Original Research

The Effect of Extremely Low-Frequency Magnetic Fields on the Morphology of Thyroid Gland Cells in Female Rats

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Abstract

The aim of this study was the evaluation of the influence of extremely low-frequency magnetic fields (ELF-MF) on the morphology of thyroid epithelial cells in female rats.

The rats were exposed to ELF–MF (frequency: 5-10 Hz, induction 10 mT), 1 hour daily for 7 days over a week for 4 months. Control animals were sham exposed. After that time the rats were sacrificed by anaesthetizing with Ketamine and decapitated.

The animals' thyroid glands were extracted. They served as microscopic preparations. In the preparations, the thyreocytes, nuclei, nucleoli and nucleo-cytoplasmatic ratio were evaluated. We concluded that changes in the location of nuclei, surface field, the number and surface of nucleoli and nucloli organizers show an increase in reply activity in the nuclei of thyreocytes and a probable increase of DNA synthesis.

Keywords: extremely low-frequency magnetic field, nucleoli organizers - Ag-NORs, nucleolus, nucleus, thyroid gland cells

Introduction

Extremely low-frequency magnetic fields (ELF-MF), which are applied for treatment of humans, are distinguished from other various types of magnetic fields. They are magnetic fields of frequency not higher than 60 Hz, induction value ranging from 1pT to 15mT, volume of 130V/m, waveform of the magnetic field both rectangular and triangular.

The essential problem raised in our article is the effect of the magnetic field on particular metabolic processes of living organisms. ELF-MF is a stress stimulus associated with the development of the adaptation mechanisms within the vegetative nervous system, as well as the endocrine system. The thyroid gland is one of the endocrine system organs that receives stress stimuli very strongly. ELF-MF influence the nervous fibres of thyroid gland follicles localized in the neighbourhood of basement membrane. This influence is described as imposing the rhythm of magnetic field on nerve endings and causing the depolarization of nerve cells and changes in cell membrane of thyreocytes [1-3].

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It is believed that the cell membrane is the primary receiver of ELF-MF. Exposure to the magnetic field may be treated as a specific stress situation, which in consequence leads to an increase of cell metabolism of neurotransmitters. Under the influence of the magnetic field, cell membranes gain the capability of signal amplification by cascade triggering of the Na, K-ATPase functioning. A cellular mechanism of signal transmission is associated with the opening and closing of ionic canals; it causes changes in the activity of intramembrane enzymes and membrane receptors [4-6]. Long-term exposure of an organism to ELF-MF intensifies the process of membrane transmission, resulting in morphological changes in the epithelial cell follicles of the thyroid gland. As a reaction to the stress stimulus in the form of ELF-MF activity, an increase of biosynthesis of cellular proteins and of transcription in a cell are observed and also changes in expression and differentiation of genes occur [7-9].

The aim of this research was to evaluate the influence of ELF-MF on the morphology of thyroid epithelial cells in female rats. The parameters of waveform, magnetic field frequency, and field amplitude applied in this work were the same as the ones used in the treatment of humans. The choice of ELF-MF parameters was partially arbitrary.

In the available specialist literature the authors of this work did not find many sources dealing with the effect of ELF-MF on thyroid gland epithelial cell morphology in female rats. Furthermore, the authors did not come across any publications concerning the influence of ELF-MF on nucleoli organizers of thyroid gland in female rats.

Experimental Procedures

36 female Wistar rats, Hannover substrain, aged 10 months, weighing 300+/- 50g each were included in the experiment. The rats came from the Medical University of Silesia in Katowice, Poland. The categorization of laboratory animals was worked out in cooperation with the international organizations: WHO – World Health Organization, FAO – Food and Agriculture Organization, ILAR – Institute for Animal Resources, and ICLAS – International Council for Laboratory Animal Science. The conventional animals CV-I – controlled, of established health standard and kept n semi barrier condition, were used in this experiment. It had significant influence on the quality and repeatability of the obtained results and allowed us to decrease the number of animals necessary for performing the experiment properly. The rats were fed with standard feed for laboratory animals of Murigran type, which was sterilized in an autoclave and produced in accordance with "Wytwórnia Koncentratów i Mieszanek Paszowych – Agropol," Motycz, Poland. The amount of iodine in the Murigran chow was 0.858 mg/ kg.

