EFFECTIVENESS OF NOISE IN BLOCKING ELECTROMAGNETIC EFFECTS ON ENZYME ACTIVITY IN THE CHICK EMBRYO

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Received February 19, 1995
Received after revision, March 17, 1995

Summary

We have previously demonstrated that exposure of the chick embryo to a 60 Hz, 4 μT split sine wave for the first 72 hours of development causes a significant reduction in the activity of the ectoenzyme 5'-nucleotidase. This reduced activity persisted, throughout the embryonic period, despite further incubation in a field free environment. We also showed that the reduction in 5'NT activity can be localized in the developing brain to the Cerebellum. The present study reveals that superimposition of an electromagnetic noise, of similar amplitude and frequency, can mitigate the effect of the field on 5'NT activity.

Key Words: chick embryo; electromagnetic fields; 5'-Nucleotidase and electromagnetic noise.

Introduction

There now appears to be little doubt that exposure to extremely low frequency electromagnetic fields can alter or induce a variety of basic biological processes including transcription and translation (1). However, the mechanism(s) inducing virtually all the biological effects observed to date remains elusive. Due in part to this uncertainty, many questions as to electromagnetic field effects have arisen (2). What factors play a role in

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Abbreviations:

5'Nt, 5'-nucleotidase (5'-ribonucleotide phosphohydrolase, EC 3.1.3.5);
ELFEMFs, extremely low frequency electromagnetic fields;
EMF, electromagnetic field.

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the conflicting results noted following exposure of developing systems to electromagnetic fields? When physiological changes occur, are they permanent or transient and are they dependent on chronic versus acute exposure? Do all affected systems react in a similar manner under all conditions? While such questions remain unanswered, the debate and the controversy as to the potential hazards from EMF induced biological changes continues unabated.

In a series of earlier studies, we showed that levels of 5'NT were markedly reduced in chick embryos exposed to ELFEMFs during early stages of development. In addition, we found that the activity levels of the affected enzyme remained reduced even when the exposed embryos were incubated further in a field free environment. We also found that the reduced 5'NT levels in the developing central nervous system were most apparent in the Cerebellum, while activity in the Cortex and Brain stem appeared to be unaffected (3&4).

While these studies were in progress, researchers at the Vitreous State Laboratory, The Catholic University of America in Washington D.C., reported that the superimposition of incoherent noise on a coherent signal appeared to negate the effect of the electromagnetic field (5,6&7). Preliminary results from our laboratory appeared to confirm this theory (8&9). We report here on a set of experiments aimed at testing this hypothesis. Our findings show quite clearly that the effect of the electromagnetic field, as used in this study, can be effectively blocked by superimposing random noise.

Materials and Methods

Embryos:

Fresh fertile White Leghorn eggs were obtained and processed as previously reported (3). Eggs were incubated in three specially designed, temperature controlled incubators. All three incubators contained a Helmholtz coil for producing the desired field (see 10). The coil was 44.2 cm in diameter and was wound with two turns of 0.82-mm-diameter wire. The two coils were connected in series. Current to the coils was adjusted by the use of a variable transformer and appropriate resistors. A single pole, 250 watt,120-V ac/dc light dimmer switch was used to split the pulse. A schematic of the exposure system for the split pulse experiment is found in (11). Control embryos were not exposed to the field (coil off). Experimental embryos were either exposed to a 4μT, 60 Hz split sine wave or to the same wave plus a 4μT noise field. For production of the noise field an additional coil was wound with two turns of 22 gauge wire using the frame of the existing coil. The additional coil was set to produce a band limited noise magnetic field with a nominal pass- band of 30 to 100 Hz. The noise generator, custom built at Catholic

University was incorporated into a 35 watt audio amplifier (Realistic model 35, Tandy corporation). A current limiting resistor,(10 ohm), was connected in series with the coil to better match the output impedance of the amplifier. The noise amplitude was adjusted such that the average noise fluctuations, viewed on an oscilloscope displaying the integrated output of a pick-up coil, were of the same order of magnitude as the oscillations of the periodic (coherent) field. The signal integrator used for this measurement was built by Integrity Design and Research, Buffalo, New York. The pick-up coil was approximately 4 inches in diameter and was built at Catholic University,Department of Physics. The amplitude of the resulting magnetic field was measured by means of a search coil, (METEX-3800 Digital Multimeter), as well as the oscilloscope and measured on average 8μT.

