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The Superposition of a Temporally Incoherent Magnetic Field Inhibits 60 Hz-induced Changes in the ODC Activity of Developing Chick Embryos

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Previously, we have shown that the application of a weak (4 μT) 60 Hz magnetic field (MF) can alter the magnitudes of the ornithine decarboxylase (ODC) activity peaks which occur during gastrulation and neurulation of chick embryos. We report here the ODC activity of chick embryos which were exposed to the superposition of a weak noise MF over a 60 Hz MF of equal (rms strength). In contrast to the results we obtain with a 60 Hz field alone, the activity of ODC in embryos exposed to the superposition of the incoherent and 60 Hz fields was indistinguishable from the control activity during both gastrulation and neurulation. This result adds to the body of experimental evidence which demonstrates that the superposition of an incoherent field inhibits the response of biological systems to a coherent MF. The observation that a noise field inhibits ODC activity changes is consistent with our speculation that MF-induced ODC activity changes during early development may be related to MF-induced neural tube defects at slightly later stages (which are also inhibited by the superposition of a noise field). *Bioelectromagnetics 19:53–56, 1998.*

Key words: ELF; ODC; EMF; Noise; embryonic development

INTRODUCTION

It has been proposed (Litovitz et al., 1991 and 1994a) that biological systems are fundamentally incapable of responding to a weak extremely low frequency (ELF) electromagnetic (EM) field unless the field exhibits the characteristic of temporal constancy for periods of time greater than about 10 secs. Thus even when the exposure time is several hours, the parameters of the EM field (e.g., peak amplitude, frequency, or waveform) must be constant over any given 10 sec time period.

This hypothesis was supported by the results of a series of experiments in which coherence parameters were varied, and the activity of ornithine decarboxylase (ODC) in L929 mouse fibroblasts was measured (Litovitz, 1994a). Since superimposing a temporally incoherent MF on a coherent (e.g. 60 Hz) MF renders the amplitude of the resulting field inconstant, the system's presumed need for constancy is not met. Therefore, it follows that superimposing such a field should inhibit any bio-response associated with the application of the coherent field alone. This inhibition effect was demonstrated both in ODC studies L929 cells (Litovitz 1994a) and in chick embryo morphology studies (Litovitz et al 1994b). In this communication, we test the ability of a superimposed MF to inhibit the 60-Hz alteration in ODC activity during the early developmental time course of the chick embryo.

MATERIALS AND METHODS

Incubation and Exposure Systems

Fertilized White Leghorn eggs (from Truslow Farms, Chestertown, MD) were used within 24 h of their being received. The apparatus and techniques followed the ''Project Henhouse'' protocols (Berman et al., 1990) with several exceptions that are noted in this section. As in the Henhouse experiment, VWR Model-

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6000 water-jacketed incubators were used. However, the fields due to the coiled heater element located below the water jacket at the bottom of the incubator were ~ 1 μT. In an effort to minimize the unintentional ambient fields in the incubator, this heater element was not used. Instead the water was heated externally using one RTE Model-110 FRC Bath/Circulator for each incubator. Disconnecting the heating elements in the incubators and using the water circulating baths, reduced the stray ELF fields within the incubators to less than 0.2 μT, which confirmed an earlier finding by Martin (1992). The water bath pump circulated temperature-controlled water through the walls of the incubator, and the temperature of each bath was monitored daily. These external baths controlled the temperature in the incubator to within ±0.4 °C. Vibrations were not measured. In addition, rather than simply monitoring the current through the Helmholtz coils (which does not ascertain the actual field at the position of the eggs), the AC magnetic field at the position of the eggs (both the intentionally applied fields and the ambient fields) was measured directly at least once per day using an Alpha Lab dosimeter (frequency-weighted, calibrated for 60 Hz sine waves).

Six incubators were used simultaneously (two incubators each for controls, 60 Hz exposed and 60 Hz plus noise) with ten eggs in each. The eggs were placed in holders such that each egg was equidistant from the axis of symmetry of the Helmholtz coils. Incubation for the early time-course experiments was for 8, 15, 17, 20, 23, or 26 hs depending on the desired stage of development.

