Insights Into Electromagnetic Interaction Mechanisms

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Low frequency (< 300 Hz) electromagnetic (EM) fields induce biological changes that include effects ranging from increased enzyme reaction rates to increased transcript levels for specific genes. The induction of stress gene HSP70 expression by exposure to EM fields provides insight into how EM fields interact with cells and tissues. Insights into the mechanism(s) are also provided by examination of the interaction of EM fields with moving charges and their influence on enzyme reaction rates in cell-free systems. Biological studies with in vitro model systems have focused, in general, on the nature of the signal transduction pathways involved in response to EM fields. It is likely, however, that EM fields also interact directly with electrons in DNA to stimulate biosynthesis. Identification of an EM field-sensitive DNA sequence in the heat shock 70 (HSP70) promoter, points to the application of EM fields in two biomedical applications: cytoprotection and gene therapy. EM field induction of the stress protein hsp70 may also provide a useful biomarker for establishing a science-based safety standard for the design of cell phones and their transmission towers.

LOW FREQUENCY EM FIELD INTERACTIONS WITH CELLS

Weak EM fields, with frequencies lower than 300 Hz and field strengths less than 1 Gauss (1,000 mG), induce a variety of effects in cells and tissues (Blank, 1995a; Goodman et al., 1995; Hong, 1995). These biological effects include bone healing (Bassett, 1995), nerve regeneration (Sisken and Walker, 1995), influences on signal transduction pathways involved in response to EM fields. Based on published evidence of the electron transport/charge flow in DNA, it is likely that EM fields interact directly with electrons in DNA to stimulate biosynthesis.

Our studies of EM field induction of the stress response proteins with the identification of an EM field-sensitive DNA sequence in the heat shock 70 (HSP70) promoter, point to the application of EM fields in two biomedical applications: cytoprotection and gene therapy. (Therapeutic use of EM fields has met with great success since the early 1970’s in accelerating the healing of bone fractures and soft tissue wounds.)

Paradoxically, it is the health risk issue that has in recent years dominated EM field research. Epidemiological studies have indicated that EM fields can also induce adverse health effects. The synthesis of stress response proteins is a protective cellular mechanism induced by a variety of potentially-noxious stimuli; the induction of these proteins by exposure to EM fields implies that these fields are perceived by biological systems as a possible hazard. In yet another manifestation, as we note below, EM field induction of the stress protein hsp70 may provide a useful biomarker for establishing a science-based safety standard for the design of cell phones and their transmission towers.

Abbreviations: HSP 70, heat shock gene; hsp, heat shock protein; HSF, heat shock factor; HSE, heat shock element; bp, base pair; mG, milligauss; microtesla, $\mu$T ($1 \mu$T = 10 mG); Hz, Hertz.

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cell calcium levels (Liburdy, 1992; Liburdy et al., 1993) and increased transcript levels for the immediate early response genes myc (Lin et al., 1994, 1996; Jin et al., 1997), jun, and fos (Phillips et al., 1992; Rao and Henderson, 1996) and the stress response gene HSP70 (Goodman and Blank, 1998). In cell-free systems, increases in enzyme reaction rates include ornithine decarboxylase (Byus et al., 1988), Na,K-ATPase, and cytochrome oxidase (Blank, 1995b; Blank and Soo, 1998).

Induction of the cellular stress response is elicited by many agents, including sudden elevated temperature ('heat shock'), changes in pH and heavy metals. The induction of stress gene HSP70 by EM fields indicates that cells respond to these fields as an environmental stress (Goodman and Blank, 1998). Heat shock genes (HSPs) represent a ubiquitous and evolutionarily conserved group of genes present in organisms as diverse as humans and Escherichia coli (Lindquist and Craig, 1988). We have used the induction of the stress response as a tool for examining how EM fields can stimulate such disparate processes as gene expression and changes in enzyme reaction rates.

Considering EM interactions from a purely physical point of view, several mechanisms have been proposed to account for the initial interactions with cells (Liboff et al., 1987; Lednev, 1991; Blanchard and Blackman, 1994), but these models have been limited by their inability to account for the wide range of experimental observations. The Mobile Charge Interaction (MCI) model (Blank, 1995a), based on simple physical interactions, grew out of a wide range of experiments. It posits that magnetic fields interact with moving charges (i.e., charge flow) in cells and change their velocities, as in the classic interaction of a magnetic field with any moving charge. If the charge flow is associated with a biological function, as in the case of an enzyme, that function will be altered. Field-induced changes in enzyme reaction rates, proportional to charge flow, have been demonstrated in Na,K-ATPase and cytochrome oxidase reactions (Blank, 1995b; Blank and Soo, 1998).

