Myocardial Protection Conferred by Electromagnetic Fields

A.L. DiCarlo, PhD; J.M. Farrell, PhD; T.A. Litovitz, PhD

Background—It has been reported that electromagnetic (EM) fields induce stress proteins in vitro. These proteins have been shown to be important in recovery from ischemia/reperfusion. It was, therefore, hypothesized that EM fields could activate stress responses in vivo and protect myocardial tissue during anoxia.

Methods and Results—Chick embryos were exposed to 4-, 6-, 8-, and 10-μT and 60-Hz EM fields for 20 minutes followed by a 1-hour rest period before placement in an anoxic chamber. Embryos were reoxygenated when survival of controls dropped to <40%, and final observations were made 30 minutes later. Data from 80 experiments (>500 EM field-exposed embryos) indicated that EM field protection was extremely significant (P<0.0001). Survival rates were 39.6% in controls and 68.7% in field-exposed embryos. In a second set of experiments, embryos were exposed for 20 minutes to several pretreatments: (1) hyperthermia (43°C), (2) 60-Hz, 8-μT EM fields, or (3) 60-Hz, 8-μT EM fields plus a random EM noise field (8 μT). Embryo survival was 37.7% (control), 57.6% (heated), 69% (60-Hz EM field only), and 41.5% (60-Hz EM field plus EM noise). To confirm that heating resulting from field exposures did not occur, thermocouples were placed into several eggs at the site of the embryo during exposure; no increase in temperature was noted.

Conclusions—We conclude that athermal EM field exposures induce stress responses that protect chick embryo myocardium from anoxia damage. These results suggest that EM field exposures may be a useful, noninvasive means of minimizing myocardial damage during surgery, transplantation, or heart attack in humans. (Circulation. 1999;99:813-816.)

Key Words: ischemia ■ heat-shock proteins ■ chick embryo ■ electromagnetic fields

Stress proteins were first discovered in 1962 by Ritossa1 in the Drosophila melanogaster larval salivary gland; after heat stress, an unusual puffing pattern was observed on the chromosomes. Thus, stress proteins are often referred to as heat shock proteins (hsp). These proteins, also known as molecular chaperones, are important in maintaining the 3-dimensional conformations of other proteins during times of cellular stress. Stimuli that can induce stress proteins include high temperature, amino acid analogs,2 hypoxia,3 and active oxygen species.4 Additional information about cellular stress responses can be found in a review by Morimoto.5

The most extensively studied of all the stressors is heat shock. The ability of prior heat stress to protect against subsequent heat stress is referred to as thermotolerance, a phenomenon that was first documented in HeLa cells.6 The protective nature of hsp was further demonstrated by showing that mild heat shock (42°C in humans) conferred cellular resistance to subsequent lethal 45°C heat shock.7 The synthesis and degradation of hsp precedes the development and decline of heat protection; this has been taken as evidence that these proteins are involved in the development of thermotolerance.

Cardiomyocytes respond to hypoxia and metabolic stress with increased hsp70 production, pointing to the possible additional role of stress proteins during ischemia/reperfusion.8 Cellular self-preservation has been studied to determine means to minimize myocardial tissue damage during surgery, transplantation, and heart attack. Cross-protection (one mild stress protecting against a different lethal stress) in particular has been investigated. Mestril et al9 showed that preheated cardiomyocytes had higher survival than nonheated cells after ischemia. In vivo, prior heating resulted in improved recovery after coronary artery occlusion and reperfusion in rat hearts.9 Marber et al10 reported that hearts from mice which overexpressed hsp70 suffered less damage than normal hearts during ischemia. It has been established that hsp70 levels, after direct heating, correlate with myocardial protection.11 In the area of transplantation, stress conditioning by heating an organ before removal from the donor has been demonstrated to increase safe cold storage time of the tissue, and thus, the probability of successful engraftment.12 This increased viability has been positively associated with enhanced expression of hsp70 in the transplanted organ.

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Correspondence to Theodore A. Litovitz, PhD, Director, Vitreous State Laboratory, Catholic University of America, 620 Michigan Avenue, NE, Washington, DC 20064. E-mail litovitz@cua.edu
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Many electromagnetic (EM) field exposure effects on cellular enzymes have been reported. In chick embryos in vivo, EM field exposures altered the activity levels of ornithine decarboxylase (ODC),\(^\text{13}\) acetylcholinesterase, alkaline phosphatase, and 5'-'nucleotidase.\(^\text{14}\) Field-induced changes in ODC activity have also been noted in many cell types,\(^\text{15,16}\) and fields have been shown to alter the enzymatic activity of the Na, K-ATPase,\(^\text{17,18}\) and cytochrome oxidase.\(^\text{19}\) Additional documented EM field effects include suppression of nighttime melatonin increases in hamster pineal glands\(^\text{20}\) and enhancement of breast cancer cell proliferation by blockage of the oncostatic action of melatonin.\(^\text{21}\)

