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Electromagnetic Field Exposure Induces Rapid, Transitory Heat Shock Factor Activation in Human Cells

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Abstract Stimulation of human promyelocytic HL60 cells by a 60Hz magnetic field at normal growth temperatures results in heat shock factor 1 activation and heat shock element binding, a sequence of events that mediates the stress-induced transcription of the stress gene HSP70 and increased synthesis of the stress response protein hsp70kD. Thus, the events mediating the electromagnetic field–stimulated stress response appear to be similar to those reported for other physiological stresses (e.g., hyperthermia, heavy metals, oxidative stress) and could well be the general mechanism of interaction of electromagnetic fields with cells. J. Cell. Biochem. 66:482–488, 1997.

Key words: cellular stress; heat shock element (HSE); heat shock factor (HSF); electrophoretic mobility shift assay (EMSA); electromagnetic (EM) field; heat shock protein (hsp)

The induction of heat shock gene expression by heat and other forms of stress creates a programmed response in the genome of all eukaryotic and prokaryotic organisms and has been a paradigm for inducible gene expression [Morimoto, 1993; Morimoto et al., 1994]. The classic heat shock response occurs via a biochemical pathway that utilizes latent heat shock transcription factors (HSFs) that translocate to the nucleus, where they trimerize and bind to heat shock elements (HSEs) in the promoters of stress-inducible genes [Sistonen et al., 1994; Nakai et al., 1995]. HSEs are composed of pentanucleotide modules, nGAAn, arranged as continguous inverted repeats, that bind HSF [Perisic et al., 1989], the inducible transcriptional activator that regulates the transcription of these stress-inducible genes [Lis and Wu, 1993].

Abbreviations: EM, electromagnetic; EMSA, electrophoretic mobility shift assay; HSE, heat shock element; HSF, heat shock factor; HSP, heat shock gene; hsp, heat shock protein; Hz, hertz.

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A number of HSFs have been discovered in recent years, and their various functions elucidated [for review see Nakai et al., 1995]. HSF1 is the primary factor involved in the response to immediate environmental changes, including such stresses as heat shock, heavy metals, and oxidative stress [Sarge et al., 1993]. Environmental conditions that activate HSF1 are thought to be those that denature proteins, necessitating increased synthesis of hsps (heat shock proteins), the molecular chaperones [Baler et al., 1992] that help proteins refold to their original structure. In contrast, HSF2 mRNA is developmentally regulated and accumulates at high levels in the testis at the spermatocyte and spermatid stages of development and during hemin-induced differentiation of erythroleukemia cells [Murphy et al., 1994]. Cooperative interactions between HSF trimers and HSEs can occur [Amin et al., 1994]. Thus, the activation state of HSF1 provides an indication of the adverse nature of changes of environmental conditions on cellular physiology.

Many biological responses to in vitro and in vivo stimulation with electric and magnetic fields at a variety of frequencies and field strengths have been reported [for review see Goodman et al., 1995; Hong, 1995]. Several lines of evidence indicate that stimulation by 60 Hz magnetic fields induces the expression of

heat-inducible genes without elevated temperature: transcriptional activation of the heat shock puff that codes for hsp70 on chromosome 3R in *D. melanogaster* salivary gland chromosomes [Goodman et al., 1992], increased HSP70 transcript levels [Goodman et al., 1994], increased SSA1 (equivalent to HSP70) in *S. cerevisiae* [Weisbrot et al., 1993], and increased synthesis of the stress protein hsp70 [Goodman and Henderson, 1988; Blank et al., 1993, 1994]. Thus, stimulation with low frequency magnetic fields (<300Hz) points to an environmental or physiological stress that induces a cellular response similar to that of increased temperature but without elevated temperature.

Although the mechanism of the interaction of magnetic fields with cells remains unknown, the induction of the stress response by 60Hz magnetic field stimulation appears to be an appropriate cellular response to a stimulus that is not normally part of its environment and may be the general interaction mechanism. To determine whether the stress response induced by magnetic field stimulation is mediated by a sequence of events similar to that reported for other physiological stresses [for review see Morimoto et al., 1994], we examined the activation of heat shock transcription factor and its DNA binding activity.