Similar data have also been reported from experiments using the Belousov-Zhabotinski (BZ) reaction. In this system, there is no cell or tissue involvement, rather only a 10-step chemical reaction. The effect in the BZ reaction is apparently due to EM field interaction with electrons being transferred during the redox steps.

Efforts to understand EM field interaction mechanisms have been, in general, largely confined to prevailing paradigms. Published reports have emphasized three experimental areas: the initial physical transduction step at the membrane level; the signal transduction pathway that carries information from the membrane to the nucleus; and the effects of the EM field waveform and waveshape. Although this approach has produced much interesting information, it has not led to an understanding of the EM field interaction mechanism.

**Initial physical transduction step at the membrane**

The cell membrane as a site of interaction with magnetic fields is demonstrated by increases in the activities of the two membrane enzymes mentioned above. However, membranes are not required for the interaction. In fact, EM-stimulated gene expression has been reported in cell-free preparations (Goodman et al., 1993; Tuinstra et al., 1997).

**Signal transduction pathways**

In eukaryotic cells, there is a steady-state level of expression of stress genes. Using the HSP70 gene as an example, Figure 1 illustrates the steady state concentration of the constitutive stress protein hsc70, shown by the fine line. Under conditions of stress, there is enhanced expression of the HSP70 gene that leads to the synthesis of the inducible form, the so-called molecular chaperone hsp70, shown by the heavy line. The concentrations of both hsc70 and hsp70 are controlled by negative feedback, indicated by the minus signs.

The box labeled TRANSCRIPTION in Figure 1 contains the complex set of processes presented in greater detail in Figure 2. Increased synthesis of the molecular chaperone hsp70 occurs in response to a variety of environmental stimuli (Lindquist and Craig, 1988). Like many of the proteins in the heat shock multigene family (hsp90, hsp70, hsp60, hsp47, hsp27, and zeta-crystallin), the hsp70 chaperone contributes to the repair, folding, and assembly of nascent proteins during stress, and to the degradation of damaged proteins. It is also involved in pre-conditioning and the development of cytoprotection. Figure 2 illustrates the signal pathway for 'heat shock' (Morimoto, 1998) and the pathways involved in EM field induction of the stress response protein hsp70. The two stresses, heat and EM fields, operate through different pathways and involve different regions of the promoter. The increased expression of the HSP70 gene in response to heat shock is primarily mediated by the specific transcriptional activator, heat shock factor 1 (HSF1); the enhancement of transcription occurs after binding of HSF1 to the heat shock elements (HSEs) (Mosser et al., 1988; Sorger, 1991). The HSEs for heat-responsiveness are located in the 5'-flanking sequences of the HSP70 genes that contain triplet repeats of GAA, a nucleotide recognition motif/sequence (Pelham, 1982). A minimum of three 5-bp modules are required to form a functional HSE (Amin et al., 1988).

**Fig. 1.** Negative feedback loop. The steady state concentration of the constitutive stress protein hsc70 is shown by the fine line. Under conditions of stress, there is enhanced expression of the HSP70 gene leading to the synthesis of the inducible form, the molecular chaperone hsp70, shown by the heavy line. The concentrations of both hsc70 and hsp70 are controlled by negative feedback, indicated by the minus signs.
The heat shock domain on the HSP70 promoter lies between −107 and −68, relative to the transcription initiation site, with the HSE centered at −100.

HSFs (1 through 4)/HSE interactions provide the basic regulatory system for induction of HSP genes in response to various forms of stress, including EM field stress. However, the induction of HSP70 by EM fields utilizes either of the two transduction pathways. One pathway contains steps that are reminiscent of the heat shock pathway, i.e., HSF1-binding to HSE. In EM field stress, HSF1 binds to an HSE upstream of the heat shock domain. Magnetic stress also uses a pathway that involves AP-1 binding. EM field exposures induce HSF1 phosphorylation by members of the MAPK subfamilies (ERK1, JNK/SAPK, and p38 protein kinase) resulting in increased protein levels for hsp70, c-Fos, AP-1 binding activity and increased MAPK/ERK1/2 phosphorylation (Jin et al., 2000).

