ORIGINAL PAPERS

PLASMA THYROID HORMONES AND CORTICOSTERONE LEVELS IN BLOOD OF CHICKEN EMBRYOS AND POST HATCH CHICKENS EXPOSED DURING INCUBATION TO 1800 MHZ ELECTROMAGNETIC FIELD

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Abstract

Introduction: This study attempted to determine the effect of a 1800 MHz electromagnetic field (EMF) (only carrier frequency) on thyroxine (T_4), triiodothyronine (T_3) and corticosterone (CORT) concentrations in the blood plasma of chick embryos, and to investigate the effect of electromagnetic field (EMF) exposure during embryogenesis on the level of these hormones in birds that are ready for slaughter. **Material and Methods:** Throughout the incubation period, embryos from the experimental group were exposed to a 1800 MHz EMF with power density of 0.1 W/m², 10 times during 24 h for 4 min. Blood samples were collected to determine T_4, T_3 and CORT concentrations on the 12th (E12) and 18th (E18) day of incubation, from newly hatched chicks (D1) and from birds ready for slaughter (D42). **Results:** The experiment showed that T_4 and T_3 concentrations decreased markedly and CORT levels increased in the embryos and in the newly hatched chicks exposed to EMF during embryogenesis. However, no changes were found in the level of the analyzed hormones in the birds ready for slaughter. Differences in T_4 and T_3 plasma concentrations between the EMF-exposed group and the embryos incubated without additional EMF were the highest in the newly hatched chicks, which may be indicative of the cumulative effect of electromagnetic field on the hypothalamo-pituitary-thyroid axis (HPT). **Discussion:** The obtained results suggest that additional 1800 MHz radio frequency electromagnetic field inhibits function of HPT axis, however, it stimulates hypothalamo-pituitary-adrenal axis by inducing adrenal steroidogenic cells to synthesize corticosterone. Further investigations are needed to elucidate the mechanisms by which radio EMFs affect HPT and HPA axis function in the chicken embryos.

Key words:
Electromagnetic fields, Mobile phone, Thyroid hormone, Corticosterone, Chicken embryo

INTRODUCTION

During the course of evolution, living organisms have developed in the constant presence of natural electromagnetic fields. Today artificial electromagnetic fields, which are a consequence of human progress, begin to play a considerable role in shaping the Earth's electromagnetic environment. Among these, the fields generated by mobile telephony deserve special attention due to their prevalence. The extent of the problem is evidenced by a recent report of the International Telecommunication Union [1] according to which, in 2012 there were 6 billion mobile phones in use worldwide, which is by 5 billion more in comparison with the year 2000.

Research on the impact of electromagnetic fields (EMF) on living organisms has been conducted for over a century. Despite many studies, the possible health consequences of exposure to weak electromagnetic fields, including EMF produced by mobile telephony, have not been conclusively established yet [2–5].

One of the research areas receiving special emphasis in recent times is the impact of electromagnetic fields on organisms at the embryo stage of development, neonatal organisms, as well as juvenile organisms [6,7]. These studies often make use of various models that allow to evaluate the impact of EMF on living organisms. Due to the rate and specific characteristics of development and the well understood process of embryogenesis, chick embryo is frequently used as a model in different kinds of biological research, including studies, which investigate the effect of EMF on living organisms [8–13].

One of the most important indicators showing that the body's homeostasis has been disrupted are hormones. Many studies showing the effect of radio frequency electromagnetic fields (RF-EMF) (900/1800 MHz) on the endocrine system of adults are available [14–17]. However, there are no data in the literature concerning the impact of RF-EMF on the hormonal system of organisms in the embryo stage.

Therefore, this study attempted to determine the effects of a 1800 MHz electromagnetic field on the concentration of thyroid hormones (thyroxine (T_4) and triiodothyronine (T_3)) and corticosterone (CORT) in domestic chick (*Gallus domesticus*) embryo blood, and to examine the effect of EMF exposure during embryogenesis on the level of these hormones in birds that are ready for slaughter.

MATERIALS AND METHODS

Materials

Fertilized eggs at the age of 50 weeks were obtained from the same parental broiler flock of Ross 308. RIA kits for T_4 and T_3 were purchased from BRAHMS (USA). The kit for determination of corticosterone was from LDN (Germany).