The rats had continuous access to sterilized water. The cages and the bedding were also sterilized in the autoclave and the cages with dirty bedding were removed outside also through the autoclave and without sterilization. At the age of six months the rats underwent ovariectomy performed from lumbar access. They were anaesthetized pharmacologically with Ketamine in the dose of 1mg/kg body mass injected intravenously. The span of time from ovariectomy to the beginning of ELF-MF stimulation of individuals was 100 days.

The rats were divided into 3 groups:

- Group '40' – 12 female rats exposed to ELF-MF of 40 Hz frequency, induction of 10mT and sinusoidal waveform impulse, for 1 hour per day, 7 days a week, for 4 months.
- Group '5' – 12 female rats exposed to ELF-MF of 5 Hz frequency, induction of 10mT and rectangular waveform impulse, for 1 hour daily, 7 days a week, for 4 months.
- Control Group 'C' – 12 female rats which did not undergo any procedural therapy, only exposure in simulated conditions was performed for 1 hour daily, 7 days a week, for 4 months; it was sham exposure.

The rats from group '40' and group '5' were exposed to the ELF-MF, of the previously mentioned parameters, and the cages with the animals placed inside the coils of the device generating the field. The sham animals were exposed to the simulated magnetic field, i.e. they were put inside the coils with no voltage. The induction value (r.m.s.) of the ELF-MF applied in the experiment was 10mT.

The ELF-MF was generated by a magnetotherapy device powered by network electricity; the device, Ambit 2000, was produced by the 'Ambit' factory in Warsaw, Poland. The device consists of a coil, serving as an applicator of the magnetic field and of appliances for changing the magnetic field parameters. The device allows for the creation of fields of rectangular, sinusoidal or triangular waveform and the respective half-cycle fields in the frequency range of 2-60 Hz and the induction of 0.001-5 mT (r.m.s. value). The regulating system located in the generator controlled in a computerized way the parameters of the field which could be watched on the monitor screen. The inductive coils of the generator were of cylindrical shape of 500 mm in diameter and the height was of 160 mm. The winding of the coils was fixed on the inner side of a cylinder wooden lining. During the experiment the induction coils of the generator were placed horizontally and the magnetic fields distribution was vertical. Inside the coils of the applicator the induction was slightly higher than near the applicator wall and the range of influence comprised the area outside the coils. The investigations of the spatial distribution of magnetic induction inside the coils of the applicator for various waveforms of the magnetic field were performed by The Institute of Physics of the Silesian University, which made the standardization and repeatability of the experiments on laboratory animals possible. The influence of other sources of magnetic fields, as well as noises and vibrations which could be noticed by rats were eliminated.

As the intensity of electric current in the solenoid making up of an applicator is very low, no sound vibration is produced by an applicator. The source of sound vibration during exposure to variable magnetic field is an action of mechano-electric elements of a generator. Taking into account that both during active and sham exposure these elements are working, it is obvious that one cannot eliminate sound vibration definitely, but in both situations the level of this vibration is comparable. The differences in analyzed parameters between active and sham-exposed animals are related to the action of magnetic field, not to the effect of sound vibration.

The Teslometer TSL-2, produced by the Technological University of Wrocław was used for measurements of the magnetic field induction inside the inductive coils of the generator.

The rats were sacrificed after four months' exposure to the Extremely Low-Frequency Magnetic Fields. The sacrifice was done by anaesthetizing the animals with Ketamine and the procedure was conducted by pitching and decapitation. The sacrifices took place in the special operation boxes protected by a hygiene barrier and located outside the breeding rooms always at the same time of the day, between 10.00 and 12.00 A.M. It emerged from the archive data that on the day of sacrifice the concentration of the FSH hormone corresponded to the values of the same hormone in the early follicular phase. During the post-mortem examination the thyroid glands were removed and after formalin fixing preparations were made out of them which, in turn, underwent microscopic evaluation.