METHODS

Cell Culture

Experiments were conducted with human promyelocytic HL60 cells [Westin et al., 1982] (provided by Dr. I.B. Weinstein, Columbia University Cancer Center). Cells were maintained in RPMI 1640 (Gibco/BRL, Gaithersburg, MD) with 10% fetal bovine (Gibco/BRL). Cells were prepared for each experiment the previous day by aliquoting cells from a single T75 flask into individual T25 flasks, ensuring that control and experimental samples derived from the same original batch of cells and that cell concentrations were identical. The medium was not changed again before exposure of cells to the 60Hz magnetic field. Cells for experimental and control samples were at cell densities of 1×10^6 cells/ml in T25 flasks (Falcon Plastics, Lincoln Park, NJ) at 15ml per flask at 37°C. All cells are maintained in mu metal containers for 2 days before experiments.

Electromagnetic Field Exposure Conditions

Magnetic fields were generated by a pair of Helmholtz coils (164 turns of 19 gauge copper wire around a 13 × 14cm Plexiglas form; Electro-Biology Inc., Parsippany, NJ). Cells were exposed to a 60Hz 8μT (peak) field for 20 min in T25 flasks and then removed from the field for 0, 10, 20, 30, and 40 min, and cell lysates were prepared. The Helmholtz coils were shielded in mu metal containers (Ammuneal Manufact. Corp., Philadelphia, PA) within the incubator. The sinusoidal field was generated by a Wavetek function generator (model 21, 11 MHz; Wavetek) connected to a power regulator. The function generator and power regulator were situated outside the incubator. Signal parameters were monitored by a calibrated inductive search coil (Electro-Biology, Inc.) with a Hitachi (V-1065, 100MHz) oscillscope. Control cells were sham-exposed at the same time in the same incubator and shielded in an identical mu metal container.

To ensure that no heating resulted from the active coils, we monitored temperatures with a thermocouple probe (PhysiTemp, Inc., Hackensack, NJ) attached to the coils throughout all exposures (sensitivity ±0.1°C).

Heat Shock Conditions

Cells in T25 flasks, wrapped in Parafilm and placed in mu metal containers, were immersed in a water bath at 43°C for 20 min, and then removed and maintained at 37°C for an additional 30 min before lysate was prepared. Temperature was monitored with the PhysiTemp thermocouple probe.

Preparation of Cell Extracts

Lysates were prepared according to Mosser et al. [1988] 0, 10, 20, 30, and 40 min following 20 min 60Hz magnetic field exposures as follows: cells were harvested and centrifuged, the pellets were washed with cold phosphate-buffered saline (PBS), and rapidly frozen at −70°C. The frozen pellets were suspended in a buffer containing 20mM HEPES, pH7.9, 25% (vol/vol) glycerol, 0.42M NaCl, 1.5mM $MgCl_2$, 0.2mM EDTA, 0.5mM phenylmethylsulfonyl fluoride, and 1.0 mM dithiothreoitol and centrifuged at 14K rpm for 20 min. The supernatants were collected, divided into individual tubes (each containing 20μl), frozen in liquid nitrogen, and stored at −70°C. Protein concentrations were

determined using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Richmond, CA).

Electrophoretic Mobility Shift Assay (EMSA)

Three pairs of complementary consensus HSE-containing sequences were used: HSE P sequence 5′-ATTCCAGAAGCTTCACAA-3′ and its reverse complement and HSE D sequence 5′-CTAGGAAGATTCCAGTCCCTGCCCAGGCCC-3′ and its reverse complement. The HSE P and HSE D oligonucleotides correspond to sequences in the promoter/enhancer regions of the stress-inducible αB-crystallin gene. In addition to the two nGAAn motifs, the HSE D also contains a metal response element–like binding sequence [Head et al., 1996]. The third pair of complementary consensus HSE-containing sequences was a self-complementary ideal HSE oligonucleotide, 5′-CTAGAAGCTTCTAGAAGCTTCTAG-3′, which contains four perfect inverted nGAAn repeats when annealed (generously provided by Dr. R.I. Morimoto, Northwestern University, Evanston, IL). Ten micrograms of each protein sample were used for binding assays, incubated for 15 min at room temperature with oligonucleotide labeled with γ[^{32}P]-ATP and loaded on a 4% polyacrylamide gel (using the variation of Mosser et al. [1990] as described in Head et al. [1996]), and exposed to film overnight.