Experimental design

Three hundred eggs used in the experiment were incubated using standard procedures in Masalles type 65 DIGIT incubators (1–18 days of incubation: temperature 37.8±0.1ºC, relative humidity 50–60%, 19–21 days of incubation: temperature 37.2±0.1ºC, relative humidity 60–75%). The eggs were divided into 3 equal groups: group I (control) – eggs developed in a standard incubator; group II (sham group) – eggs developed in an incubator equipped with a switched-off generator of electromagnetic field; groups III (experimental) – eggs incubated in an incubator equipped with a switched-on generator of electromagnetic field.

Throughout the incubation period, embryos from the experimental group were exposed to EMF power density ranging from 0.090 to 0.110 W/m² (±0.001 W/m²) 10 times during 24 h, 4 min each. During every exposition period sinusoidal shape of a signal which was transmitted as constant wave was used.

To determine the effect of electromagnetic fields applied during embryogenesis on endocrine activity and hormone concentrations in birds, the chicks from each group

(N = 80) were wing banded and transported to a poultry house, where they were kept according to the manufacturer's instructions until ready for slaughter [18].

EMF generator

The electromagnetic field source was a specially designed generator of radio frequency electromagnetic waves (only carrier frequency GSM 900/1800 MHz) (Figure 1). The maximum power output of the generator that could be emitted was 330 mW. The output power was delivered to a Yagi GSM ceiling antenna with omnidirectional characteristics. Antenna parameters: indoor 8dBi, omni ceiling antenna bands: GSM-900 MHz, GSM-1800 MHz – a linear polarized antenna which is produced by IAPT. The generator was computer controlled via USB interface. The installed software allowed to choose the generated signal strength and generator's operation time.

During the experiment, the antenna remained at a fixed distance from the egg tray (24 cm above the eggs). Embryos developing in eggs were situated in the area where electric and magnetic fields were included in the ranges from 4.23 V/m to 6.25 V/m (± 0.01 V/m) and from 0.010 A/m to 0.014 A/m (± 0.001 A/m) respectively, whereas power density was from 0.090 W/m² to 0.110 W/m² (± 0.001 W/m²) and frequency was 1800 MHz.

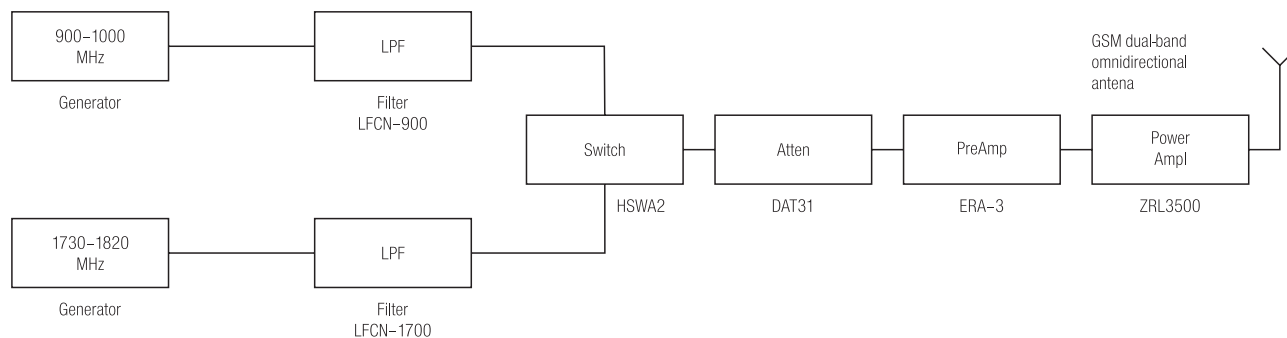Power density of electromagnetic wave was measured with a Tenmars TM-195 3-axis meter. This device allows, among others, to perform isotropic measurement of intensity of electric fields (0.01–20.0 V/m) and intensity of magnetic fields (0.1–532.6 mA/m) as well as power density (10.0–106.94 mW/m²) in the range of frequency from 50 MHz to 3.5 GHz.

Determinations of hormones

Blood samples from 15 chicks from each group were collected by cardiac puncture of developing embryos on days 12 (E12) and 18 (E18), newly hatched chicks (D1), and on the last day of fattening from the wing vein of chickens. Sampling was performed into Eppendorf test tubes with Coaparin® (Polfa Warsaw Ltd, Poland). The blood samples were centrifuged for 10 min (2000×g) and individual plasma samples from hatched chicks were kept at –20°C until hormones determination.