In order to obtain the repeatability of results of the quantity tests, standard places of taking sections were established and the description of the position of the cutting plane in regard to the anatomical point of the thyroid gland was defined. The female rats' thyroid glands preserved in formalin were later rinsed thoroughly and dehydrated in alcohol solutions of increasing concentrations from 50 to 100%. After dehydration the tissues were put in xylene and afterwards embedded in paraffin of 56 degrees Celsius. A paraffin block was made out of thyroid gland tissue block and sequence of the obtained sections was marked, starting with the upper cartilages of trachea. The tissues were cut into a series of sections of 4 μm thickness. The cutting into sections was performed by means of the ultramicrotome produced by Reichert Company (Germany), with the use of a knife of established cutting angle and a flat, concave blade. Micro slides with paraffin-coated sections of female rats' thyroid gland stuck to them were deparaffinated at a temperature of 36 degrees Celsius in the incubator and afterwards left in xylene and later in a mixture of alcohol and xylene. Next, the sections were gradually hydrated by dipping them in a series of alcohols of decreasing concentrations, from 100% to 50%. Finally, the sections were rinsed with water. After taking the preparations out of water they were stained in a solution of silver nitrate, and again rinsed in water and after that they were put, one by one, in 5% alkaline solution of sodium tiosulphate. After having been rinsed in water the preparations were stained in a solution of hematoxylin and eosin. Then the preparations were gradually dehydrated in a series of alcohols of increasing concentrations.

After dehydration the sections were put into xylene and topped with a cover glass by means of DePeX. The reagents used for these investigations were produced in Gliwice, Poland by Polskie Odczynniki Chemiczne.

A Docuval light microscope made by Carl-Zeiss in Jena (Germany), with a picture analysis computer programme, was applied in the study.

The microscopic slides were estimated with the use of 400x magnification of the light microscope, maintaining the calibration scale before each evaluation of a slide. For measurements the pore size mesh was used, with pores of 500 μm and the size of the mesh was 15x15 mm. Fifty visual fields in each of the examined rats and for each parameter were evaluated and in each visual field 20 cells were estimated. Surface areas of nuclei, nucleoli and nucleoli organizers were calculated by means of the direct measurement of the 2-dimensional area occupied by an object in the microscope field of vision. The areas were calculated moving the slide clockwise.

In the preparations of rats' thyroid glands the following parameters were evaluated: height (H) and surface area of cytoplasm of thyreocytes (SAC), axis of the nucleus height (ANH), axis of the nucleus base of thyreocytes (ANB), the surface area of nucleus (SANU), the location of the nuclei of thyreocytes (LN), the nucleo-cytoplasmatic ratio (SAN/SAC), the number of nucleoli (NNU), the surface area of nucleoli (SANU) and the number of nucleoli organizers AgNORs (NAgNOR), and the surface area of AgNORs nucleoli organizers (SAAgNOR).

The investigation was performed by means of a double-blind trial: one person randomly divided all the rats into the three examined groups, the second one exposed the rats to ELF-MF, and the third person made preparations and evaluated them with the use of the microscope. The third person did not know which preparations she was evaluating. The obtained results were analyzed using the methods of statistical calculation.

In the statistical analysis the following methods were applied: Cochran-Cox variance homogenity test, the analysis of variance heterogenity - F statistics tests, the determination of significance of the differences between mean values (post-hoc analysis) with Tukey test.

For calculations the parametric statistics module of the computer program Statistica 5.0 was applied.

The use of animals in the study was approved by the Ethical Committee for Conducting Experiments on Animals at Silesian Medical University in Katowice.

Results

In Table 1 the mean values of the epithelial height of thyreocytes and the surface area of cytoplasm of thyreocytes are presented. The mean epithelial heights of thyreocytes in the three investigated groups of female rats showed a significantly characteristic difference for comparison between groups.

The mean values of the variable of the surface area of cytoplasm of thyreocytes do not differ significantly depending on the field frequency (no differences between groups '40' and '5,' but there was a significant difference between group '40' and 'C,' and '5' and 'C').