Given the importance of stress proteins in ischemia protection, it is critical to note the study done by Goodman and Henderson\(^\text{22}\) which showed that EM field exposures changed transcript levels of several proteins. In 1994, Blank et al\(^\text{23}\) showed that EM stimulation yielded the same pattern of protein synthesis as heat shock. Both stimuli affected the same stress pathway and feedback inhibition occurred. Lin et al\(^\text{24}\) later reported that EM fields activate heat shock factor (HSF), a primary step in stress protein induction. Exposures also caused measurable changes in the transcription and translation of hsp70 genes in various cells (HL-60,\(^\text{23}\) yeast,\(^\text{25}\) and \(D.\) melanogaster salivary gland\(^\text{26}\)) and in the immediate early response genes \(myc,\(^\text{27,28}\) jun,\) and \(fos.\(^\text{29}\)) Finally, EM fields have been used to cross-protect fertilized \(S.\) coprophila eggs from lethal hyperthermia.\(^\text{30}\) These findings strongly suggest that cells respond to EM field exposures as an environmental stress.

Accepting that stress proteins play a role in cardiac protection during ischemia/reperfusion and that EM fields induce stress proteins in vitro, we hypothesize that these fields will confer protection against anoxia damage in vivo. To test this, an experiment was designed to determine whether EM field exposures protect against cardiac anoxia in chick embryos. In this kind of experiment, it is logical to be concerned that any observed protection could be due to heating of the embryo by the fields and induction of the classic heat shock response. To address this concern, (1) thermocouples were placed into eggs at the site of the embryo to record any increases in temperature during EM field exposures, and (2) the total energy deposited into the embryo during the EM field exposure period and the corresponding increase in temperature were calculated.

**Methods**

Fertilized White Leghorn eggs (Truslow Farms, Chestertown, Md) were incubated (37.8°C) for 96 hours (developmental stage 24\(^\text{\text{30}}\)) in water-jacketed incubators (VWR). Incubators were modified to minimize stray magnetic field emissions, and ambient measurements indicated that magnetic fields were <0.5 \(\mu\)T at all egg placement positions.

**Field Exposures**

Fields were produced by passing current through Helmholtz coils wound and connected as described previously.\(^\text{31}\) For the first set of data presented, 60-Hz EM fields of 4-, 6-, 8-, and 10-\(\mu\)T were used (separate experiments with all data combined). In the second experiment, 4 20-minute preexposure conditions were examined: (1) control (no field), (2) 43°C hyperthermia, (3) 60-Hz, 8-\(\mu\)T magnetic field, and (4) exposure to the magnetic field described in (2) plus a superimposed random magnetic noise field (band width range, 30 to 90 Hz, 8 \(\mu\)T). Superposition of a noise field was done to confirm that EM fields were not heating the embryo. If heating occurred with the 60-Hz field alone, additional heating effects would be seen with the 60-Hz plus noise condition. All embryos were contained within an intact egg.

Signals were generated using a 15-MHz function/arbitrary waveform generator (Hewlett-Packard model 33120A), and 35-W audio amplifier (Radio Shack MPA-46). Noise fields were produced with a random noise generator built at Catholic University, incorporated into a 35-W audio amplifier. Magnetic fields were measured using 60-Hz calibrated magnetic dosimeters (Model IDR-109; Integrity Design and Research Corp).

**Heat Shock**

Embryos were heated by placing eggs into a steel-enclosed sand bath sitting in 43°C water. A thermocouple (HH82 digital thermometer, Omega) was inserted into 1 egg to monitor temperature. When embryo temperature reached 43°C, timing was begun and embryos were held at 43°C (±0.2°C) for 20 minutes.

**Anoxia**

Embryos were maintained at 37.8°C for 1 hour after treatment, during which time a portion of the shells were removed and vitelline membranes drawn back to reveal the embryos. Abnormal, bleeding, or incorrect stage embryos were discarded. Embryos were coded and placed into plastic, virtually air-tight bags (10 to 12 embryos per bag), with a distribution of embryos such that each treatment was represented. Coding insured blind evaluation of results. Air was evacuated by use of gentle suction, and bags were filled with argon and sealed. Sensors (Model STX70, single gas monitor, Industrial Scientific) placed within the bags confirmed that oxygen levels remained <1% during the experiment. Bags were returned to the incubators, and temperature was maintained at 37.8°C.

Embryo survival was evaluated by observing heartbeat. Observations (beating or stopped) were entered into a computer which provided percent survival for each condition. When <40% of control embryos were still beating, bags were opened to re-oxygenate. Final observations were made after 30 minutes at 37.8°C.

**Temperature Controls**

To eliminate the possibility that heating by EM field exposure was responsible for observed protection, thermocouples were placed into 8 eggs to record temperature changes during 20-minute, 60-Hz, 10-\(\mu\)T EM field exposures. To position the thermocouples, eggs were candled (a light source was placed behind the egg to visualize the location of the embryo) while in the incubator, and a small hole was made in the shell, just large enough to insert the thin wire of the thermocouple. The end of the thermocouple was placed adjacent to the embryo. The same procedure was repeated in control embryos to insure there was no heat lost because of the hole in the shell. The thermocouples used had an accuracy of measurement to within ±0.1°C.

