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Effect of 50 Hz Electromagnetic Fields on the Induction of Heat-Shock Protein Gene Expression in Human Leukocytes

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Although evidence is controversial, exposure to environmental power-frequency magnetic fields is of public concern. Cells respond to some abnormal physiological conditions by producing cytoprotective heat-shock (or stress) proteins. In this study, we determined whether exposure to power-frequency magnetic fields in the range 0–100 μT rms either alone or concomitant with mild heating induced heat-shock protein gene expression in human leukocytes, and we compared this response to that induced by heat alone. Samples of human peripheral blood were simultaneously exposed to a range of magnetic-field amplitudes using a regimen that was designed to allow field effects to be distinguished from possible artifacts due to the position of the samples in the exposure system. Power-frequency magnetic-field exposure for 4 h at 37°C had no detectable effect on expression of the genes encoding HSP27, HSP70A or HSP70B, as determined using reverse transcriptase-PCR, whereas 2 h at 42°C elicited 10-, 5- and 12-fold increases, respectively, in the expression of these genes. Gene expression in cells exposed to power-frequency magnetic fields at 40°C was not increased compared to cells incubated at 40°C without field exposure. These findings and the extant literature suggest that power-frequency electromagnetic fields are not a universal stressor, in contrast to physical agents such as heat.

INTRODUCTION

Environmental exposure to power-frequency electromagnetic fields continues to be of public concern. Epidemiological studies provide no firm evidence of a carcinogenic hazard; however, a possible increased risk of childhood leukemia associated with magnetic-field amplitude greater than 0.4 μT and prolonged exposure has been highlighted (*1*). The experimental evidence from animal and cellular studies is controversial, and no consensus regarding the biological models and exposure conditions that can elicit consistent measurable effects has yet emerged. One way in which the biological effects of power-frequency magnetic-field exposure and its significance can be evaluated is by comparing the effects of power-frequency magnetic-field exposure to those induced by physical stimuli known to cause a cellular response.

Cells respond to a variety of stresses, the precise nature of the response being determined by the insult encountered. For several types of stressors, such as ionizing radiation, heat and some chemicals, the response involves the production of stress proteins (heat-shock proteins) (*2, 3*). These proteins are highly conserved families of molecules that serve a range of intracellular functions including protecting cells against injury. Heat-shock proteins are sensitive markers of protein damage, and increased levels after exposure to power-frequency magnetic fields would reflect abnormal intracellular conditions and a potential for cellular damage.

The regulation of heat-shock protein gene transcription is mediated by the interaction of heat-shock factor (HSF) transcription factors with heat-shock elements (HSEs) in the heat-shock protein gene promoter regions (*4, 5*) Under normal circumstances, HSF1 is present in the cytoplasm as a latent monomeric molecule that is unable to bind to DNA. On exposure to stress, an intracellular flux of newly synthesized non-native proteins activates HSF1 (*5*), which is hyperphosphorylated by members of the mitogen-activated protein kinase subfamilies (*6, 7*). HSF1 is converted to inducibly phosphorylated trimers that have the capacity to bind DNA, and the phosphorylated trimers translocate from the cytoplasm to the nucleus (*8*). The consequences of HSF1 binding to its target, and the events that result in the ensuing transcription of heat-shock protein genes, have been reviewed extensively (*9, 10*).

The evidence that exposure to power-frequency magnetic fields induces heat-shock protein expression is equivocal, and as yet there have been few independent confirmatory replication studies. However, reports that power-frequency magnetic fields induce heat-shock proteins in a variety of biological models from bacteria (*E. coli*) to nematodes (*C. elegans*) and chick embryos (*11–15*) continue. The expression of HSP16, HSP27 and HSP70 has been reported to be

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increased at magnetic-field strengths ranging from 8 μT to 500 mT (*15–17*). However, other studies have reported that magnetic-field strengths of a similar range (6.3 μT–50 mT) have no effect on heat-shock protein expression in a variety of biological models (*18–21*). The physiological implications of field-induced heat-shock protein expression are unknown, but they could range from therapeutically beneficial to detrimental. Hence it is important to establish whether the reported effects are robust and to define the exposure limits and the biological conditions of any induced effect.