Competition Assays

Extracts were incubated with a 100-fold molar excess of the unlabeled HSE oligonucleotide (15 min at room temperature) before adding the [^{32}P]-labeled HSE oligonucleotide.

Supershift Assays

In experiments involving addition of antibodies to whole cell extracts prior to gel shift analysis, the sera were diluted in 1× PBS + 1mg/ml bovine serum albumin (BSA) + 0.02% sodium azide. One microliter quantities of anti-HSF1 or anti-HSF2 (diluted 1:500) were added to each 10μl of whole cell extract and incubated for 20 min in a final volume of 15μl of binding buffer at room temperature prior to the addition of the [^{32}P]-labeled olignucleotide probe [Sarge et al., 1993; Head et al., 1996]. [Anti-HSF1 (10H8) and anti-HSF2 antibodies from Dr. R.I. Morimoto.]

RESULTS

Induction of HSE Binding Protein by 60 Hz Magnetic Field Stimulation

HL60 cells were exposed to a 60Hz 8μT magnetic field in six individual experiments. Initial experiments determined that 8μT was the most effective range of field strength by exposing cells to higher and lower ranges of field strengths (0.8μT and 80μT). The lower and higher field strengths were also effective with respect to HSE–binding activity, giving results similar to those in Figure 1 but of lower magnitude. Electrophoretic mobility shift assays (EMSAs) were performed to determine activation of heat shock transcription factor and its DNA–binding activity. Three pairs of complementary consensus HSE-containing sequences were tested differing in the number of nGAAn motifs and flanking sequence. The DNA protein complexes formed during incubation at 25°C were separated from free radiolabeled HSE oligonucleotide by electrophoresis on native polyacrylamide gels. Figure 1 shows an EMSA of protein extracts from HL60 cells exposed to magnetic fields using the HSE P oligonucleotide. The free HSE oligonucleotide migrated to the bottom of the gel, while specific and nonspecific complexes migrated more slowly. Lysates prepared of samples exposed to 60Hz magnetic fields for 20 min plus a further 20 minutes recovery showed a mean increase of 3.4±.18 in binding activity (Fig. 1, lane 2), somewhat less than that seen in the heat-shocked sample (Fig. 1, lane 5). HSE binding activity was reduced to unexposed sham-control levels (Fig. 1, lane 1) by 40 min following the initial 20 min of 60Hz magnetic field exposure (Fig. 1, lanes 3,4). In further experiments the use of oligonucleotide HSE D gave the same results as those with HSE P.

In other experiments HSE binding activity was tested with a self-complementary ideal HSE oligonucleotide (Fig. 2). Peak binding activity (3.6±.26) of the magnetic field–stimulated sample was seen at 20 min plus 20 min of recovery (Fig. 2, lane 2) and was similar to the binding activity of the heat-shocked sample (Fig. 2, lane 5). Longer and shorter times following magnetic field exposure showed less binding activity (Fig. 2, lanes 1,3,4) and were similar to that seen in the sham-exposed protein sample (Fig. 2, lane 6). Constitutive binding activity was also evident.