The two domains, thermal and magnetic, function separately. The HSE in the heat shock domain is not interchangeable with the HSE in the EM field domain; site-specific mutagenesis in either domain removes the response to that stress only. Inserting nCTCTn sites are homologous to the c-Myc protein complex sequence in the c-myc gene (Taira et al., 1992; Lin et al., 1999, 2001). The level of response is proportional to the number of nCTCTn modules present (Lin et al., 1998, 1999, 2001).

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Magnetic stress also uses a pathway that involves AP-1 binding. EM field exposures induce HSF1 phosphorylation by members of the MAPK subfamilies (ERK1, JNK/SAPK, and p38 protein kinase), resulting in increased protein levels for hsp70, c-Fos, AP-1 binding activity, and increased MAPK/ERK1/2 phosphorylation (Jin et al., 2000). Figure 2 illustrates the differences in signaling pathways between magnetic stress and heat shock.

An important characteristic of the chaperone hsp70 is its participation in preconditioning for the development of cytoprotection (known as ‘acquired thermotolerance’ in the case of heat shock). Basically, cytoprotection is induced by preconditioning with a nonlethal stimulus (e.g., heat, hypoxia, EM fields). These preconditioning treatments lead to transient resistance to the cytotoxic effects of a potentially lethal metabolic stress, such as reperfusion ischemia. Cytoprotection induced by pre-conditioning with EM field exposures (Carmody et al., 2000) has important advantages over ischemic or thermal pre-conditioning, including among others:

- preconditioning with EM fields is noninvasive, safe, comfortable for the patient and simple to administer;
- higher hsp70 levels can be restimulated with EM field exposures at 20-min intervals (e.g., before, during, and following by-pass surgery) (Han et al., 1998);
- hsp70 levels induced for preconditioning are retained for more than 3 h (Han et al., 1998; Carmody et al., 2000).

Waveform and waveshape

Early research on the effectiveness of EM fields for accelerating bone-healing focused on the characteristics of the EM field waveform and wave shape. These studies showed that the EM frequency was important, that simple sine waves could be as effective as complex waveforms, and that a coherent signal was needed for a minimal duration. Studies using EM noise fields (a signal containing a random mixture of frequencies 30–300 Hz) showed that cells must be exposed for at least 10 sec to elicit a response (Litovitz et al., 1991). Superimposing a noise field on a 60 Hz signal eliminates the effect of that signal, but to be effective, the energy level of the noise must at least match the energy level of the stimulating signal. The authors suggest that a 10 sec exposure is the minimum time needed by the receptors at the cell membrane to transduce the signal, but this explanation would be true of any transducer, in the membrane or not.

Evidence for direct interaction of EM fields with DNA

Our discussion of mechanism has observed the custom of ‘rounding up the usual suspects’. Mechanisms involving membranes and/or signal transduction pathways...
are implicated in many biological processes, and may very well play a role in interaction with EM fields. Certainly, some enzyme studies demonstrate interaction at the level of the membrane. However, our observations as well as reports from laboratories studying charge transport through DNA, have led us to consider the possibility that EM fields influence certain biosynthetic events directly at the level of the DNA.

DNA-mediated charge transport and the oxidative damage that results are extremely sensitive to variations in the sequence and conformation-dependent stacking of the intervening bases (Dandliker et al., 1997). Protein binding to DNA modulates long-range charge transport both negatively and positively depending upon the specific protein/DNA interactions in play (Rajski and Barton, 2001). This represents a new methodology for examining protein–DNA binding interactions and hints at mechanism related to DNA-mediated charge transport (Ratner, 1999), which may play a role in transcriptional activity induced by EM fields. The following observations support this hypothesis:

- The assumption that magnetic fields stimulate changes in cells by first interacting with cell membranes is based on the belief that they act only indirectly through their induced electric fields, which are very small because of cell dimensions. In fact, low-frequency magnetic fields penetrate the cell (unlike electric fields), and the field strength at the nucleus is comparable to that at the membrane. Magnetic field interactions are, therefore, just as likely at the nucleus.

- The magnitude of an effective magnetic stimulus is very small compared to a thermal stimulus. EM fields induce the synthesis of hsp70 at an energy density 14 orders of magnitude lower than heat shock (Table 1). The effectiveness of an EM stimulus at such a low level of energy input suggests a qualitatively different molecular process.

- Despite cell and tissue differences (e.g., mammalian, diptera, yeast, bacteria), approximately the same EM field exposure, 60 Hz, 80 mG for 20 min, (Goodman and Blank, 1998) induces hsp70 synthesis in all systems studied. Figure 3 presents data from threshold studies using rodent cardiomyocytes (Carmody et al., 2000). The relatively narrow range within which EM fields induce increased levels of hsp70 suggests that the mechanism involves interaction with the same cellular components, e.g., the same promoter region on the DNA.

