Acta Biologica Hungarica 56 (1–2), pp. 43–51 (2005)

STEREOLOGICAL ANALYSIS OF THYROID MAST CELLS IN RATS AFTER EXPOSURE TO EXTREMELY LOW FREQUENCY ELECTROMAGNETIC FIELD AND THE FOLLOWING "OFF" FIELD PERIOD

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(Received: July 18, 2003; accepted: March 26, 2004)

Influence of extremely low frequency electromagnetic field (ELF-EMF) on thyroid gland mast cells was investigated on male Mill Hill rats. Animals were exposed to EMF (50 Hz, 50 μT to 500 μT, 10 V/m) from 24 hours after birth, 7 hours/day, 5 days/week for three months when a part of animals (group I) was sacrificed, while the rest of them were subjected to recovery evaluation and sacrificed after one (group II), two (group III) and three (group IV) weeks following the exposure. Stereological analysis on toluidine blue-stained paraffin sections showed increased volume density of degranulated mast cells in all groups and, except in group III, and numerical density as well, implicating the sensitivity of thyroidal mast cells to power frequency EMFs. Since in our previous investigations, morphofunctional alterations of thyroid gland in rats exposed to ELF-EMF were found the contribution of released mast cell mediators to these changes could be presumed.

Keywords: Electromagnetic field – mast cell – thyroid gland – rat – stereology

INTRODUCTION

Mast cells present a constitutive part of the thyroid gland stroma and are localized around blood vessels and thyroid follicles. Mast cells release a broad range of mediators found to be important for thyroid function in euthyroid states and during thyroidopaties. Histamine released from mast cell granules is known to increase thyroid blood flow and capillary permeability, while serotonin influences follicular cells by stimulating the release and synthesis of thyroid hormone [15, 16]. Mast cells are demonstrated to play a role in some pathological conditions as goiter, autoimmune thyroiditis, subacute thyroiditis, etc., affecting the extracellular matrix via released chymase or modulating thyroid folliculogenesis and angiogenesis by a variety of growth factors [2, 22, 25].

Literature data regarding the bioeffects of the extremely low frequency electromagnetic fields (ELF-EMFs), points to EMF influence on various cell types. Proliferation and differentiation of granulocyte-macrophage progenitor cells were

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decreased after exposure of murine bone marrow cells to 80 μT, 50 Hz electromagnetic field compared to non-exposed cells [24]. Human fibroblast exposed to 50 Hz EMF in culture showed increase in DNA strand breaks after 24 h of exposition to intermittent field [8). In mature mice exposed to 1 mT, 60 Hz magnetic field for 13 weeks the suppression of natural killer cell activity was found [6]. Investigation of ELF-EMF influence on thyroid gland [11) demonstrated no changes of perifollicular mast cell number in rats after exposure to 0.5 Hz EMF during adulthood or perinataly. However, later studies showed that mast cells were sensitive to EMF exposure under certain experimental conditions [3, 7]. In our previous studies, changes in intrathyroidal mast cell population in male rats exposed postnatally to low-intensity ELF-EMF during four months were observed, as indicated by histological analysis and stereological measurements [12]. Changes in the thickness of thyroid follicular epithelium, intrafollicular colloid content and thyroid capillary bed in male rats were also demonstrated after various duration of exposition to ELF-EMF [13, 20]. In the present study we aimed to evaluate the response of thyroid gland mast cells in male rats exposed to ELF-EMF from birth until the end of the third month of their postnatal life by using the methods of stereology. Following the exposure period, an additional screening of this cell population was performed at the end of one, two and three weeks in "off" field conditions, we named recovery period(s). As mentioned above, thyroid parenchymal and stromal elements were previously shown to be influenced by the applied field and, therefore, in this work a particular emphasis was attributed to degranulated mast cells in the light of their possible influence on thyrocites and capillary endothelium of the thyroid gland.