This study was designed to determine whether exposure of human peripheral blood to power-frequency magnetic fields induces heat-shock protein gene expression in leukocytes and to compare this response to that induced by heat alone. The study also examined whether cells subjected to mild heat (40°C) become more sensitive to a concomitant exposure to power-frequency magnetic fields.

MATERIALS AND METHODS

Power-Frequency Magnetic-Field Exposure System

A purpose-built three-coil exposure system was used to provide magnetic-field exposures in the range 0–100 μT at 50 Hz. Each coil was 25 cm in diameter and spaced 15 cm apart with the center axis vertical (Fig. 1). The coils were wound with multiple taps connected in series and driven by a single amplifier fed with a 50 Hz sinusoid from a master oscillator. In these studies, six 96-well plate holders were stacked in an open frame, vertically above each other, with gaps of several centimeters between them to ensure good air circulation. This structure occupied the 30-cm vertical span of the coil system. Sixteen samples were placed in a cluster of wells at the center of each plate such that they were all close to the central magnetic axis of the coil system. The number of active turns within each individual coil and their relative polarity were preselected before each experiment. This enabled a variety of magnetic-field spatial distributions to be created, and hence different plates within the stack were exposed simultaneously to different field strengths based on their position and the coil configuration.

The field distributions that can be generated by the exposure system include: zero field at the center of the stack with maxima at the top and bottom, maximum field at the center of the stack with zero at top and bottom, and zero at one end of the stack with a maximum at the other (Fig. 1). The finite depth of the blood sample (5 mm) and positioning variances result in a magnetic-field exposure variation of approximately 2 μT through the sample at each nominal exposure amplitude. The ability to vary the relative magnetic-field exposure at each plate allows any effects due to magnetic fields to be distinguished from possible artifacts relating to the physical position of the plates. The two field distributions used in this study were maximum (100 μT) at the bottom and zero at the top, and maximum at the top and zero at the bottom. These field distributions were alternated between sequential experiments.

The coil system was housed in a wooden incubator (to avoid distortion of the magnetic fields), and temperature control was maintained using a custom-designed, proportional control system. Temperature was logged throughout the course of a 4-h exposure and varied by less than ±0.1°C at any one position. The spatial variation of temperature within the coil exposure system was less than ±0.2°C. The magnetic-field intensity was not logged continuously throughout the experiments due to the intrinsic stability of the technology; however, the coil current was monitored and maintained at a constant value. The field distributions as measured using a Hall Effect probe agreed with theoretical calculations to within 1% of the maximum fields.

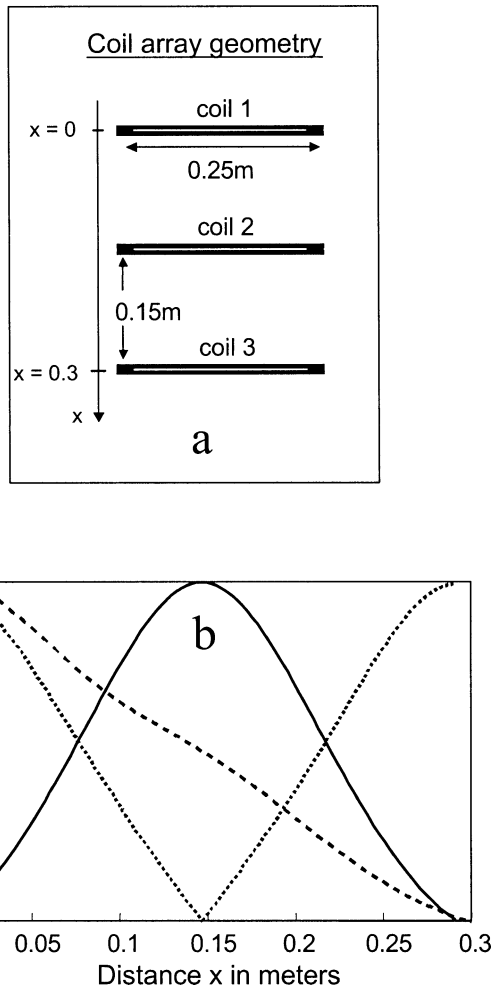

FIG. 1. The coil array geometry (panel a) and absolute magnitude of magnetic-field strength along the central axis of the coil array for three different configurations of active turns (panel b).

