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Ornithine Decarboxylase Activity in Developing Chick Embryos after Exposure to 60-Hertz Magnetic Fields

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Fertilized white leghorn eggs were exposed to a 4 micro-Tesla (μT) 60 Hz horizontal magnetic field for 15, 18, 23 and 28 h. After exposure to the magnetic field, the embryos were isolated and assayed for ornithine decarboxylase (ODC) activity. ODC activity in magnetic field-exposed embryos was compared to ODC activity in sham-exposed embryos. ODC activity in magnetic field-exposed embryos was not statistically elevated above sham-exposed embryos.

The ability of biological systems to sense and respond to extremely low frequency (ELF) electromagnetic fields (EMF) has yet to be conclusively demonstrated. Replication of reported results help solidify the body of evidence and aid in reaching a consensus. Farrell *et al.* have reported an enhancement of ornithine decarboxylase (ODC) activity in developing chick embryos during gastrulation, after exposure to weak electromagnetic fields (1). Ornithine decarboxylase (EC 4.1.1.17) catalyzes the conversion of ornithine to putrescine and is the rate-limiting enzyme in the synthesis of the polyamines spermidine and spermine (2). Polyamines play an essential role in DNA synthesis and cell division (3). ODC activity can be elevated in biological systems by the addition of growth hormones and tumor promoting agents (4). Elevated levels of ODC activity are associated with uncontrolled cell growth and have been proposed as a clinical marker for some types of cancer (5). Because of the important role ODC plays in cell proliferation, a biological response to EMF that results in elevated levels of ODC activity has far reaching implications. We have attempted to replicate findings that show an EMF-induced ODC response. We here report our efforts to replicate the findings reported by Farrell *et al.* (1) of an EMF-induced increase of ODC activity in chick embryos.

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MATERIALS AND METHODS

Eggs. Fertilized White Leghorn eggs were obtained from the same source used by the original investigator (Truslow Farms Inc., Chestertown, MD). Eggs were collected within two hours of being laid and stored at 12°C until delivery. Upon receiving eggs in our laboratory eggs were again stored at 12°C until the start of the experiment.

Electromagnetic exposure facility. The regional ELF magnetic field exposure system used has been previously described (6). In brief magnetic field exposures were carried out a Helmholtz coil-based exposure system with a central incubator supplying air of uniform temperature and relative humidity to two satellite chambers. Two vertically oriented Helmholtz coils surround each exposure chamber. Coils were energized using function generator/power amplifier combination under computer control. The chambers were mechanically and electrically isolated. Each chamber was equipped with a thermocouple and a three-axis AC magnetic field sensor which in conjunction with a custom written data acquisition software continuously monitored and recorded chamber temperature and flux density.

Exposure condition. Eggs were exposed to 4 μT 60 Hz horizontal magnetic field. Thirty eggs were used for each experiment, fifteen exposed and fifteen sham-exposed. Exposure to the magnetic field was started at the same time as the beginning of the 37°C incubation.

Eggs were oriented inside the exposure chamber with the long axis of the eggs parallel to the direction of the magnetic field. Temperature in the chambers was set at 38 ± 0.2°C and relative humidity was set at 80%. Eggs were placed in the exposure chamber after the coil was energized and removed from the chamber prior to turning the coils off to avoid switching transients. Flux density, temperature, relative humidity and orientation of eggs in the magnetic field were all identical to those used by Farrell *et al.* (1).

Positive control experiments. Twelve fertilized eggs were incubated in a Revco water-jacketed incubator for 26 h at 38 ± 0.2°C and 80% relative humidity. After incubation six eggs were injected with 0.1 units of somatotropin (Sigma, St. Louis, MO) in 50 μl of saline solution. The remaining six eggs were injected with 50 μl of physiological saline solution and used as controls. The eggs were then incubated for an additional two hours before harvesting.

Embryo harvesting. After exposure embryos were harvested from each fertilized egg. This was accomplished by standing each egg vertically on a stage and cutting along the top of the shell to expose

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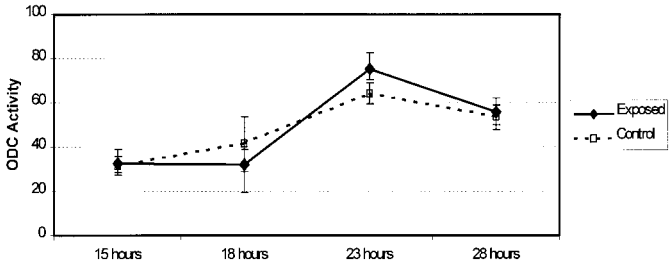

FIG. 1. ODC activity in 60 Hz 4 μT magnetic field-exposed and sham-exposed embryos. Each time point represents the average of 6 experiments each containing 15 embryos, for a total of 90 embryos for exposed and 90 for sham-exposed condition.

the yolk with embryo floating on top. The albumin surrounding the yolk was carefully pipetted out. A filter paper annulus cut to the circumference of the embryo with the center punched out was then placed on top of each embryo and the yolk was cut around the filter paper. The filter paper with embryo attached was removed and placed in a 60 mm tissue culture dish containing physiological saline solution. With the aid of a dissecting microscope and small surgical scissors the filter paper and the extraembryonic membranes were trimmed away leaving only the embryo proper. Embryos were pooled according to exposure conditions, washed twice with cold saline, pelleted by centrifugation at 500 g and stored at −70°C until ODC assay.