Table 1. Average values and standard deviations for: height of thyroid gland cells (H), surface area of cytoplasm of thyroid (SAC), axis of the nucleus height (ANH), axis of the nucleus base (ANB), surface area of nucleus (SAN), the nucleo-cytoplasm ratio (SAN/SAC), surface area of nucleoli (SANU), the number of nucleoli (NNU), the number of nucleoli organizers (NAgNOR), and the surface area of AgNOR nucleoli organizers (SAAgNOR).

Parameters	Examined groups of rats X +/- SD			Level of significance		
	'40'	'5'	'C'	'40' and '5'	'40 and 'C'	'5' and 'C'
H [μm]	4.57(+/-0.05)	3.13(+/-0.06)	2.02(+/-0.08)	p<0.0001	p<0.0001	p<0.0001
SAC[μm²]	6.71(+/-0.08)	6.69(+/-0.07)	7.24(+/-0.07)	0.88249	p<0.0001	p<0.0001
ANH [μm]	3.17(+/-0.06)	2.81(+/-0.07)	1.78(+/-0.05)	p<0.0001	p<0.0001	p<0.0001
ANB [μm]	1.65(+/-0.08)	2.58(+/-0.06)	3.51(+/-0.08)	p<0.0001	p<0.0001	p<0.0001
SAN [μm²]	5.17(+/-0.08)	4.32(+/-0.07)	3.27(+/-0.09)	p<0.0001	p<0.0001	p<0.0001
SAN/SAC	0.80(+/-0.06)	0.66(+/-0.07)	0.46(+/-0.07)	p<0.0001	p<0.0001	p<0.0001
SANU[μm²]	0.41(+/-0.07)	0.26(+/-0.06)	0.17(+/-0.07)	p<0.0001	p<0.0001	p<0.0001
NNU	5.17(+/-0.08)	4.32(+/-0.07)	3.27(+/-0.09)	p<0.0001	p<0.0001	p<0.0001
NAgNOR	3.72(+/-0.08)	2.05(+/-0.07)	1.46(+/-0.07)	p<0.0001	p<0.0001	p<0.0001
SAAgNOR[μm²]	0.19(+/-0.06)	0.25(+/-0.05)	0.12(+/-0.05)	p<0.0001	p<0.0001	p<0.0001

Table 1 also presents the results of statistical analyses for comparison of measurement features: axis of nucleus height, axis of nucleus base, surface area of nucleus and the nucleo-cytoplasmatic ratio – between the groups of the analyzed rats. The results of particular parameters were significantly different depending on the type of field volume.

After exposure to the field of 5Hz and 40 Hz frequencies, an increase in the measured parameters of the nucleus took place. Under the influence of ELF-MF the nuclei presented different locations in relation to basement membrane and two arbitrarily selected cellular division areas.

In Table 1 the mean numbers of nucleoli in thyreocytes and the mean surface areas of nucleoli are shown. In the investigated groups of female rats, '40', '5', and 'C,' the mean values of nucleoli in thyreocytes and mean surface areas of nucleoli differed significantly, the mean numbers of nucleoli in thyreocytes and the mean surface areas of nucleoli increased after the exposition to ELF-MF, the highest value presenting in group '40.' In Table 1 and 2 the mean numbers of nucleoli organizers in thyreocytes and the mean surface areas of nucleoli organizers are displayed. It was shown that these values differed significantly in the investigated groups of rats.

The Cochran-Cox variance homogeneity test was used. The values of the Cochran-Cox test and the p level for all the examined parameters allow the conclusion that on the significance level = 0.05 only in the case of the number of nucleoli variable (NNU) the hypothesis of variance homogeneity can be accepted. In the rest of the cases this hypothesis must be rejected because the p value is lower than the assumed significance level. The analysis of variance can be done even in such a case. In order to verify the results an additional analysis of variance heterogeneity was carried out using statistics with Fischer-Snedecor distribution with 2 and 1797 degrees of freedom (the F test statistics). The value of the F test statistics for the 10 examined parameters was many times higher than the critical level given in the tables (its value is 3.00). Therefore, the hypothesis concerning the lack of significant differences in the location of features in particular groups was rejected and the alternative hypothesis of the significance of ELF-MF influence on the 10 examined parameters was accepted. By means of Tukey test the hypothesis about the lack of significant differences between any two average values of a particular parameter was verified. The post-hoc probability values are lower than the assumed significance level (0.05), which proves that a particular pair of average values shows a significant difference (Table 2). This conclusion does not concern only the surface area of cytoplasm of thyroid gland (SAC) variable as the value did not vary according to the frequency of ELF-MF, but depended solely on being exposed to ELF-FM (lack of differences between groups 1 and 2, distinct difference between 1 and 3 as well as between 2 and 3).