Following three days of exposure, eggs were either removed and embryos recovered or the eggs were placed in an ordinary egg incubator for a further three days of field free incubation. At the completion of the subscribed exposure period embryos were removed and examined under a dissection microscope for gross abnormalities. Normal appearing embryos from each of the three groups were harvested, pooled separately and stored at -70C until required for biochemical analysis. Biochemical analyses were carried out within 72 hours of embryo removal. Each experiment consisted of ten runs with each run having 10 eggs per group for a total of 300 eggs per experiment.

Preparation of homogenate:

Frozen whole embryos were thawed out slowly on ice to minimize the effect of proteolysis on enzyme activity, and gently homogenised immediately in a hypotonic buffer medium, pH 8. One gram wet weight of embryo was homogenised in 10 to 30 ml of homogenising buffer using a hand held teflon/glass homogeniser. The homogenate was centrifuged at 1500 x g for 10 minutes in a clinical laboratory centrifuge. Supernatant was removed and retained. The pellet was manually resuspended in 1 ml of buffer and centrifuged as before. Both supernatant fractions were pooled and diluted 2-fold with homogenising buffer and used for enzyme determination. All steps were carried out at 4°C.

Biochemical Assay:

Enzyme activity was determined spectrophotometrically as previously described using a Sigma Reagent Kit (Sigma Diagnostics, St. Louis, MO 63178,USA) (3). The Centrifichem Analyzer (Baker Instruments Corporation, Pleasantville, NY 10570, USA) was used to quantify 5'NT activity.

Data analysis:

Mean specific activities were utilized and standard deviation, one way analysis of variance and comparison of means were performed using a computer program (Lotus 1-2-3) as well as standard statistical methods.

Results

A total of 600 eggs were processed in the two experiments. Overall mean specific activities (nmol/min/mg protein) of 5'NT are reported in Fig 1. Confirming our earlier studies, the values of 5'NT were significantly reduced in both experiments following exposure and remained so even after an additional three days of field free incubation. As in our preliminary studies, mean specific activity of 5'NT in embryos exposed to the field plus noise were virtually identical to control levels. The difference in activity levels of 5'NT between control and field + noise exposed embryos while small, is not statistically significant. The average raw data per run for both experiments is reproduced in Table 1. The mean values for field + noise never exceeded control values but did on two occasions attain identical levels. In both experiments, the difference between values for field exposed embryos compared to control or field plus noise exposed embryos was statistically significant $P<0.01$ in the 3 day and $P<0.001$ in the 3 day on 3 off experiments).

Discussion

The results of the present study confirm our earlier findings that exposure to a 60Hz split sine wave for the first 72 hours of development affects biochemical values of enzymes such as 5'NT in morphologically normal appearing chick embryos. The reduction in activity levels of 5'NT in exposed embryos, as in the previous studies, is again between 40 to 45% and the effect is still apparent even after a further three days of field free incubation. Both results confirm our earlier findings (3&4). The present study also reveals that superimposition of a band limited noise of equal amplitude results in the levels of 5'NT being maintained with no statistically significant change from control values.

These results appear to assume significance well beyond the single finding that exposure to ELFEMFs reduces enzyme activity and that superimposed noise apparently negates the effect of such field exposure. Recent clinical laboratory findings have reported that 5'NT levels are abnormally low in patients suffering from a variety of immunodeficiency diseases and blood disorders such as chronic lymphocytic leukaemia and B thalassaemia, a type of anaemia (12). In addition, a mutant type of 5'NT, characterized by fast electrophoretic mobility, has been identified in a case of chronic haemolytic anaemia (13). As well, an increased incidence of leukaemia and other blood cell disorders has been reported in individuals exposed to EMFs (14). More recently, an epidemiological study by

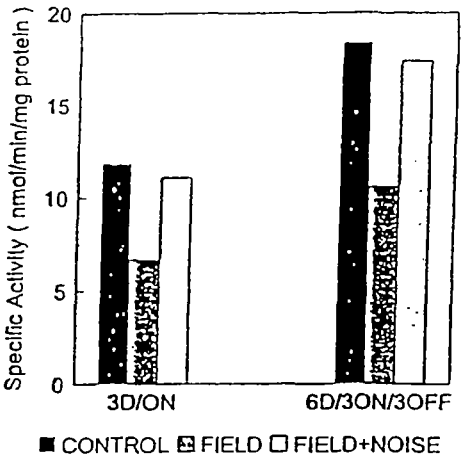

FIGURE 1
Mean Specific Activity of 5'NT in Control Embryo and embryos exposed to a 4 uT Electromagnetic Field or to the Field plus Random Noise.

