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STRUCTURAL AND STEREOLOGICAL ANALYSIS OF RAT THYROID GLAND AFTER EXPOSURE TO AN ELECTROMAGNETIC FIELD

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Considering that the thyroid gland is one of the most sensitive organs to ionizing radiation, we decided to investigate the effects of a low frequency electromagnetic field (LF-EMF) as non-ionizing radiation on the thyroid structure of male rats exposed for five months to a 50 Hz magnetic field of decreasing intensity 500 μT to 50 μT along their cages. Stereological analysis showed a significant reduction in: volume density of the follicles ($p<0.05$), volume density ($p<0.01$) and thickness ($p<0.01$) of the follicular epithelium, as well as the thyroid activation index ($p<0.01$) while the volume density of the colloid ($p<0.01$) and interfollicular tissue ($p<0.05$) were significantly increased compared to the control. The most prominent cytological changes in the thyroidal follicular cells were the absence of apical protrusions, the rare presence of intracellular colloid droplets and the extremely low follicular epithelium in some areas of the glands.

These morphometrical and morphological alterations indicate that long-term exposure to LF-EMF induced disturbance of normal thyroidal structure.

Key words: thyroid gland, low frequency electromagnetic field, stereology, morphological alterations

INTRODUCTION

Recent studies have demonstrated that electromagnetic fields (EMF) can have a profound effect on living systems. It is generally accepted that EMFs are capable of producing effects at the organismal, tissue, cellular and membrane levels (Glaser, 1992; Graham et al., 1994; Goodman et al., 1995.)

It is well-known that the thyroid gland, which produces hormones involved in a broad range of functions, is one of the most sensitive organs to ionizing radiation. Non-ionizing radiation in the form of an EMF has been suggested to have an effect on this gland (Katola et al., 1981; Kasyanova et al., 1992.) but this has not yet been supported with sufficient data.

Our purpose in this study was to determine the effect of non-homogeneous LF-EMF on thyroid morphology using intensities to which humans can be exposed in their homes and work environment.

MATERIALS AND METHODS

A total of 10 male Mill Hill rats of 280-370 g body weight were used in these experiments. They were maintained under controlled laboratory conditions. Five animals were exposed to the influence of LM-EMF for 7 hours a day, 5 days a week for a period of five months of postnatal life. Control animals (n=5) were housed in identical conditions except for the LF-EMF.

The LF-EMF was produced in a single coil of 4 mm thick wire placed in 1320 turns. The coil was energized from standard 220 V, 50 Hz and 16 A outlets via an autotransformer. The autotransformer provided 60 V output and was used in order to reduce the electric field which was measured to be less than 10 V/m everywhere in the room. One half of the coil had a south-east/north-west orientation of axes, and the other half was orthogonal to that direction. Cages with animals were placed symmetrically on both sides of the coil. The coil produced a magnetic field of decaying intensity along the cages from 500 μT to 50 μT.

After sacrifice, rat thyroids were removed and fixed in Bouin's solution. Paraffin-embedded thyroids were cut serially in four-micrometer sections and stained using the method of PAS alcian blue and Florentine. Every fourth section from the middle of the gland to the periphery was subjected to stereological analysis. We determined the volume density of follicles (Vvf), follicular epithelium (Vve), colloid (Vvk), interfollicular tissue (Vvi), capillary network (Vvs) with grid M42 (Weibel et al., 1966). Some other characteristics were derived from the above mentioned parameters: thyroidal activation index (Ia) defined by the ratio of the Vve to the Vvk (Kališnik, 1971; 1981). The thickness of the follicular epithelial cells (D) was estimated by the formula described in Bogataj et al. (1977) The results were statistically analyzed by Student's t-test.

RESULTS

Five months exposure to the LF-EMF decreased significantly the volume density of thyroid follicles (Vvf; $p<0.05$; Figure 1), volume density (Vve; $p<0.01$; Figure 2) and thickness (D; $p<0.01$; Figure 3) of the follicular epithelium, while the volume density of colloid (Vvk; $p<0.01$; Figure 4) as well as the interfollicular tissue (Vvi; $p<0.05$; Figure 5) was significantly increased. The index of activation of the thyroid gland (Ia) was significantly decreased ($p < 0.01$; Figure 6). Volume density of the capillary network (Vvs) was increased compared to the control but not significantly (Figure 7).