ODC assay. Ornithine decarboxylase (ODC) activity in pM of $^{14}C\text{-}CO_2$/mg of protein/30 min was determined by our previously described method (6) modified for chick embryo assay in our laboratory. Embryo pellets were homogenized and lysed by adding 380 μl of lysis buffer (6) and then sonicating for 20 s at 4°C using a Kontes Micro-Ultra Cell Disrupter Model KT50 (Kimble Kontes, Vineland, NJ). Lysates were centrifuged at $10^4 g$ for 30 min at 4°C. Two 100 μl aliquots of lysate were incubated for one hour at 37°C in the presence of ODC assay mix (6). A third 100 μl aliquot was incubated in an identical manner with the addition of 1.0 mM difluoromethylornithine (DFMO, Hoechst Marion Roussel, Cincinnati, OH); an irreversible enzyme activated inhibitor of ODC (7), and was used as a blank in determining ODC activity in the two aliquots.

Statistical analysis. For each experiment, ODC activity of magnetic field exposed samples and sham exposed samples were calculated by averaging the result of duplicate assays. Averaged results from an experiment were used to calculate ODC activity ratio for that experiment. ODC activity ratio is expressed as exposed over control for experimental treatment and Chamber A over Chamber B for sham verses sham experiments. Average ODC activity and standard error of the mean, and average of ODC activity ratio and standard error of the mean were calculated for each set of experiments.

Instat computer program (GraphPad Software, San Diego, CA) was used to calculate paired, two-tailed Student's t-test for statistical comparison.

RESULTS

Magnetic Field Exposure

EMF exposures were conducted at 4 μT for 15, 18, 23 and 28 h. Six experiments were performed for each time point. In addition six sham-sham experiments were performed at 15 and 23-h time points. Figure 1 illustrates the variation in ODC activity at different stages of chick embryo development in both exposed and sham-exposed embryos. The peak in ODC activity observed at 23 h matches that found in the literature (8, 9) and by Farrell *et al.* (1). In all cases the p value was greater than 0.05 indicating there was no statistical difference between exposed and sham exposed values. Table 1 shows the mean ODC activity ratio for each time point as well as sham-sham and positive control experiments. The ODC activity ratio for each ELF exposed time point was not statistically different from one.

Positive Control Experiments

Three sets of positive control experiments, using twelve eggs in each set, were run using 0.1 units of the growth hormone somatotropin to increase ODC activity in the chick embryos. ODC activity in somatotropin injected chick embryos increased 2.41 fold ± 0.14 compared to ODC activity in control embryos.

DISCUSSION

Farrell *et al.* reported statistically significant changes in ODC activity in embryos after exposure to a 4 μT magnetic field at 15 and 23 h (1). ELF exposure for 15 h resulted in a two-fold increase in ODC activity over control embryos, and an ELF exposure of 23 h gave a 65% suppresion of ODC activity in exposed embryos. In our effort to replicate these findings we have exposed embryos to 4 μT magnetic field for 15, 18, 23, and 28 h. We were able to observe changes in ODC activity due to duration of incubation as observed by Farrell *et al.* but the magnetic field effect on ODC they reported has not been reproducible in our laboratory. We have been able to demonstrate induction of ODC activity in chick embryos after exposure to somatotropin thus confirming our ability to detect small changes in ODC activity similar in magnitude to that attributed to magnetic fields. Our exposure methodol-

TABLE 1

ODC Activity Ratios[a]

	n	Mean ratio	SEM
15 h sham-sham	6	1.25	±0.11
15 h field exposed	6	1.07	±0.20
18 h exposed	6	0.94	±0.14
23 h sham-sham	6	1.03	±0.13
23 h field exposed	6	1.19	±0.12
28 h field exposed	6	1.04	±0.09
28 h positive control (Somatotropin)	3	2.41	±0.14

[a] Ratio is expressed as exposed/control for field experiments and chamber A/chamber B for sham-sham experiments.

ogy was finalized after discussion with members of the original investigating group. We believe the magnetic field flux density and vector orientation with respect to the eggs were as nearly identical as possible to those used by the original investigators, as were humidity and temperature during the incubation. Critical variables including integrity of sine wave, flux density, temperature and humidity were continuously monitored during all field and sham exposures. In spite of this attention to details of exposure conditions we were unable to reproduce the increase in ODC activities in exposed embryos reported by the original investigators.

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