Cell and Culture Conditions

Peripheral blood was collected from healthy male volunteers ($n = 3$, age range 30–50 years) into Vacutainers containing EDTA anticoagulant (BD Biosciences, UK). Volunteers gave informed consent, and the study complied with the University of Sheffield's REH Ethics policy on research involving human participants, data and tissue; UK National Health Service ethical approval was not required for the study. The rationale for using whole blood for these experiments, rather than leukocytes isolated from whole blood, was to minimize the stress to the cells before exposure. Whole blood was diluted 1:1 with RPMI 1640 growth medium prewarmed to 37°C and divided into aliquots (200 μl/well) into the four center wells of each of the four central strips in 96-well Stripwell plates (Costar). The lids were modified to allow air flow around the wells while in the exposure system. Samples were maintained at 37°C for 1 h before the exposure period. Six plates were exposed simultaneously for 4 h, each plate to a different magnetic-field strength. Additional samples were maintained in a separate incubator at 37°C for the same period, a subset of which was heat-shocked at 42°C for the final 2 h of the 4-h experimental period (positive control).

At the end of the exposure period, samples were transferred to Eppendorf tubes on ice, and erythrocytes were lysed using SV RNA Red Blood Cell Lysis solution (Promega Corporation). Samples were washed and leukocytes were pelleted by centrifugation prior to mRNA isolation and analysis of heat-shock protein gene expression.

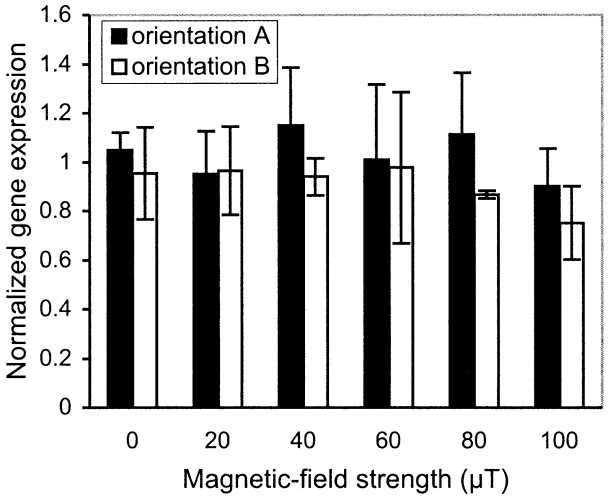

FIG. 2. Expression of the gene encoding HSP27 normalized to control at 37°C for power-frequency magnetic-field orientations A and B (means ± SEM), after 4 h exposure.

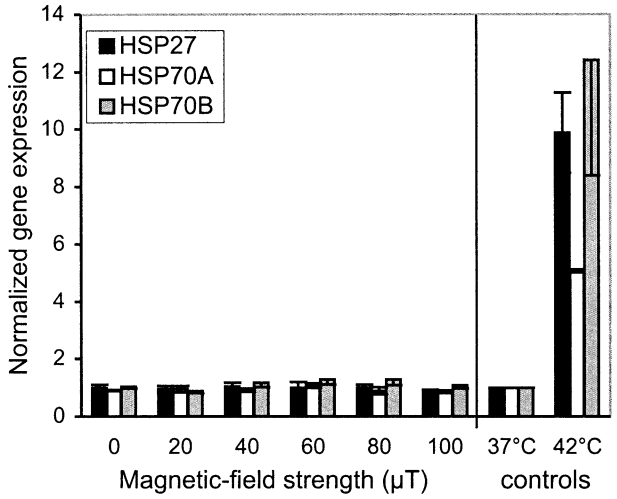

FIG. 3. Gene expression normalized to control at 37°C. Data are means ± SEM for six experiments. Power-frequency magnetic-field exposure was 4 h. Heat was applied for the last 2 h of the experimental period for the 42°C control samples.