**Results**

The Table gives results of 80 experiments. A total of 957 eggs were used. Results represent data from all exposures ≥4 \(\mu\)T. \(P\) values were determined with the \(\chi^2\) test.
Anoxia protection conferred by EM field exposure and heat shock. Percent survival after anoxia of chick embryos preexposed to the following 20-minute treatments: Control (no field exposure); heating at 43°C; 60-Hz, 8-μT rms (root mean square) exposure; or 60-Hz, 8-μT rms plus noise, 8-μT rms exposure. Mean and SEM for n experiments (7<n<14) are given. P values indicate the significance level achieved by comparison of individual treatment data with control using χ² test.

In the Figure, results for heating and field exposures represent 10 and 9 experiments, respectively, with 10 to 12 eggs used per exposure condition per experiment. A total of 456 embryos were used: 146 controls, 66 heated, 180 60-Hz field-exposed, and 64 60-Hz plus EM noise–exposed. Data indicate that embryos preexposed to 60-Hz EM fields have greater survival (69%) than controls (37.7%). This treatment is significant at P<0.0001 according to the χ² test. Heated embryos had greater survival (57.7%) than controls (P<0.01). Hyperthermic protection was not significantly different than field-exposed (P>0.13). Superposition of random EM noise on the 60-Hz EM field reduced embryo survival rates from 69% to 42.2%, which was not different from control (P>0.6) but was significantly different from 60-Hz EM field exposure (P<0.0003).

Embryos that were exposed to 60-Hz, 10-μT fields for 20 minutes with a thermocouple in place at the site of the embryo experienced no measurable increase in temperature. Control embryos that were not exposed to fields but were opened, to allow for the introduction of the thermocouple, experienced no change in temperature as a result of the presence of the thermocouple.

**Discussion**

Consistent with the cross-protection studies discussed in the Introduction, we find that prior heat treatment of chick embryos provides protection against subsequent oxygen deprivation. What is new is the finding that 60-Hz EM field exposures also protect during anoxia. In fact, there is a tendency for field exposures to provide greater benefit than heating in conferring protection. This may be due to stress suffered by embryos during heat shock. Heated embryos (n=8) exhibited tachycardia (184 bpm on average) in comparison with both control (n=4; 131 bpm) and field-exposed embryos (n=4; 130 bpm). These elevated rates are indicative of the severity of whole-body stress. Field exposures seem to be capable of inducing protective responses without obviously stressing the embryos.

We have previously demonstrated that cellular responses to EM fields require that fields are coherent (parameters are predictable) over some minimum time (~10 seconds). It was determined that superimposing incoherent magnetic noise fields on 60-Hz coherent EM fields could block biological effects induced by coherent EM fields alone. Inhibition of EM field–induced effects by EM noise was noted in chick abnormalities, c-myc transcription, 5′-nucleotide activity, and ODC activity in murine cells, and chick embryos. The noise plus 60 Hz magnetic field results are significant because they eliminate the possibility that protection observed is a result of embryo heating. More energy is deposited in the embryo during the 60-Hz plus noise than the 60-Hz field-only experiment. If heating were a factor, 60-Hz plus noise field exposures would also give increased survival; this clearly did not occur. This result is consistent with calculations of embryo heating caused by a 60-Hz, 10-μT magnetic field, which predict a rise in temperature of <10⁻⁶ °C. Adding to this body of evidence, which indicates that field exposure of the embryo does not increase the temperature, are the results of the thermocouple experiment which show no measurable increase in temperature during EM field exposures. In the literature, there is consensus that an increase in temperature of at least several degrees above normal is needed to induce accumulation of hsp36,37; in chick embryo fibroblasts, temperature elevations of at least 2°C are required to activate HSF1.

The beneficial use of EM fields is not new. For some time, EM stimuli have been used to heal damage, for example, in the treatment of bone fractures, wound healing, and inflammation reduction. To our knowledge, this report represents the first indication that field exposures may be used to precondition and minimize myocardial damage resulting from anoxic stress. This EM field preconditioning treatment could be effective in increasing myocardial preservation when applied before surgery or transplantation. We also believe that there is a high probability that this protection will occur even if the EM field is applied immediately after the start of hypoxia (mimicking treatment following onset of heart attack).

Most of the damage incurred by ischemic events is suffered at reperfusion, when blood flow and oxygen supplies resume. High concentrations of oxygen radicals (oxidative stress) during this period are responsible for extensive cellular damage and are, themselves, initiators of stress response. It has been found that increased synthesis of hsp70 enhances survival against oxidant stress. However, the cell’s innate stress responses (HSF activation and hsp synthesis) are induced primarily at reperfusion, when it is often too late to prevent significant damage. It is therefore reasonable to presume that the mechanism behind the enhanced survival observed in EM field preexposed embryos may be partly because protection against these oxidative stresses was induced before reoxygenation.

This assumption is consistent with data obtained by Lin et al, showing that activation of HSF and AP-1 transcription...
factors are induced within minutes in response to EM field exposures.

If this research can be replicated in mammalian systems, many clinical applications for increasing myocardial preservation during surgery and transplantation and minimizing damage during myocardial infarction could be anticipated.

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References

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