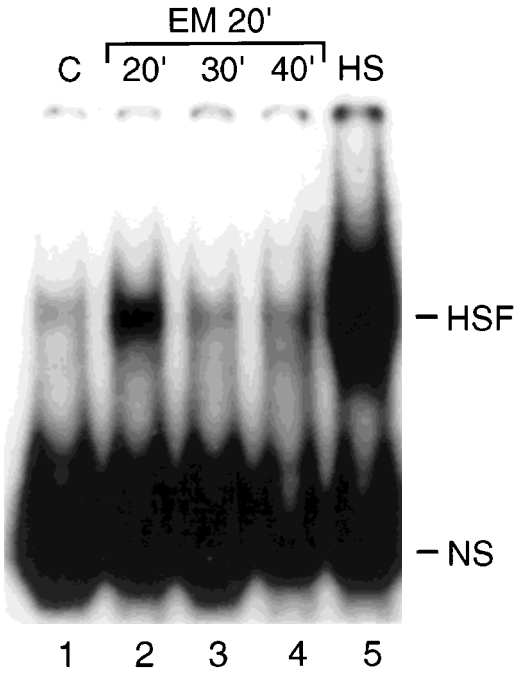

Fig. 1. Electrophoretic mobility shift assay (EMSA) of protein extracts from HL60 cells exposed for 20 min to a 60Hz 8μT magnetic field (EM), sham-exposed (C) or heat-shocked (HS) using the HSE P oligonucleotide. Lysates were prepared 20, 30, and 40 min following EM field stimulation. Cells were heat-shocked (HS) at 43°C for 20 min and incubated an additional 20 min at 37°C, and lysates were prepared. Control cells were sham-exposed for 20 min and lysates prepared after 20, 30, and 40 min. The positions of heat shock factor (HSF) retarded bands and nonspecific (NS) retarded bands are marked. **Lane 1:** Sham-exposed control 20 min plus 20 min recovery. **Lane 2:** EM field exposed 20 min plus 20 min recovery. **Lane 3:** EM field exposed 20 min plus 30 min recovery. **Lane 4:** EM field exposed 20 min plus 40 min recovery. **Lane 5:** HS at 43°C for 20 min plus 20 min recovery at 37°C. NS, nonspecific free probe.

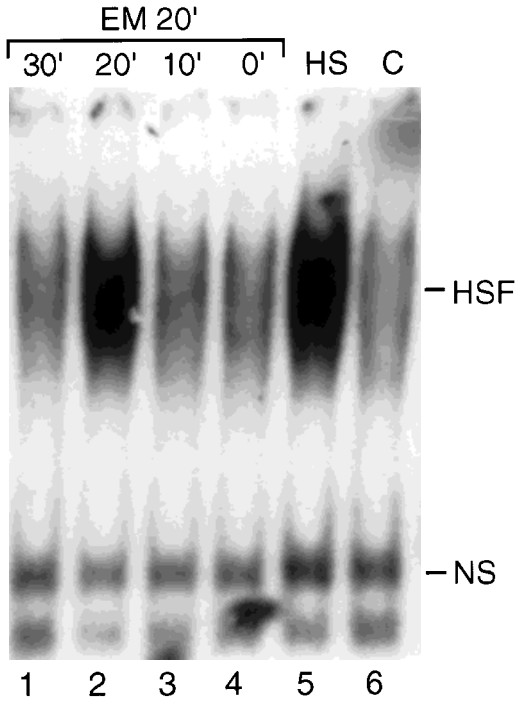

Fig. 2. EMSA of same protein extracts using ideal HSE oligonucleotide [Sarge et al., 1993] (details in Methods). Cells exposed to 60Hz 8μT magnetic fields (EM) and lysates prepared at 0, 10, 20, and 30 min following magnetic field stimulation. Cells heat-shocked (HS) at 43°C for 20 min and incubated additional 20 min at 37°C, and lysate prepared. The positions of heat shock factor (HSF) retarded bands and nonspecific (NS) retarded bands are marked. **Lane 1:** EM field exposed 20 min plus 30 min recovery. **Lane 2:** EM field exposed 20 min plus 20 min recovery. **Lane 3:** EM field exposed 20 min plus 10 min recovery. **Lane 4:** EM field exposed 20 min plus 0 min recovery. **Lane 5:** HS 20 min plus 20 min recovery at 37°C. **Lane 6:** Sham-exposed control 20 min plus 20 min recovery (C). NS, nonspecific free probe.