- DNA is known to conduct electrons, and studies on ATPase, cytochrome oxidase, and the BZ reaction, show that EM fields accelerate electron transfer rates. We have suggested that EM fields activate DNA by generating repulsive forces when accelerating electrons within the DNA double helix (Blank and Goodman, 1997, 1999, 2001). The velocity of charge movement calculated from our Na,K-ATPase measurements, \(10^7 \text{m/sec}\), is similar to ultrafast electron transfer in DNA of \(10^8 \text{m/sec}\) (Wan et al., 1999). At these velocities, the forces at low field strengths affect enzyme reactions, and may be large enough to initiate changes in DNA.

- Because EM fields penetrate the cell, they could theoretically interact directly with the DNA in the nucleus (or even the mitochondria). The nCTCTn sites, the EM response elements (EMREs) on the promoters of the c-myc and HSP70 genes, could be acting as sensors or antennae.

- Initiation of transcription by EM fields (Goodman and Blank, 1998) offers an approach to mechanism based on EM field interaction with moving electrons in DNA to generate repulsive forces that in turn cause DNA chain separation. In investigating the possibility that nCTCTn sequences can generate large repulsive forces between DNA chains, we estimate the forces of repulsion between chains, assuming that electron affinity is a measure of electron density at each base (A = 0.97, G = 1.51, T = 0.81, C = 0.57), and that the velocity of electrons at each base is inversely related to electron density. When an electron current flows through the DNA, the electron velocity determines the force for a particular value of EM field. The repulsive force is opposed by the attraction between chains due to hydrogen bonds: for A-T bonds, \(~10\text{kcal/mol}\), and for C-G bonds, \(~15\text{kcal/mol}\). From the balance of forces (repulsion minus attraction), we deduce that sites rich in C and T, as in the identified EM field-sensitive sequences, would be more likely to initiate transcription.

**TABLE 1.** Differences in energy densities between magnetic stress and heat shock stress

<table>
<thead>
<tr>
<th>Energy</th>
<th>Input</th>
<th>Energy density (joules/m³)</th>
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<tbody>
<tr>
<td>Magnetic</td>
<td>0.8 (\mu)T</td>
<td>(2.6 \times 10^{-7})</td>
</tr>
<tr>
<td>Thermal</td>
<td>+5.5 C</td>
<td>(2.3 \times 10^{-7})</td>
</tr>
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EM fields induce the synthesis of the stress protein hsp70 at an energy density 14 orders of magnitude lower than heat shock. The effectiveness of an EM stimulus at such a low level of energy input suggests a qualitatively different molecular process.
come apart. These calculations offer a plausible mechanism for initiation of transcription by EM fields, and a rationale for the specific sequences and the low level of energy input.

**BIOMEDICAL APPLICATION OF EM FIELDS**

We have utilized EM field-induced stress proteins to develop two new potential medical applications. Under noninvasive procedures of preconditioning, the synthesis and levels of these proteins are markedly increased so that they are available to serve as protective agents, i.e., 'chaperones' that bind to denatured or damaged proteins and facilitate their refolding.

We have demonstrated that EM fields induce gene expression (Goodman and Blank, 1998; Lin et al., 1999) and that activation of the gene by EM fields requires specific EMREs, which control genes when placed upstream of reporter constructs. Their ability to confer EM field responsiveness suggests the use of EMREs in the control and regulation of gene therapy. The characterization of a cellular promoter system that can be regulated provides a novel, noninvasive technique for the regulation of transgene expression in humans without interfering with normal physiologic function.

**Induction of hsp70 for use prior to cardiac surgery**

The protective roles that stress proteins play in a variety of normal cellular processes have interested clinicians concerned with minimizing the stresses of cardiovascular surgery on their patients, in particular, the problems of reperfusion and ischemia (Donnelly et al., 1992; Mestril et al., 1996; Plumier and Currie, 1996; Benjamin and McMillan, 1998). At the level of individual cells, prior heat stress by forced expression of hsp70 on mammalian cells protects them from cell injury resulting from heat, severe metabolic stress, or simulated ischemia (Yellon et al.; Heads et al., 1994; Mestril et al., 1994). However, such an approach in a patient undergoing by-pass surgery, for example, could be distinctly deleterious, since the body naturally resists changes in body temperature. An abundance of hsp70 would be important to limit myocardial injury, improve recovery from cardiac surgery by reducing infarct size, increase contractile function, and limit myocardial injury following coronary occlusion. But it is important to develop modalities that are safe as well as effective within the early and late phases of the preconditioning response, the so-called golden window of preconditioning (Williams and Benjamin, 2000).