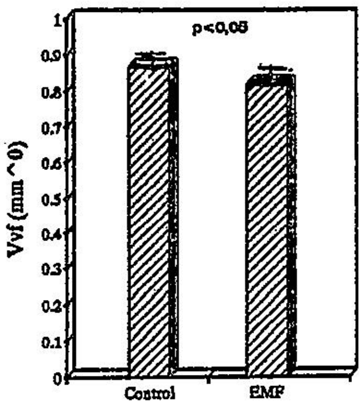

Figure 1. Volume density of the thyroidal follicles (Vvf) in control animals and animals exposed to LF-EMF. The mean ± SE (vertical bar) are given.

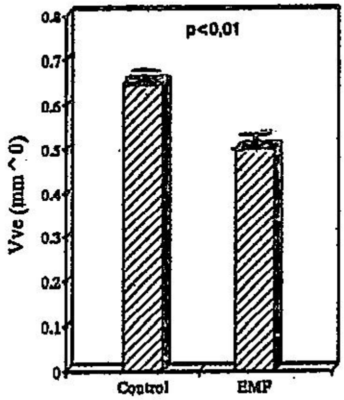

Figure 2. Volume density of the follicular epithelium (Vve) in control animals and animals exposed to LF-EMF.The mean ± SE (vertical bar) are given.

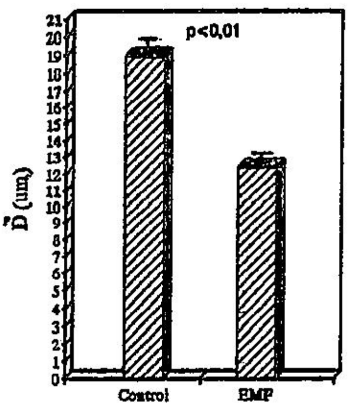

Figure 3. Thickness of the epithelium of thyroid follicles (D) in control animals and animals exposed to LF-EMF. The mean ± SE (vertical bar) are given.

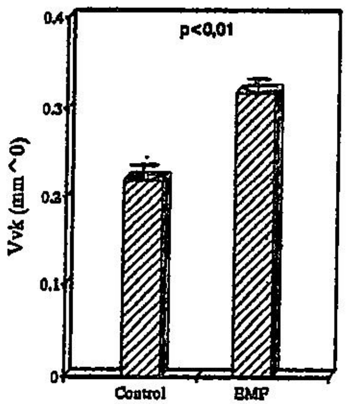

Figure 4. Volume density of colloid (Vvk) in control animals and animals exposed to LF-EMF. The mean ± SE(vertical bar) are given.

Thyroid glands from animals exposed to the LF-EMF showed less regular arrangement of follicles, with large and small follicles scattered throughout the

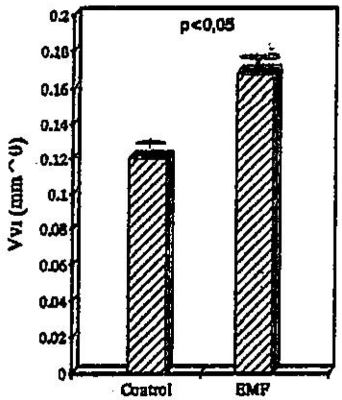

Figure 5. Volume density of interfollicular tissue (Vvi) in control animals and animals exposed to LM-EMF. The mean ± SE (vertical bar) are given.

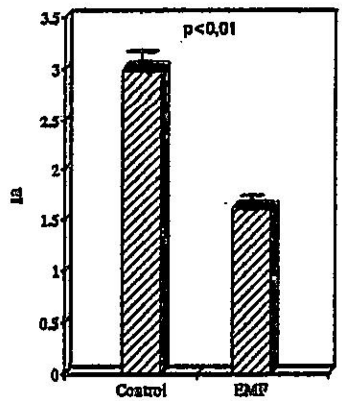

Figure 6. Activation index of the thyroid gland (Ia) in control animals and animals exposed to LM-EMF. The mean ± SE (vertical bar) are given.