Thyroid hormones determination
Thyroxine (T_4) and triiodothyronine (T_3) concentrations in plasma samples were determined radioimmunologically using T_4 and T_3 kits (BRAHMS, Germany). The lowest limits of T_4 and T_3 assay sensitivity were 1.5 ng/ml and 0.09 ng/ml, and mean recoveries (performed in our laboratory) 96.3% and 95%, respectively. The intra- and inter-assay coefficients of variation for T_4 and T_3 analysis were 4.0% and 5.3%, and 3.5% and 6.3%, respectively. The cross-reactions of T_4 antibodies with L-T_3 and L-rT_3

LPF – low-pass filter; PreAmp – preamplifier; Power Ampl – power amplifier.

Fig. 1. Block diagram of the generator

were < 0.2% and 5%, respectively, while with other iodothyronines and iodothyronine-like compounds they were below 0.5%. The cross-reaction of T_3 antibodies with L-T_4 was 0.06%, and with other iodothyronines and iodothyronine-like particles it was below 0.2%.

Corticosterone

Corticosterone was measured by enzyme-immunoassay using a commercial kit. All samples were run in duplicate and kit calibrators and controls were included in each analysis. Absorbance was measured at 450 nm, with a reference wave length of 650 nm, in an ELISA microplate reader (MRX® II Dynex Technologies, USA). The lowest limits of COR assay sensitivity were 1.573 ng/ml, and mean recoveries (performed in our laboratory) 98.2%. The intra- and inter-assay coefficients of variation for corticosterone were 2.4% and 6.4%.

Ethics

The experiment received permission of the Local Ethical Committee for Experimentation on Animals in Kraków (Resolution No. 113/2010).
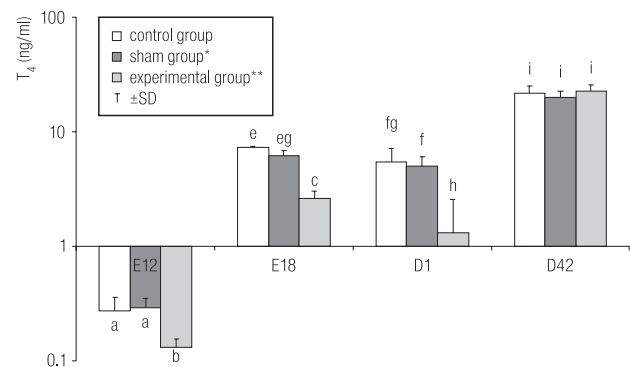
STATISTICAL ANALYSIS

The results were analyzed statistically by the use of two-way analysis of variance for repeated measures, while significant differences between the means were evaluated using Student's t-test. Because the analyses did not show significant differences in plasma thyroid hormone and corticosterone levels between male and female embryos during incubation process and after hatching, the data from both sexes were combined. The results were presented as means ±SD and considered significant at $p \leq 0.05$. Normality of distribution was assessed using Shapiro-Wilk's test. Log transformations were performed as needed in order to maintain homogeneity of variance. Probability of 0.05 was considered an important indicator of statistical differences between the means.

Statistical analyses were performed using Sigma-Stat 2.03 (SPSS Science Software Ltd., USA), while the figures were made with Grapher 8.0 (Golden Software Inc., USA).

RESULTS

In all groups, the lowest mean T_4 concentration was found in E12 embryos, and the highest in the birds from D42 group. The respective values were 0.27±0.084 ng/ml and 21.75±3.265 ng/ml in the control group, 0.29±0.059 ng/ml and 20.12±2.569 ng/ml in the sham-exposed group, and 0.13±0.026 ng/ml and 22.49±3.427 ng/ml in the experimental group ($p < 0.05$) (Figure 2). Statistical analysis showed that in E12 and E18 embryos and in the newly hatched chicks exposed to additional EMF, plasma T_4 concentration was significantly lower than in the control and sham-group embryos and chicks ($p < 0.05$) (Figure 2). EMF exposure during the embryo stage had no significant effect on plasma T_4 levels in the birds ready for slaughter (Figure 2).

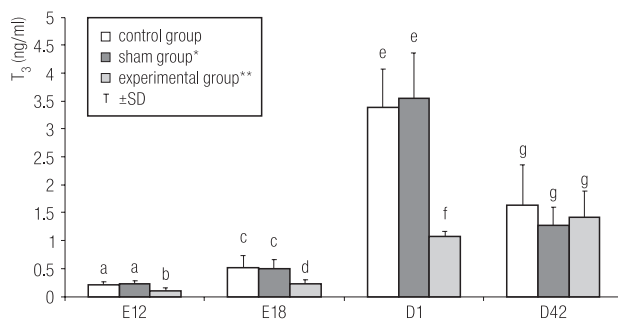* Egg incubation in the incubator with a switched-off generator of EMF.
** Eggs incubated in the incubator with a switched-on generator of EMF; means marked with different letters differ significantly ($p \leq 0.05$).
EMF – electomagnetics fields; SD – standard deviation.