MATERIALS AND METHODS

Experiment was performed on 89 male rats of Mill Hill strain. Animals were housed in laboratory conditions and subjected to a natural photoperiod. Access to food and water was unlimited. Forty-seven animals were exposed to the influence of ELF-EMF from 24 h after birth, 7 hours a day, 5 days a week for a period of three months. After this period, 12 animals were sacrificed (group I) while the rest of them were subjected to recovery evaluation and sacrificed after one week (12 animals) (group II), two weeks (12 animals) (group III) and three weeks (11 animals) (group IV) after three months of exposure to ELF-EMF. Forty-two animals served as controls to corresponding exposed groups, and were maintained in a similar environment, but without the presence of artificially produced ELF-EMF. The investigation was made with permission of the Ethical Committee on Animal Experiments of the University of Novi Sad.

The exposure system, by which ELF-EMF was produced, 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 symmetri-

cally on both sides of the coil. The coil produced the EMF of decaying intensity along the cages with 500 μT value on the side of the cage near the coil to 50 μT on the opposite side.

After sacrifition, removed thyroid glands were fixed in Bouin's solution, embedded in paraffin and cut on a rotation microtome in 5 μm thick sections. Analysis of the thyroid mast cells was performed on paraffin slices stained with toluidine blue and after the method of Dominici [4].

Stereological analysis was performed on every fourth serial paraffin section of the thyroid gland using on M42, eyepiece graticule in a Reichert light microscope. The numerical and volume density of total thyroidal mast cells were determined on 100 test fields per animal with ocular magnification of 10 and objective magnification of 40. The numerical and volume density of three morphological types of mast cells were also determined, according to Bani-Sacchi [1] on 140 test fields per animal using immersion objective. The author's morphological classification of thyroidal mast cells comprises type A mast cells which are compact with abundant cytoplasmatic granules; type B mast cells are characterized by numerous cytoplasmatic extensions that implements the initiation of degranulation and type C mast cells are degranulated cells with extruded granules in intercellular space.

In this investigation, the following stereological parameters were determined: numerical density of total mast cells (Nvm), volume density of total mast cells (Vvm), numerical density of type A (Nvm type A), B (Nvm type B) and C (Nvm type C) mast cells as well as the volume density of type A (Vvm type A), B (Vvm type B) and C (Vvm type C) mast cells. Additionally, the ratio of the Nvm type B to Nvm type C and Vvm type B to Vvm type C was calculated. The stereological analysis was made by the same researcher.

All obtained results were expressed as mean ± SE. Statistical significance of differences between control animals and ELF-EMF exposed animals were determined using Student's *t*-test.

RESULTS

Mast cells in thyroid gland of control and EMF exposed animals from all four investigated groups revealed characteristic localization in the interfollicular tissue around blood vessels and between thyroid follicles, sometimes in close proximity to follicular cells (Fig. 1).

In exposed animals, stereological analysis showed the progressive decrease in numerical density of total mast cell volume (Nvm), compared to controls, as indicated on plotted results (Fig. 2a). On the contrary, the total mast cell volume density (Vvm) roughly followed the pattern of corresponding controls (Fig. 2b). The similar tendency could be observed for investigated stereological parameters regarding type A and B mast cells as well (Nvm type A and B, Vvm type A and B), with divergence in the last investigated group of the recovery period (Figs 2c–2f). Numerical and volume density of type C (degranulated) mast cells (Nvm and Vvm of type C) were the