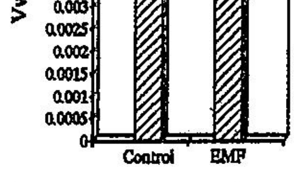

Figure 7. Volume density of blood vessels (Vvs) in control animals and animals exposed to LF-EMF. The mean ± SE (vertical bar) are given.

gland. Colloid in the follicles was generally homogenous although there were numerous follicles with areas of darker staining, explicitly PAS positive colloid.

The most prominent cytological changes in the thyroid follicular cells were the absence of apical protrusions, the rare presence of intracellular colloid droplets and extremely low follicular epithelium in some areas of the glands (Figures 8a and 8b).

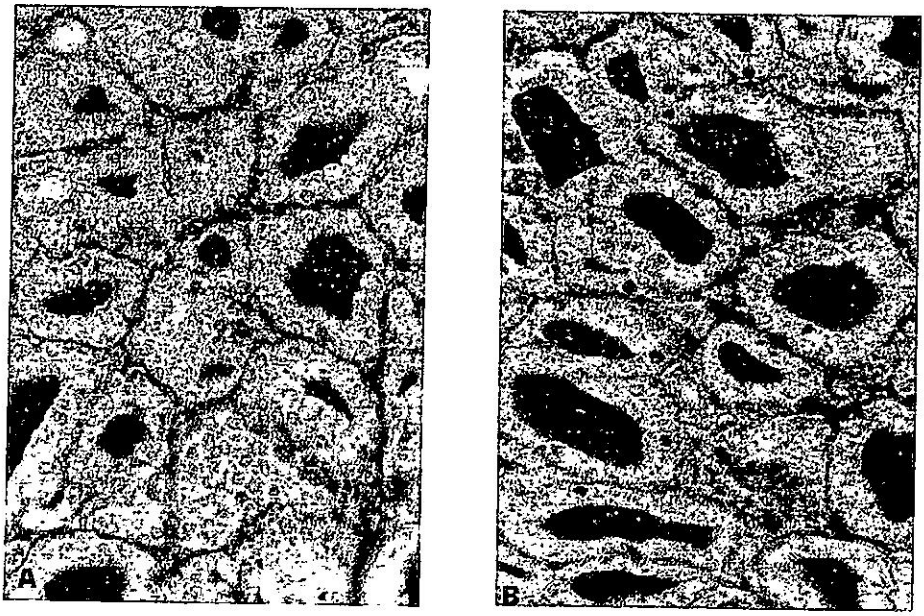

Figure 8. Thyroid gland follicular epithelium: A) control animal B) animal after five months exposure to LF-EMF. PAS alcian blue, x 1600

DISCUSSION

As our results showed, long-term exposure to a LM-EMF can disturb the normal structure of the thyroid gland. This is substantiated by the significant decrease in the thyroidal activation index which reflects the marked increase in volume density of colloid and decrease in volume density of epithelium in rats exposed to the EMF compared to the control animals.

Previous studies have indicated that an EMF interrupts many biological processes by acting on some essential metabolic substances such as ATP, cAMP, DNA, mRNA, proteins (Berg, 1992), and cell respiration processes (Gorczynska et al., 1986.). For these reasons mechanisms involved in the effects observed in the present study could be very different. The question arises: are the morphological changes of the thyroid gland the consequence of direct influence of EMF the on one of the components of the hypothalamic-pituitary-thyroid axis or an indirect consequence of changes in some other organs associated with the thyroid?

Since the thyroidal activation index, which is positively correlated with plasma TSH level (Kališnik, 1971, 1981), significantly decreased, the observed morphological changes such as the rare presence of intercellular colloid droplets as well as the absence of apical protrusions, which are the signs of decreased endocytic process, can be considered to be the result of a decrease in TSH level or more specifically the absence of its stimulative effect on the follicular epithelium. These morphological signs of a decrease in thyroid function are in agreement with earlier observations that a constant MF caused a significant decrease in thyroxine concentration (Katola et al., 1981).

