate by thyroid follicular cells used in different physiological processes, including thyroid hormone synthesis.

CGRP localized within thyroid nerve fibers is shown to have no effect on basal or thyroid-stimulating hormone (TSH)-stimulated thyroid hormone secretion, but it enhances VIP-stimulated iodothyronine secretion (Grunditz et al., 1986). CGRP from PF cells is also without an effect on thyroid hormone secretion, but when administrated together with two other PF cell-derived peptides, calcitonin and katacalcin, the TSH-induced increase in thyroid hormone release in mice is inhibited (Ahren, 1989). Since CGRP is found around blood vessels in the thyroid and its role as a potent vasodilator is well known, it is possible that CGRP is involved in the regulation of local microcirculation, as noted earlier (Grunditz et al., 1986). According to our results, the number of CGRP-positive nerve fibers was increased in rats exposed to ELF-EMF, suggesting CGRP as a possible neuronal modulator of NPY

vasoconstrictor action on thyroid vasculature in these animals.

The results of our study show a significantly increased number of serotonin-positive MCs in skin and NPY-containing nerve fibers in the thyroid gland of rats exposed to ELF-EMF. According to our observations, the effect on other investigated mediators is moderate. Since the physiological role of serotonin and NPY is connected to their vascular effect, it could be presumed that a 50-Hz EMF affects blood vessels in the skin and thyroid gland through MC-derived amines and mediators released from peptidergic neurons.

Acknowledgments

This work was supported by the Cancer och Allergifonden, by the Karolinska Institute, and, partly, by the Serbian Ministry of Science and Technology Grant No. 1334. The authors thank professor Tomas Hökfelt at the Department of Neuroscience, Karolinska Institute, Stockholm, Sweden, for general support. The authors also thank Ms. Marianne Ekman for skillful technical assistance and Ms. Margareta Krook-Brandt (at the Department of Learning, Informatics, Management and Ethics, The Medical Statistics Service Group) for expert support with the statistical evaluation.

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