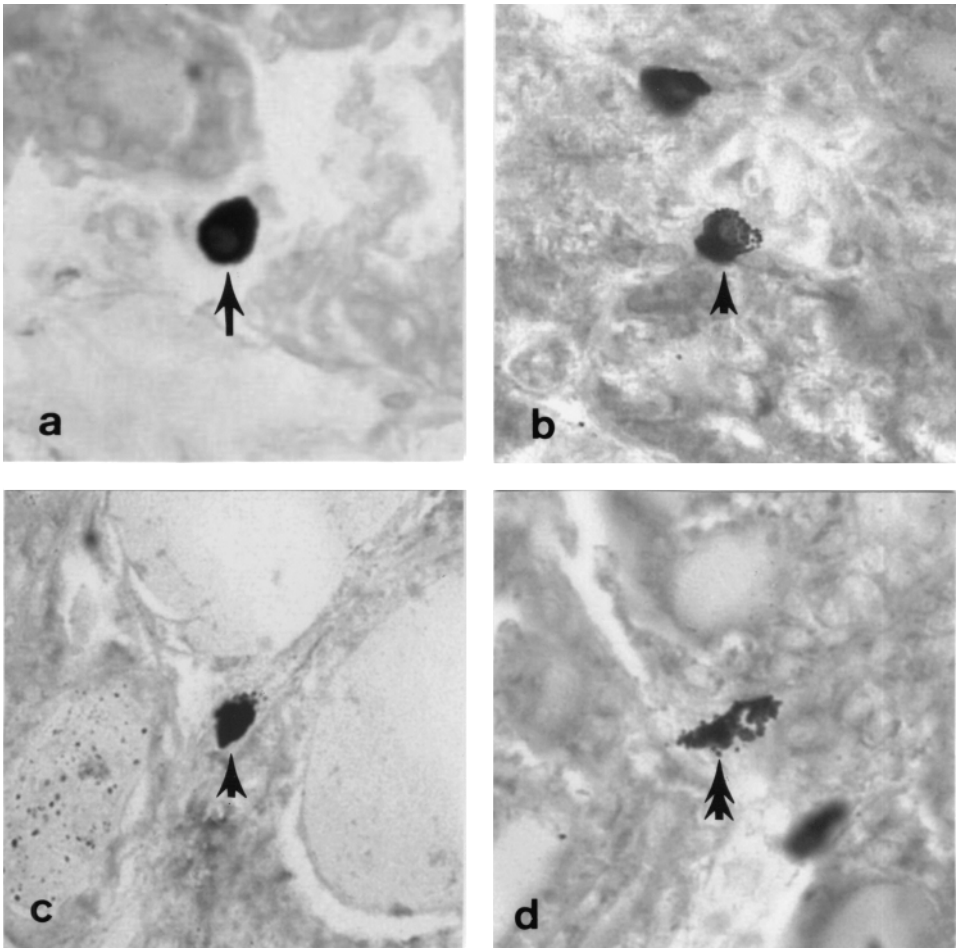

Fig. 1. Three morphological types of mast cells in the thyroid gland stroma, according to the Bani-Sacchi classification [1]: type (**a**), type B (**b** and **c**) and type C (**d**). Type A (**a**) shows a resting mast cell with compact cytoplasm (arrow). Type B (**b, c**) represents degranulating mast cell with filopodia containing granules (arrowheads). Type C (**d**) represents a fully degranulating mast cell which becomes elongated and displays separate granules at the edges (double arrowheat). Toluidine blue staining. Magnification ×630