We propose using magnetic fields for preconditioning by inducing stress proteins prior to surgery through exposure to magnetic fields (Carmody et al., 2000). Magnetic fields offer the advantages of being effective at extremely low energy levels (Table 1), and can be applied under conditions that are convenient for both physician and patient. EM fields are easily applied and noninvasive; they penetrate all cells and have considerably longer-lasting effects than hyperthermia. An 80% increase in hsp70 levels is induced within 20 min, and remain elevated for up to 3 h. Unlike hyperthermia, protein levels can be augmented by reapplication of EM fields during extended surgical procedures. Because of the ubiquity of stress proteins and ease of induction by magnetic fields, the potential for their use in other medical applications is considerable.

**Use of EM field response elements for controlling delivery of genetic information**

The use of EMREs in gene therapy offers a simple, noninvasive, and precise technique for gene activation through exposure to EM fields. Insertion of EMREs into the promoter of a gene that is otherwise unresponsive to EM fields renders that gene EM field responsive, and gene expression is induced (Lin et al., 2001). The applied EM field can be directed to the region where the gene product is needed and, since the EM field intensities needed to affect EMREs are well below the human perception threshold, their introduction and presence would not be felt by the patient. An example of such application would be the introduction of an exogenous insulin gene containing one or more EMREs placed upstream of the gene. Control would be provided by the simple and safe regulation of EM field exposure. The procedure would be automated by having the EM field generating circuit activated by an implanted glucose sensor responsive to set blood glucose levels. This noninvasive medical application could be beneficial in regulating and programming activation of any gene.

**STRESS PROTEINS AS MARKERS FOR BIOLOGICAL RESPONSES TO ENVIRONMENTAL EM FIELDS**

Research on biological interactions of EM fields has largely been driven by the health risk issue, which has been a source of continuing controversy. Our finding that weak EM fields can stimulate the synthesis of stress proteins indicates that cells view EM fields as potentially harmful, rather than benign. In that sense, cellular studies have provided important evidence to complement the epidemiological studies.

The results of those cellular studies have also pointed to a molecular mechanism, thereby, neutralizing a frequent argument in this controversy. (In discussions of the safety issue, the 'absence of a plausible mechanism' is often cited as an indication that EM field exposures are probably benign and ineffective, and therefore, safe). While understanding and defining mechanism is desirable, it is not necessarily essential in discussing human safety. A case in point are the results of recent epidemiological studies on childhood leukemia risks, where exposures to low-frequency EM fields exceed 3–4 mG (Ahlbom et al., 2000; Greenland et al., 2000), which have been provided with mechanistic bases by laboratory research.

The stress response activated by low-frequency EM fields is also relevant to the issue of cell phone safety. The EM field frequencies associated with cell phones are higher than the low frequency fields discussed above, but living cells demodulate low frequency components from the complex cell phone signals (Mullins et al., 1999), and induction of the cellular stress response could be the initial biological effect on exposure to cell phone signals. [Stress proteins have been reported in C. elegans on exposure to cell phone signals dePomerai et al., 2000.]

The current safety standard applied to cell phone use is based on specific absorption rates (SARs), i.e., the
heating of tissue. However, long before changes in temperature are detected, there are significant changes in hsp70 levels. Since the stress response proteins, as noted, are induced by magnetic fields at 14 orders of magnitude lower energy densities than by heat, the effects of EM field-induced stress vs. thermal stress is significant, and is underscored by a number of additional clear biological differences (Goodman and Blank, 1998). The EM field-induced stress response offers a more reliable and realistic biological criterion for establishing cell phone safety standards than tissue heating.

CONCLUSIONS

Our view of biological mechanism(s) of EM field interaction, summarized in this report, is derived from a wide variety of experimental observations. Although these ideas are based largely on our own investigations, they also include analyses of data from other systems, particularly, the recent reports on electron flow in DNA (Dandliker et al., 1997; Wan et al., 1999). In our view, the same mechanism that accounts for effects on a simple electron transfer reaction would account for the complex interaction with electrons in DNA leading to gene expression. It is our hope that these insights into EM interaction mechanisms will encourage and stimulate other investigators to approach questions of mechanism outside the confines of the prevailing paradigms.

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LITERATURE CITED