Sequence Specificity and Identity of the 60Hz Magnetic Field–Inducible HSE Binding Protein

To determine whether activation of HSF1 and/or HSF2 could account for transcription from genes containing heat shock elements, sequence specificity and identity of the magnetic field–inducible HSE binding protein were examined. Polyclonal antibodies against HSF1 and HSF2 were used in supershift assays to identify HSF1 and/or HSF2 activation. In these experiments HSE–binding activity used the oligonucleotide containing four perfect inverted nGAAn repeats when annealed. Protein samples from EM field–exposed cells exhibited HSF1 DNA binding activity, as seen by a supershifted band (SS), and possibly HSF2 binding activity as judged by partial reduction of the HSF/HSE band in a "wipeout" assay (Fig. 3). Protein samples from 3T3 cells were positive controls for HSF1 DNA–binding activity and showed a supershifted (SS) band with HSF1 antibody but not HSF2 antibody (Fig. 3, lanes 1–3). Protein samples from F9CNTL cells served as positive controls for HSF2 DNA–binding activity, showing an absence or wipeout of the HSF/HSE shifted band with HSF2 antibody and no supershift with HSF1 antibody (Fig. 3, lanes 4–6). The protein samples from EM field–exposed cells (Fig. 3, lanes 7–9) exhibit HSF1 DNA binding activity, as seen by a supershifted band, and possibly HSF2 binding activity in addition as judged by partial reduction/wipeout of the HSF/HSE band. For competition assays (Fig. 3, lanes 10–12), all the components were added at the same time with no preincubation. The unlabeled self-competitor oligonucleotide elimi-

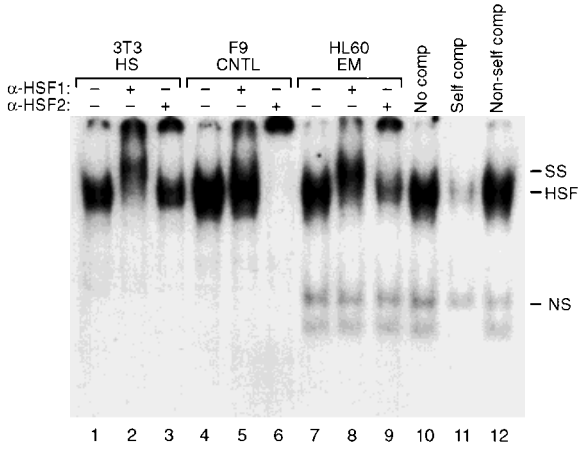

Fig. 3. EMSA of protein extracts from HL60 cells exposed to 60Hz magnetic (EM) fields (**lanes 7–9**); 3T3 heat-shocked cells (positive controls for HSF1 DNA–binding activity) (**lanes 1–3**); F9 CNTL cells (positive controls for HSF2 DNA–binding activity (**lanes 4–6**) using ideal oligonucleotide containing four perfect inverted nGAAn repeats when annealed [Sarge et al., 1993]. Binding reactions were performed in the presence or absence of polyclonal antibodies against HSF1 and HSF2. The HSE/HSF retarded band is marked HSF, and the HSE/HSF complex supershifted by addition of HSF1 or HSF2 antibody is marked SS. NS, nonspecific free probe. **Lanes 1–3:** Protein samples from 3T3 heat-shocked cells (positive controls for HSF1 DNA binding activity). **Lanes 4–6:** Protein samples from F9 CNTL cells (positive controls for HSF2 DNA–binding activity). **Lanes 7–9:** Protein sample from EM field–exposed cells (20 min EM field plus additional 20 min poststimulation). **Lanes 10–12:** HL60 EM field–stimulated sample in competition assays using a 200-fold excess of unlabeled perfect HSE (self, lane 11), an unrelated sequence (non-self, lane 12), or an absence of competitor (no competition, lane 10).

nated the HSE/HSF shifted band (Fig. 3, lane 11), whereas a 200-fold excess of unlabeled sequences (Fig. 3, lane 12) did not alter the HSF/HSE shifted band.

Together these data demonstrate that in HL60 cells the magnetic field–inducible heat shock element–binding activity we noted is HSE sequence specific and that it contains HSF1.