Heat-Shock Protein Gene Expression

Heat-shock protein gene expression was determined by reverse transcriptase-polymerase chain reaction (RT-PCR). Messenger RNA was isolated from the leukocyte cell pellet using the PolyATract System 1000 according to the manufacturer's recommended protocol (Promega Corporation, technical manual No. 228). Isolated RNA was reverse transcribed and the cDNA was amplified using Promega's single-buffer Access RT-PCR system and primer pairs for HSP70A, HSP70B and HSP27 (Stressgen Biotechnologies Corp, Canada). The cycling conditions were: initial denaturation 94°C for 2 min, denaturation at 94°C for 30 s, annealing at 54°C for 1 min, extension at 72°C for 2 min, with the final extension at 68°C for 7 min. Preliminary experiments determined the number of cycles that were required to ensure that the PCR products for each of the target genes lay within the linear portion of the amplification curve. RT-PCR products were electrophoresed through agarose gels containing ethidium bromide, and the fluorescence intensities of the amplicon bands were referenced to those of the housekeeping gene β-actin using Scion Image analysis software (Scion Corporation, Frederick, MD). Data are expressed as a ratio of these densities. The genes encoding HSP27 and HSP70A are constitutively expressed, and expression is increased after stress, whereas the gene encoding HSP70B is expressed only in stressed cells.

Data Analysis

All experiments were blinded to the individual performing the exposures and the assays, and they included negative (maintained at 37°C in a separate incubator) and positive (heat-shocked at 42°C for 2 h) controls. To compare results between experiments, the HSP/β-actin ratio was normalized to that of control samples that were maintained at 37°C and not exposed to power-frequency magnetic fields. In the case of the experiments in which samples were exposed to power-frequency magnetic fields and heat concomitantly, normalization was made to the 0-μT exposure samples.

The null hypothesis that all populations had identical means was tested using one-way analysis of variance. Based on six experiments and the observed standard deviation, there was a 90% power to detect a 50% change in heat-shock protein gene expression at the $P = 0.05$ level. For the concomitant power-frequency magnetic fields and mild heat experiments ($n = 3$), there was a 90% power to detect a 60% change in gene expression at the $P = 0.05$ level.

RESULTS AND DISCUSSION

A total of six experiments were performed. In three, the maximum magnetic field was at the bottom of the stack (orientation A); the maximum field was at the top of the stack in the remainder (orientation B). Samples at physically different positions could therefore be exposed to identical magnetic-field strengths. The effects of six magnetic-field strengths (0, 20, 40, 60, 80, 100 μT rms at 50 Hz) were investigated in each experiment. The vertical position of the samples within the exposure system had no discernible influence on heat-shock protein gene expression (Fig. 2), which would be anticipated given the small spatial temperature variation (±<0.2°C) within the coil stack. Given that there was no difference between the results from the two magnetic-field orientations, the two data sets were combined for the subsequent analysis of magnetic-field effects.

There were no significant differences (at $P = 0.05$) in gene expression at the end of a 4-h exposure for any of the heat-shock proteins at any of the six magnetic-field strengths (Fig. 3). The expression of the gene encoding HSP70B (the inducible form of HSP70) in cells from control samples indicated that some degree of stress was occurring, despite the fact that experiments were performed using whole blood rather than isolated leukocyte populations. Incubation at 42°C for 2 h (as a positive control) induced a 12-fold increase in expression of the gene encoding HSP70B, thus demonstrating that cells remained responsive to appropriate stimulation.