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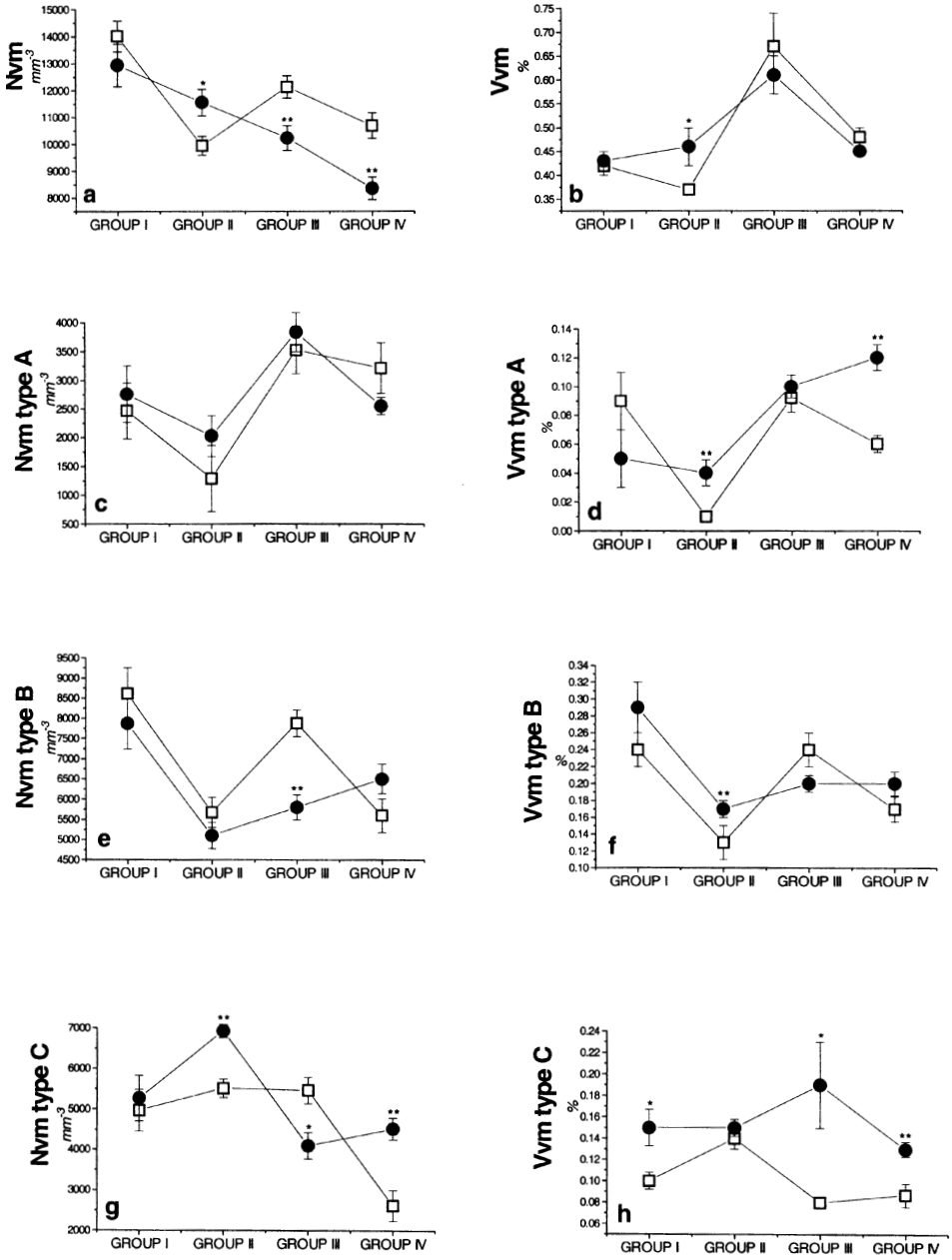
Fig. 2. Plots of stereological analysis of rat mast cells in thyroid gland of control animals (- G -) and animals exposed to EMF (- M -) for three months (group I) and during the restitution period of one (group II), two (group III) and three (group IV) weeks after exposure. Mean ± SE are given. Exposed means indicated by * are significantly different at $p < 0.05$ or by ** at $p < 0.01$ according to Student's *t*-test. **a:** Numerical density of total mast cells (Nvm). **b:** Volume density of total mast cells (Vvm). **c:** Numerical density of type A mast cells (Nvm type A). **d:** Volume density of type A mast cells (Vvm type A). **e:** Numerical density of type B mast cells (Nvm type B). **f:** Volume density of type B mast cells (Vvm type B). **g:** Numerical density of type C mast cells (Nvm type C). **h:** Volume density of type C mast cells (Vvm type C)


Nvm
mm-3
a
GROUP I
GROUP II
GROUP III
GROUP IV
Vvm
%
b
Nvm type A
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Nvm type B
e
Vvm type B
f
Nvm type C
g
Vvm type C
h

most variable parameters, as compared to corresponding controls, in all investigated groups and were found to be significantly altered by the end of the investigated recovery period (Figs 2g and 2h).

Table 1
Ratio of numerical and volume density of B type mast cells to C type

Ratio	Group I		Group II		Group III		Group IV	
	control	exposed	control	exposed	control	exposed	control	exposed
Nvm B/ Nvm C	1.73	1.49	1.03	0.74	1.44	1.41	2.14	1.44
Vvm B/ Vvm C	2.4	1.93	0.93	1.13	3.0	1.05	1.89	1.54

Ratio of the numerical density of the type B mast cells of the C type was lower in all exposed groups as compared to controls (Table 1). This tendency also applied to the two mast cell type volume density ratio. The exception was the second investigated group (first recovery week) characterized by similar values of the ratio of the volume density in the control and the exposed group.