The electromagnetic fields were established by passing current through Helmholtz coils of radius 22.1 cm, separated by 22.1 cm. These coils were wound with 2 turns of 0.82 mm wire. For sinusoidal fields, a Tenma function generator was used to produce a signal which was then fed into a Realistic Model MPA-90 audio amplifier. The current then drove a pair of Helmholtz coils. A 60-Hz AC current of 0.5 A (peak) passed over a 10 ω resistor produced a sinusoidal field of 4 μT on the coil axis. For exposures in which noise magnetic fields were used, the superposition of the noise field over the sinusoidal field used two sets of Helmholtz coils. One set established the sinusoidal field by the procedure outlined above. The second set established the noise field using a General Radio Model 13090-B random signal generator in conjunction with a Krohn-Hite Model 3323 bandpass filter, driving a Realistic Model MPA-90 audio amplifier. The filter was set for a nominal 30-90 Hz bandpass, and the amplitude of the noise was adjusted to produce an rms magnetic field strength of 4 μT (the same as the peak value of the sinusoidal field).

ODC Assay

To eliminate variability due to premature incubation before arrival at the laboratory, only embryos of the proper developmental stage were retained for analysis. For most stages of development, the number of embryos per data point was between 5 and 7. There was one exception to this, the three data points (control, 60 Hz, and 60 Hz + noise) at 26 hs of incubation. There were relatively few embryos of the desired stage (Hamburger and Hamilton stage 6) at 26 hs, and so data at these points typically contained only three embryos for each exposure condition.

To prepare embryos for the ODC-activity assay, they were placed in a physiological saline solution; and a scalpel was used to trim the area opaqua from the blastoderm (Lowkvist, Heby, and Emanuelsson, 1980), leaving only the area pellucida which contains the the embryo proper. This procedure ensured consistency, since only the embryo area proper was analyzed. The embryos were then pooled in groups according to stage of development and exposure condition. They were then centrifuged and frozen.

ODC activity was determined by minor modifications of the method of Seely and Pegg [1983]. Protein analysis was performed by the Bradford method using a BioRad Kit (BioRad Laboratories, Melville, NY 11747). Samples of frozen, pelleted embryos were disrupted by addition of 105 μl of lysis buffer (25 mM Tris-HCl, pH 7.4; 2.5 mM DTT, 100 μM EDTA, 0.1% Nonidet P-40, 50 μM pyridoxal-5-phosphate, 50 μg leupeptin), followed by 30 s of vigorous vortexing. Lysed samples were then centrifuged at 10,000 × g at 4 °C for 20 m to pellet debris. 100 μl of the supernatant was transferred to an assay tube containing 50 μl of a solution of 2.0 mM L-ornithine, 200 μM pyridoxal-5-phosphate, 6.3 mM DTT, 25 mM Tris-HCl, pH 7.4, and 2.75×10^5 cpm of L-[1-$^{14}CO_2$]-ornithine. This yielded final concentrations of 0.67 mM unlabeled ornithine, and 1.44×10^{-2} mM ^{14}C-labeled ornithine in each assay tube. Tubes were transferred from ice to a 37 °C shaker-bath and the reaction was allowed to proceed for 1 h with gentle agitation. $^{14}CO_2$ generated by ODC activity was absorbed with 150 μl of 1.0 N NaOH held in a plastic well at the top of each, sealed assay tube. At the end of the 1 h incubation period enzymatic reactions were terminated by the addition of 400 μl of 20% trichloroacetic acid (TCA), and the NaOH was transferred to a scintillation vial for counting. Background activity was determined by the use of

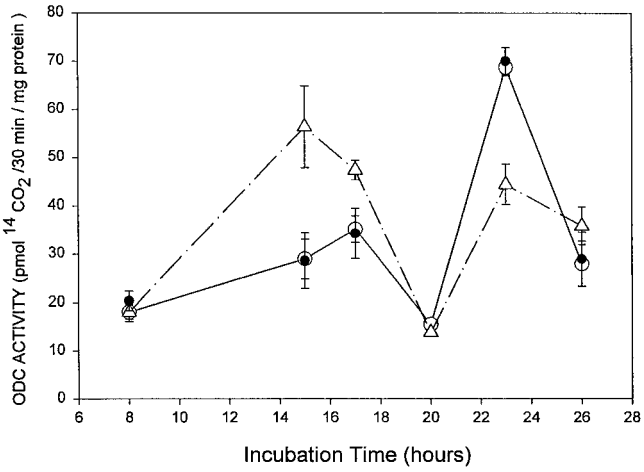

Fig. 1. ODC activity in chick embryos exposed to a magnetic field. 1) Open circles and solid line describe the control time course; 2) Open triangles and dashed line describes the time course of embryos exposed to 4 μT, 60 Hz sinusoidal MF; 3) The time course for embryos exposed to the superposition of an incoherent MF and 60 Hz MF with the same 4 μT rms magnitude is described by the solid circles (with no connecting line). Data plotted is the mean, error bars are ± SD.

samples in which ODC activity was eliminated by acid denaturation with TCA prior to incubation at 37 °C.