Discussion of Results

The parameters of the magnetic field accepted in the investigation corresponded to the typical values applied for magnetic field therapy in humans [10-12]. In order to eliminate the effect of activation of sexual glands hormones on metabolism of female rats' thyroid glands with the rats being a source of thyroid gland for investigation, the rats underwent ovariectomy in the time preceding the experiment. As a result of the above-mentioned operation, the level of

Table 2. Cochran-Cox test, Test F, Tukey test for: height of thyroid gland cells (H), surface area of cytoplasm of thyroid (SAC), axis of the nucleus height (ANH), axis of the nucleus base (ANB), surface area of nucleus (SAN), the nucleo-cytoplasm ratio (SAN/SAC), surface area of nucleoli (SANU), the number of nucleoli (NNU), the number of nucleoli organizers (NAgNOR), and the surface area of AgNOR nucleoli organizers (SAAgNOR).

Parameters	Cohran – Cox Test	p	Test F F (2,1797)	Tuckey Test		
				‘40’ and ‘5’	’40 and ‘C’	‘5’ and ‘C’
H [μm]	0.620	0	4485.760	0.00002	0.00002	0.00002
SAC[μm²]	0.480	0	66.693	0.88249	0.00002	0.00002
ANH [μm]	0.708	0	1705.342	0.00002	0.00002	0.00002
ANB [μm]	0.493	0	3263.302	0.00002	0.00002	0.00002
SAN [μm²]	0.452	0	1959.825	0.00002	0.00002	0.00002
SAN/SAC	0.924	0	168.415	0.00002	0.00002	0.00002
SANU[μm²]	0.535	0	2929.908	0.00002	0.00002	0.00002
NNU	0.353	0.0729	739.374	0.00002	0.00002	0.00002
NAgNOR	0.402	0	1720.355	0.00002	0.00002	0.00002
SAAgNOR[μm²]	0.471	0	2351.111	0.00002	0.00002	0.00002

steroid hormones in the investigated rats corresponded to the level of these hormones in the early follicular phase and was also observed in menopausal women. At the same time, it can be stated on the basis of the available literature that in physiological conditions menopause does not cause a significant involution of the thyroid gland [13-15].

The significant increase in the height of the axis of thyreocytes corresponding to the changes of frequency of ELF-MF was observed. In the group exposed to the field of 40 Hz value, the increase was significantly higher in comparison to the group exposed to the magnetic field at a frequency of 5Hz. A significant difference between groups ‘5’ and ‘40’ in relation to the control group was found.

According to Pershin et al. [16], the outer magnetic field influences metabolic processes by change of the energetic state and functions in cell membranes of endocrine glands, including the thyroid gland: this is proved by results of investigations performed by Matavulj et al. [17], who applied a magnetic field of the frequency of 50Hz with the exposition time of rats being 7 hours daily, 5 days per week.

The previously mentioned authors observed changes of thyreocytes and colloid density under the influence of the magnetic fields.

Evidence of the influence of the field intensity on the morphology of thyreocytes was shown by research of Matavulj et al. [17], where after the application of ELF-MF of value 0.5 Hz and a markedly shorter time of activity, morphological changes of thyroid gland epithelium were observed. On the basis of the data from the specialist literature it may be believed that the cause of the changes of epithelial form was stimulation of the cell membrane which is the primary receiver of the ELF-MF activity. Exposure to the magnetic field may be treated as a specific stress situation, the consequence of which is the increase of membrane metabolism of neurotransmitters. Under influence of the field, cell membranes become capable of signal amplification by triggering the cascade activity of Na,K-ATPase [18]. This is proved by the results of investigations performed by Blank and Soo [19], who evidenced the influence of the magnetic field of 60 Hz frequency and induction value of 3-10 mT on Na,K-ATPase.