Savitz and Loomis(15), has reported that occupational exposure of electrical workers to magnetic fields, while not supporting a link to leukaemia, did reveal that with increasing magnetic field exposure there is twice the occurrence of certain cancers over control samples. As a growing number of experimental, clinical laboratory and epidemiological findings appear to indicate a relationship between exposure to EMFs, lowered 5'NT enzyme activity and certain forms of cancer, the following question can be raised. Are ELFEMFs somehow implicated in the abnormal levels of 5'NT found in certain forms of cancer, and by inference could EMFs be a causal co-factor in such cancers?

By establishing blood levels of 5'NT in those routinely exposed to electromagnetic fields, can we determine when and if such exposure is adversely affecting enzyme activity? Such testing could at the very least indicate prior exposure. If the findings of this study can be validated in humans, and if we can establish a difference between chronic versus acute exposure, then such exposure could be minimized. This kind of procedure is already in place when dealing with occupational exposure to radioactive isotopes. Once the maximum permitted annual dose rate from all external sources is reached, determined for example by blood analysis or thyroid scanning, the person is removed from the exposure source for a period of time established by the regulatory body.

Finally, a recent study has reported that nurses in an Neonatal Intensive Care Unit received intermittent high exposure to ELF electromagnetic fields (16). One can only

TABLE 1.

Effectivenes of Noise in Blocking EMF Effects on Enzyme Activity in the Chick Embryo

Mean Levels of 5'Nucleotidase (nmol/min/mg protein)

3 Days Exposed				3 Days Exposed/3 Days Non-Exposed			
Run	Cntrl	Fld + N	Fld	Run	Cntrl	Fld + N	Fld
1*	23	23	14.5	1	23	21	17
2	12	11	9	2	26	23	14.5
3	10	9	2.5	3	18	17	5
4	11	10	7	4	20.5	20	14
5	8	7.5	5	5	12	11	4
6	9	8.5	6.5	6*	17	17	13
7	10.5	9.5	4	7	19.5	19	7
8	11	9.5	6.5	8	20	19	13
9	12.5	12	8	9	15.5	15	10
10	12	11	4	10	13	12	8
Total	119	111	67		184	174	106
Mean	12	11	7		18	17	11
SEM	1.3	1.39	1.07		1.36	1.21	1.39

* Runs for which mean levels were identical in the treated (field + noise) and control groups. SEM is the standard error of mean. A total of 10 runs per group, 10 eggs per run, were performed as described in the Method Section. Fld: exposure to 4 uT, 60 Hz electric field; Fld + N: exposure to 4 uT, 60 Hz field plus 4 uT electrical noise field. Cntrl: control group.

speculate as to the possible effects such exposure has on the newborn and often pre-term infants in these units who are chronically exposed to the same fields. The enzyme 5'NT has been reported to be one of many involved in normal cellular proliferation and differentiation in the developing central nervous system. It has been postulated that 5'NT, due to its glycoprotein properties,assumes adhesive or recognition functions in neuron-glia and neuron-neuron interactions especially during cerebellar synaptogenesis (17 &18). The timing of such events, in experimental animals as well as man, occurs both pre and post natally. Exposure of a pre-term infant is occurring therefore; not only during a critical period of development of the Cerebellum, but also in a period when 5'NT is postulated to be of importance in the orderly sequencing of cellular connections. If 5'NT activity levels are reduced in the pre-term infant, as we have clearly demonstrated in the chick embryo, then one can envision possible alterations in the normal development of the central nervous system. Studies are presently underway in the chick embryo to examine this possibility.

Acknowledgements: This work was supported by a grant, #252/R, from the Workplace Health and Safety Agency, Ontario, Canada.

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