It has been suggested that sensitization might be a prerequisite for biological effects of electromagnetic fields to be observed, or that a co-stress enhances the power-frequency magnetic-field effect (*22, 23*). It was for this reason that whole blood rather than isolated leukocytes was used in this study, since the separation of leukocytes from blood

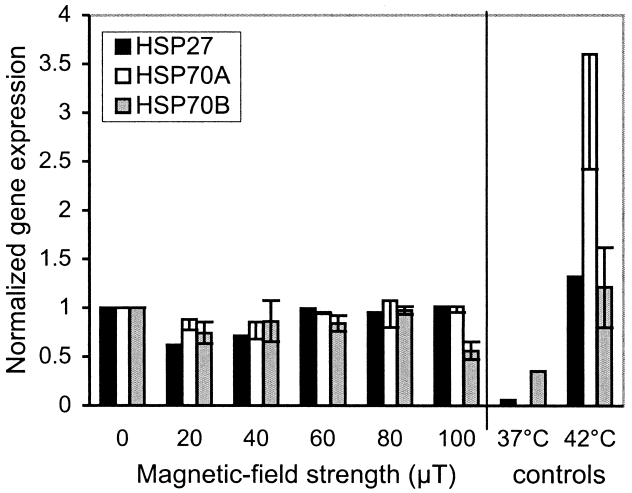

FIG. 4. Gene expression (normalized to value for 0 μT) for concomitant heat (40°C) and magnetic-field exposure (4 h). Heat was applied for last 2 h of the experimental period for the 42°C control samples. Data are means ± SEM for three experiments.

by differential centrifugation might elicit physical stress on the isolated cells. While the presence of erythrocytes, platelets and their lysis and secreted products in whole blood might influence the observed responses, we opted to use whole blood to minimize the stressing of the leukocytes.

We studied the potential influence of a concomitant stress on the response to power-frequency magnetic fields by raising the temperature of samples (40°C) during exposure to power-frequency magnetic fields. The elevated temperature alone increased expression of the genes encoding HSP27, HSP70A and HSP70B compared to that of cells maintained at 37°C; however, the superimposition of power-frequency magnetic fields had no additional effect (Fig. 4). Similar findings have been reported for higher field strengths by Miyakoshi *et al.* (*19*). However, these investigators have also reported a decrease in HSP70 expression when HL60RG cells were simultaneously exposed to 50 mT power-frequency magnetic fields and 40°C or 42°C for longer than 5 h. Although the lower levels of heat-shock protein expression, particularly HSP70B at 100 μT (Fig. 4), in our study might be interpreted as an inhibitory trend, the differences did not reach statistical significance, and the small sample size in these experiments does not allow such a conclusion to be drawn.

Published data on the effects of power-frequency magnetic-field exposure on heat-shock protein expression are equivocal, the reasons for which are currently unclear. However, the difficulties in interpreting the literature can be illustrated by studies that have used HL60 (human promyelocytic leukemia) cells to investigate heat-shock protein expression. Pipkin *et al.* (*17*) found that magnetic-field strength was an important factor: 1 mT would elicit a heat-shock protein response, whereas 100 μT was insufficient. In contrast, Lin *et al.* (*16*) found an effect at low intensities (8 μT), and Miyakoshi (*19*) found no effect with intensities up to 50 mT. Whether cells respond to power-frequency magnetic fields will be influenced by a number of factors, including the intensity and duration of the exposure and the cell type used to assess the response. At present it is not possible to predict which factor or combination of factors will elicit a biological response.

Based on the extant literature and our results, it is clear that power-frequency magnetic-field exposure is not a universal physical stressor of cells in the same way as heat. If power-frequency magnetic fields do indeed elicit changes in the intracellular environment that are sufficient to induce heat-shock proteins, then factors other than the magnitude of the magnetic-field strength and exposure time appear to be involved. The primary aim of our investigation was to examine the effects of environmental power-frequency magnetic-field amplitudes that encompass those encountered by the general public. In that context, our upper limit of 100 μT is considerably higher than normal exposure levels. It is thus perhaps reassuring that normal leukocytes appear not to be stressed by such fields.

CONCLUSION

In contrast to some studies (*11–17*) which have reported on the induction of heat-shock proteins in a range of cell types after exposure to power-frequency magnetic fields but in agreement with others (*18–21*), we have found no evidence that 50 Hz magnetic fields of amplitudes 0–100 μT induce expression of genes encoding heat-shock proteins in human peripheral blood leukocytes. In addition, exposure to mild heat at 40°C did not sensitize the cells to a concomitant exposure to power-frequency magnetic fields.

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