On the basis of several findings that implicate the plasma membrane as the primary site of interaction between EMF-s and the cell (Chiaberera et al., 1979; Inoue et al., 1985; Marron et al., 1986; Sowers, 1986; Markov, 1990; Dacha et al., 1993.), we have considered the possibility that the follicular cell membrane could also be a target for LF-EMF influence. This is particularly important for their apical membrane. It is well known that disturbance of the normal structure leads to changes in exo- endocytotic processes which enhance the sensitivity of thyroidal cells to TSH stimulation as in some other experimental procedures (Engström et al., 1976, Ericson and Engström, 1978)

Long-term exposure to the LF-EMF can be also considered as a specific stress situation. An electric field (50 Hz, 40 kV/m) acting on the whole organism during postnatal ontogeny in mice, resulted in stress of all the systems of hormonal regulation: the thyroid, adrenal gland and testes (Kartashev and Ivanova, 1992). As a result of such stress the hormonal control of development processes was weakened, which delayed the maturation of the organism. Similarly to this, exposure to an electric field postnatally every day during 7 months, led to an increased lipid content in zona glomerulosa of the adrenal cortex (Jager et al., 1992) which was evidence that these animals were exposed to a chronic stressor (Nussdorfer, 1986). In this regard, it is possible to interpret our results from the stressogenic aspect of this experimental procedure. It is also of interest that EMFs have been shown to influence the release and levels of a number of neurotransmitters (Kavaliers and Ossenkopp, 1987) and these effects may involve actions on the thyroid gland too.

Our findings that an EMF increases the volume of interfollicular connective tissue are in accordance with previous studies which have shown that an EMF modifies membrane transport and affects both the proliferative and functional capacity of connective tissue cells. Also, genetic transcription in these cells can be altered by pulsatile EMF stimulation with a resultant massive increase in DNA and messenger RNA production (Hinsenkamp et al., 1978; Murray and Ferndale, 1985).

In summary, the present results indicate that exposure to LF-EMF, produces an impairment in the thyroidal structure as masured by stereological and cytological parameters. In view of the broad range of functions in which thyroid hormones are involved, alterations in thyroid activity induced by an EMF could initiate a wide spectrum of subtle biological effects. Further studies are needed to clarify the mechanisms by which the EMF disrupts the hypothalamic-pituitary-thyroid axis.

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STRUKTURNA I STEREOLOŠKA ANALIZA TIREOIDNE ŽLEZDE PACOVA POD DEJSTVOM ELEKTROMAGNETNOG POLJA

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SADRŽAJ

Polazeći od činjenice da je tireoidna žlezda jedan od najosetljivijih organa na delovanje jonizujućeg zračenja želeli smo da ispitamo efekte niskofrekfentnog magnetnog polja (NF-EMP) kao nejonizujućeg zračenja na strukturu ove žlezde u mužjaka pacova koji su bili pet meseci izlagani delovanju nehomogenog NF-EMP-a (50 Hz) čiji je maksimalni intenzitet bio 500 μT, a minimalni 50 μT.

Stereološka analiza tireoidne žlezde je pokazala da je hronično izlaganje delovanju NF-EMP-a dovelo do statistički značajnog smanjenja volumenske gustine folikula ($p<0.05$), volumenske gustine ($p<0,01$) i debljine folikularnog epitela ($p<0,01$), kao i indeks aktivacije tireoidne žlezde ($p<0,01$), dok je volumenska gustina: koloida ($p<0,01$) i interfolikularnog veziva ($p<0.05$) značajno povećana. Volumenska gustina kapilarne mreže je povećana, ali to povećanje nije bilo statistički značajno.

Najistaknutije citološke promene tireoidnih folikularnih ćelija pod dejstvom NF-EMP bile su: odsustvo apikalnih protruzija, retko prisustvo koloidnih kapljica i izrazito smanjena visina ovih ćelija u folikulima pojedinih žlezdanih regiona.

Ove morfometrijske i morfološke promene ukazuju da dugotrajno izlaganje delovanju NF-EMP narušava normalnu tireoidnu strukturu, a time i sintetičke i sekrecione procese u njenim ćelijama.