Units of ODC activity were expressed as pmol $^{14}CO_2$ generated/30 min/mg protein at 37 °C. All assays were done under blind conditions; each sample was coded, and the identity of the coded samples was not known to the person performing the ODC assay. The code was broken after all ODC activities were calculated. Activities for each ELF exposure condition were assayed in duplicate, and a mean ± SD was obtained from the duplicate readings. Since the embryos for each stage and exposure condition were pooled (following the method used by Lowgvist), the SD's for each stage are rather small because they reflect only the variability in the assay itself, the variability in chick embryo behaviour.

RESULTS

The time courses for 1) control embryos, 2) 60 Hz exposed embryos, and 3) embryos exposed to a temporally incoherent noise field superimposed over a 60-Hz sinusoidal field are shown in figure 1. The imposition of a 60 Hz MF altered the magnitude of both the first and the second peaks of ODC activity. The first peak in ODC activity occurs during gastrulation (15 hs of incubation). This peak was enhanced by a factor of about 2 due to the imposition of a 60 Hz MF, relative to the control activity. The second peak in ODC activity occurs during neurulation and the onset of organogenesis. The ODC activity of the MF-exposed embryos was only about 65% of the control activity at this peak.

The ODC activity time course which was obtained when a temporally incoherent MF was superimposed over the 60 Hz MF was identical to the control activity in all stages of development observed. For example, during gastrulation, the 60-Hz field alone (i.e. with no superimposed noise) roughly doubled the activity. However when an incoherent MF was superimposed over the 60 Hz MF of equal magnitude, the resulting activity, during gastrulation, (29 ± 6 pmoles $^{14}CO_2$ generated/30 min./mg protein) was statistically indistinguishable from the control activity (29 ± 4 pmoles $^{14}CO_2$ generated/30 min./mg protein).

During neurulation, application of the 60 Hz field alone decreased the activity of the ODC by about 35%. The superimposition of a noise field resulted in an ODC activity in the exposed which was statistically indistinguishable from the controls. The ODC activity was 70 ± 3 pmoles $^{14}CO_2$ generated/30 min./mg protein in the exposed (with noise) compared to 69 ± 2 pmoles $^{14}CO_2$ generated/30 min./mg protein in the controls.

DISCUSSION

In a previous study which involved the morphological assessment of over 2500 chick embryos (Farrell et al., 1997a), an increased morphological abnormality rate was observed after a 48 hr exposure to either a 60 Hz MF or to a pulsed MF. We also reported in previous work (Farrell et al., 1997b) that a 60 Hz MF alters the activity of the growth-related enzyme ornithine decarboxylase (ODC) during the time course of gastrulation and neurulation in developing chick embryos. The present work agrees with this earlier ODC result. We find that application of a 60 Hz field yields roughly a doubling in ODC activity during gastrulation, and a decreased activity during neurulation.

Guided by these earlier studies, we hypothesize that the MF-induced neural tube defects observed at 48 hrs are related to the MF-induced alteration of ODC activity during the first 24 hrs of incubation. This hypothesis is based upon the work of Lowkvist and colleagues (Lowkvist et al 1980, 1983a, 1983b). They showed that in the chick embryos changes in ODC activity are accompanied by changes in the polyamines. For example the administration of an ODC activity inhibitor prior to the first major increase in ODC activity (which normally occurs during gastrulation) prevented the accumulation of polyamines and inhibited development during gastrulation. Those authors con-

cluded that this was clear evidence of a decisive role for the polyamines in this development event. In our experiments the 60 Hz magnetic field caused a significant decrease in ODC activity during the neurulation stage. In light of the data and conclusions of Lowkvist and co-workers it seems reasonable to suggest that the MF-induced decrease in ODC activity during the neurulation peak led to a decrease in polyamines and partially inhibited complete neural tube development.

Litovitz et al (1994b) showed that the superposition of a temporally incoherent MF over a coherent MF eliminates the MF-induced increase in abnormality rate. In the present work we find that the superposition of an incoherent field eliminates the MF-induced changes in ODC activity caused by a coherent MF. Therefore our present study, though clearly not a proof, is consistent with our hypothesis that the inhibition of alterations in the magnitudes of the ODC activity peaks by the superposition of the incoherent MF should lead to inhibition of neural tube defects at later stages.

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