In the present research it was proved that the increasing frequencies of the magnetic field (5Hz, 40Hz) were accompanied by increase of the height of nucleus axis and the decrease of the basal axis of thyreocytes. Simultaneously, the nucleus increased its surface area at the cost of the decrease of the surface area of cytoplasm of thyroid gland cells.

Also an increase of the nucleo-cytoplasmatic ratio of thyreocytes was noticed. Besides, the change of arrangement of nuclei in thyreocytes in each of the investigated groups in relation to the control group was observed.

The obtained results confirm the observations of Iatropoulos et al. [20] and Zagorskaia et al. [21], who analyzed in their investigations the effect of ELF-MF on the nuclei of thyreocytes and found increase of the surface area of nuclei and the nucleo-cytoplasmatic ratio of thyreocytes in rats.

Ossenkopp et al. [22] and Pershin et al. [16] also observed changes of the location of the nucleus in the thyreocytes as an effect of activity of ELF-MF. But these authors did not present any detailed morphometric characteristics of the location of nuclei in the thyreocytes.

In the stimulated-to-produce colloid thyroid gland cells, the nucleus and the Golgi apparatus become enlarged and change their location, becoming the secretory surface of the cell. The cellular nucleus is the final place of the activity of thyroid hormones. After activation of the DNA domain, known as 'thyroid-hormone response element – TRE,' the increase of nuclear concentration of RNA occurs. The nuclear receptors are peptides built as typical transcription factors, which means that they possess the linking DNA domain responsible for oligomerization and the domain linking the DNA of zinc finger structures [23-25].

It also emerges from specialist literature that in the response to the stress stimulus which, obviously, is the activity of the ELF-MF, changes in biosynthesis of protein and the regulation of the cell cycle and stimulation of transcription in the cell occur. The cellular response to the magnetic field signal is also expressed by the number and surface areas of nucleoli and nuncleoli organizers.

In the present investigation, simultaneously with the increase of the magnetic field, an increase of the number and surface areas of nucleoli and nucleoli organizers was observed. The surface areas of nucleoli were biggest after exposure to the highest achieved values with the application of the field of 5 Hz value.

The above is confirmed by Canet et al. [13] and Neuman [26], who showed that ELF-MF influence the increase of the number of nucleoli and nucleoli-formation AgNOR regions. These authors stated that AgNOR numbers increase during the G1 phase of the cellular cycle and remain up to the G2 phase. Simultaneously, AgNOR surface area gets higher. The presentation of the structure of chromatin fibres in AgNOR and the presence of proteins stained by silver nitrate AgNOR during the cellular division is the expression of the readiness of rDNA genes for transcription, which will take place after the ending of the mitosis in the next interphase. At the end of prophase when the AgNOR action finishes, the material is partially transported to cytoplasm. In metaphase and anaphase in take places of AgNOR remain only not numerous silver spots pointing at the relationship with the transcription process during the interphase preceding the division. Only in the late telophase, together with the activation of AgNOR, the quantity of the silver absorptive material increases once again and the nucleoli appear [27, 28].

The observed changes of the location of nucleus, its surface area and the number and surface areas of nucleoli and nucleoli organizers point to the increase of the replicative activity in nuclei of thyreocytes and possible increase of DNA synthesis.

The obtained results suggest that the cause of change in morphology of the epithelium of thyreocytes was stimulating the metabolism with the activity of ELF-MF. The increasing frequency of ELF-MF stimulated the increase of the number and surface areas of nucleoli and the interphase nucleoli organizers localized in the nucleoli [29].

Conclusions

Changes in the location of nucleus, surface field, the number and surface of nucleoli and nucleoli organizers show the increase of the reply activity in the nuclei of thyreocytes and probable increase of DNA synthesis. The obtained results suggest that the changes are due to the activity of extremely low-frequency magnetic field. Frequency of ELF-MF stimulates the increase of the number and surface field of nucleoli and interphase nucleoli organizers localized in the nucleoli. Changes of the parameters are a good indicator of cell activity.

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