DISCUSSION

The activation of HSF1, the predominant stress-responsive factor, involves multiple steps, including translocation to the nuclear compartment, oligomerization from the latent monomer to a trimer, acquisition of DNA–binding activity, inducible serine phosphorylation, and subsequent acquisition of transcriptional activity. Cooperative interactions between HSF trimers and HSEs have been reported [Amin et al., 1994; Kroeger and Morimoto, 1994; Morimoto et al., 1994]. HSF1 and HSF2 exhibit complementary roles that extend the regulatory potential of the HSF family to respond to diverse conditions of physiological and environmental cell signaling and stress [Kroeger and Morimoto, 1994; Nakai et al., 1995]. Our studies have established that trimerization of HSFs and binding to HSE occur in cells exposed to 60Hz magnetic field stimulation *at normal growth temperatures.* These results are similar to effects reported under conditions that cause protein damage, such as heat shock and heavy metal exposure [Morimoto, 1993; Head et al., 1996]. Our data indicate that 60Hz magnetic fields induce activation of heat shock factor and binding to heat shock element, integral steps in the stress response demonstrated for other environmental/physiological stresses. Generally, 60Hz 8μT magnetic fields are thought to be not energetic enough to cause damage to proteins or other cellular structures [for review see Goodman et al., 1995]; therefore, the stress-response proteins are probably induced for reasons other than denaturation of proteins but may involve direct interaction with DNA [Blank and Goodman, 1997]. This has become a more plausible possibility since the demonstration that DNA is capable of selective ion conduction along the base pairs [Dandliker et al., 1997].

Although previous results indicate that 60Hz magnetic field stimulation results in increased HSP70 [Goodman et al., 1994; Blank et al., 1994], there is no direct evidence regarding 60Hz magnetic field induction of serine phosphorylation of heat shock factor such as that seen in heat shock. The alternative could involve activation of HSF without serine phosphorylation, implying involvement of other transcriptional mechanisms [Jurivich et al., 1992]. Given the variability of electric and magnetic field effects on cells and tissues [Goodman et al., 1995; Hong et al., 1995] and the observation that cell state may be an important mediator of magnetic field effects, direct analysis of the phosphorylative status of HSF is necessary to verify that transcriptional activation is in fact taking place.

The cell membrane is currently thought to be the primary region for sensitivity to electric and magnetic fields, and temporary distortions might account for HSF activation in 60Hz magnetic field–stimulated cells [for review see Goodman et al., 1995]. The induction of the magnetic field response in lymphocytes depends on Ca^{2+} signal transduction activation,

suggesting that calcium concentration might account for HSF1 activation in the presence of EM fields [Walleczek and Liburdy, 1990; Walleczek, 1992; Walleczek and Budinger, 1992]. Treatment with calcium ions results in concentration- and time-dependent activation of HSF in vitro [Mosser et al., 1990]. The findings presented here indicate a potential route for electromagnetic "signals" that has a broad range of reception and a correspondingly large number of possible effects at the cellular level, without contradicting the evidence for membrane sensitivity to electric and magnetic fields. If direct interaction with DNA is possible, then interaction with the cell membrane may not be the only mechanism.

Our results to date demonstrate heat shock element binding activity in 60Hz magnetic field–stimulated human HL60 cells and provide evidence for activation of heat shock factor in mediating the stress-induced transcription of HSP70 [Goodman et al., 1992, 1994; Weisbrot et al., 1993] and the increased synthesis of hsp70kD in cells exposed to 60 Hz magnetic fields [Goodman and Henderson, 1988; Blank et al., 1993, 1994; Hinson et al., 1996]. Our studies of transcriptional activation of HSP70 by magnetic fields show that at least one region of the promoter, −155 to −230 relative to the initiation site, is sensitive to 60Hz magnetic field stimulation. This region contains two HSE binding sites at ∼−190 and ∼−100 [Lin et al., 1996].

The identities of the sensors for physiological stress as well as the initial molecular target that responds to the electromagnetic field remain to be determined. These may or may not be identical, but they stimulate the same sequence of reactions, since the data reported for 60Hz magnetic field stimulation are consistent with what is known about the regulation and control of other cellular stresses. The events following trimerization are well documented, whereas the events leading directly to HSF1 trimerization are relatively uncharted. Our data place electromagnetic field stimulation in the context of other environmental/physiological stresses and what is known about their regulation and control. These results comprise an important first step in defining the interactions between electric and magnetic fields and organisms and should contribute to the database needed for the discussion of biological effects of electromagnetic field stimulation.

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