Fig. 2. Effect of chick embryo exposure to 1800 MHz electromagnetic field on plasma thyroxine (T_4) concentration of chick embryos (12 and 18 days of incubation – E12 and E18), newly hatched chicks (D1) and chickens on the last day of fattening (D42) (N = 15) (logarithmic scale)
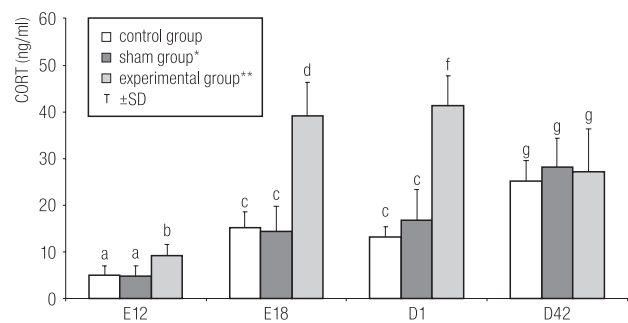
In the control group, the lowest T_3 concentration (0.212±0.055 ng/ml) was found in E12 embryos and the highest in the newly hatched chicks (D1) – 3.396±0.673 ng/ml (p < 0.05). Mean T_3 level in the blood of birds from D42 was 1.96-fold lower compared to that obtained in the case of D1 (p < 0.05). Similar changes in T_3 concentration were observed in the sham-exposed group, in which embryos developed in an incubator equipped with a switched-off generator of electromagnetic field. In the experimental group, the lowest T_3 concentration was also found in E12 embryos (0.105±0.057 ng/ml), but unlike the control group, it increased gradually to reach the highest level in the birds ready for slaughter (1.422±0.422 ng/ml; p < 0.05) (Figure 3). Concentration of this hormone gradually increased to reach the highest level in the birds ready for slaughter (1.422±0.461 ng/ml; p < 0.05) (Figure 3). Plasma T_3 concentration of embryos from the experimental group at E12, E18 and D1 was significantly lower compared to embryos from the control and sham-exposed groups (p < 0.05). There were no significant differences in plasma T_3 concentration of the birds ready for slaughter from different groups (Figure 3).

The lowest mean concentration of CORT in all the groups was noted in E12 embryos (5.12±1.905 ng/ml in the control

Abbreviations as in Figure 1 and 2.

Fig. 4. Effect of chick embryo exposure to 1800 MHz electromagnetic field on plasma corticosterone (CORT) concentration of chick embryos (12 and 18 days of incubation – E12 and E18), newly hatched chicks (D1) and chickens on the last day of fattening (D42) (N = 15)

group, 4.95±2.031 ng/ml in the sham-exposed group, 9.27±2.287 ng/ml in the experimental group). The highest CORT concentrations in the control and sham groups (25.30±4.229 ng/ml and 28.19±6.22 ng/ml, respectively) were noted in birds on the last day of fattening (p < 0.05). In the experimental group, the highest CORT concentration was noted in D1 (41.35±6.297 ng/ml) (Figure 4). Statistical analysis revealed that plasma CORT concentrations of E12, E18 embryos and the newly hatched chicks from the experimental group were significantly higher compared to those found in the control and sham-exposed groups (p ≤ 0.05). No significant differences in CORT concentrations were found between the analyzed groups in the case of D42 (Figure 4).

DISCUSSION

The present study is probably the first to show a significant decrease in plasma T_4 and T_3 concentrations of embryos exposed to a 1800 MHz electromagnetic field (Figure 2 and 4). Our results are highly similar to those obtained in the study investigating the effect of RF-EMF on thyroid hormone levels in adult rats [15] and humans [16,19]. However, our findings contradict the earlier

SD – standard deviation. Other abbreviations as in Figure 1.