DISCUSSION

Results suggest that after three months exposure of rats to ELF-EMF the volume density of degranulated mast cells in the thyroid gland significantly increased as compared to controls, while other investigated stereological parameters in this group (after three months of exposure) was not significantly affected by the treatment. However, regarding recovery periods, it seems that the population of intrathyroidal mast cells was more disturbed at the end of these periods, as seen from the calculated stereological parameters.

The significantly increased volume density of degranulated mast cells noted in our study is opposite to the previous finding in our laboratory of decreased value of this stereological parameter in male rats exposed for four months to EMF of the same characteristics and exposure dynamics [12]. The volume density of type B mast cells was also opposite to the former results, but the volume density of type A mast cells and the numerical density of type B mast cells showed the similar tendency with the results of the four month duration experiment.

Results of the presented investigation are in accordance with the study of Iurina et al. [7] reporting the sensitivity of mast cells in mice, rats and rabbits from dermal, intestinal and popliteal lymph node to power frequency EMF (50 Hz and 2 kA/m, 16 kA/m or 32 kA/m) after 4 hours of exposure during 5 days. Also, Cook et al. [3]

found the number of mast cells in brain parenchyma affected by weak ELF-EMF (7 Hz or 40 Hz and 50 nT or 500 nT) applied during 15 nights from midnight to 8 hours. The experimental study conducted on healthy volunteers exposed to ordinary TV or PC screens demonstrated the alteration in number and morphology of mast cells in skin biopsies taken after 2 or 4 hours of provocation [9]. But, no alterations were noted in rat peritoneal mast cells exposed *in vitro* to 5 mT 60 Hz magnetic field for 30 minutes to 2 hours, as measured by histamine release and sensitivity to degranulating compound 48/80 [18].

The number of mast cells in the thyroid gland is thought to be correlated with the level of circulating thyroid stimulating hormone (TSH) [14]. TSH induces thyroid mast cell granule content release in rats after administration of a single dose of TSH followed by a reduced concentration of histamine and serotonin in the thyroid gland [5]. The increased levels of circulating TSH were found by Udintsev et al. [23] in rats exposed to EMF (50 Hz, 20 mT) for 18 hours, while later studies showed no significant field effect on TSH level [19, 21]. Thus, alterations in the population of mast cells found in our study might be linked to the possible influence of increased TSH on these cells in exposed animals. This could be related with the ratio values of type B to type C mast cell numerical and volume densities in groups exposed to ELF-EMF. These calculations also showed that the population of thyroidal mast cells is shifted towards degranulated cells in the exposed animals and in favor of partially degranulated cells in the control groups. However, our results of the calculated numerical density of total mast cells in animals exposed to EMF and from recovery periods, had an opposite weekly tendency in respect to our previously reported values of thyroid activation index [20]. Thyroid activation index is considered correlated to the plasma level of TSH [10] thus indicating the decreased value of TSH in animals exposed to EMF and thereafter subjected to recovery periods [20]. This further points to possible TSH-independent mast cells alterations in exposed animals as a direct action of ELF-EMF on these cells. Consequently, EMF might interfere in some of the steps in the mechanism of mediator release through degranulation, a process observed in thyroid samples taken from exposed animals.

Our previous investigations of ELF-EMF effects on thyroid gland showed either increased or decreased thyoid activity, depending on the duration of exposure [12]. We also showed the morphological recovery of the thyroid within three weeks after the three month of exposure to ELF-EMF [20]. The results of the presented study showed significantly altered numerical and volume density of degranulated mast cells during recovery periods. Therefore, we could hypothesize that mast cells contributed to the mentioned recovery of the thyroid via paracrine action of their mediators on intrathyroid targets. Further, certain literature data indicates to a greater EMF effect may occur after removal of the field [17] and to which our data are in agreement with.

In conclusion, changes in number and volume of a mast cell population in rat thyroid gland were observed after three month exposure to ELF-EMF as well as in the investigated recovery periods, as shown by the stereological analysis. However, this

requires further investigation in order to determine which mast cell mediator(s) is(are) released under the EMF influence. Subsequently, it would provide additional data for definition of possible consequences of its action on thyroid gland parenchymal and/or stromal structural elements.

ACKNOWLEDGEMENTS

This work was supported by the Serbian Ministry of Science and Technology grant No 1334. The authors would like to thank the anonymous reviewers for their helpful suggestions.

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