Fig. 3. Effect of chick embryo exposure to 1800 MHz electromagnetic field on plasma triiodothyronine (T_3) concentration of chick embryos (12 and 18 days of incubation – E12 and E18), newly hatched chicks (D1) and chickens on the last day of fattening (D42) (N = 15)

results of Magin et al. [20], who showed that blood T_4 and T_3 concentrations increase in dogs exposed to a 2400 MHz field. The increase in hormones concentrations observed in the experiment could be caused by the fact that in their experiment Magin and his contributors used a field with a much bigger density than in the presented paper, which entailed significant temperature growth in tested dogs thyroid glands.

Meanwhile, in the experiment with rats, Black and Heynick [21] observed no effect of 2450 MHz EMF on T_4 levels. The effect of weak electromagnetic fields (ELF EMF) (50 Hz) on blood iodothyronine concentration of chick embryos was also investigated by Sechman et al. [22], who demonstrated that ELF EMF significantly elevate the levels of T_4 (between 11th and 18th day of incubation) and T_3 (between 18th day of incubation and hatching) in the blood of the analyzed birds. Such effects can result from the fact that 50 Hz frequency field has got other characteristics and different biological influence than the ones that were used in the presented experiment. One of the factors that may reduce blood T_4 and T_3 concentrations in the embryos exposed to 1800 MHz electromagnetic fields is the negative impact of RM-EMF on iodine uptake by the thyroid gland [15], which may decrease the synthesis and secretion of T_4 by thyrocytes, followed by T_3, the main source of which in avian blood peripheral deiodination of T_4 is catalyzed by D1 and D2 deiodinases [23,24].

It is known that one of the main indicators of stress reactions in chickens is an increase in their blood corticosterone levels [25–27]. In our study we found the level of this hormone to increase considerably in both, embryos as well as in the newly hatched chicks exposed to electromagnetic field during incubation (Figure 4). The stress-inducing action of electromagnetic field was implicated in the studies with rats, where corticosterone concentrations were also observed to increase as a response to RF-EMF [17,28,29]. It cannot be excluded that the increase in plasma CORT levels of the RF-EMF exposed embryos results from

a negative influence of this field on the hypothalamic–pituitary–thyroid (HPT) axis and/or the thyroid gland function. Several lines of evidence suggest that T_3 is an important negative modulator of avian adrenocortical steroidogenic function [30,31]. Moreover, it has been shown that in one-day-old chickens thyroidectomy (TX) increased relative adrenal weight and plasma levels of corticosterone [32].

In addition, *in vitro* TX increased basal and adrenocorticotropin (ACTH)-induced corticotropin production by isolated adrenal steroidogenic cells. Therefore, it seems likely that the significant decrease in plasma T_4 and T_3 concentrations in the RF-ELF exposed embryos evokes an elevation in sensitivity of adrenal gland steroidogenic cells to ACTH, which in turn increases CORT concentration in blood. On the other hand, the increase in blood CORT level may inhibit synthesis and secretion of corticotrophin-releasing hormone (CRH) and/or ACTH at the hypothalamic and hypophyseal levels, respectively. It has been shown that in chickens the glucocorticoid feedback becomes functional already between E15 and E18 [33,34]. Since in birds CRH is a potent stimulator of TSH secretion [35,36], it cannot be excluded that the decrease in T_4 secretion from the thyroid gland following RF-EMF exposure of the chicken embryo is accelerated not only by a negative effect of CORT on thyroid hormone synthesis but is also associated with inhibitory effect of CORT at the hypothalamic level.

The present study is probably the first to show that the difference in T_4, T_3 and CORT concentrations between the control and experimental groups, and between the sham and experimental groups is highest in the birds that were exposed the longest to RF-EMF (D1) (Figures 2–4). The results suggest that embryo exposure to electromagnetic field may cause the effects induced by this factor to add up over time and be indicative of the cumulative effect of this field on the hypothalamic-pituitary-thyroid axis.

On the other hand, no significant differences were found in the plasma concentrations of thyroid hormones and corticosterone in the birds ready for slaughter (D42), which had been exposed to an additional electromagnetic field during the embryonic period. Such results may suggest the time limited effect of electromagnetic field on the HPT and HPA axis, which disappears as the effect of the additional field is terminated.

To sum up, the obtained results suggest that additional 1800 MHz radio frequency electromagnetic field stimulates HPA axis function by inducing adrenal steroidogenic cells to synthesize CORT, while inhibiting thyroid hormone synthesis and secretion. Further investigations are needed to elucidate the mechanisms by which radio electromagnetic fields affect HPT and HPA axis function in chicken embryos.

ACKNOWLEDGMENTS

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