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PROSPECTS

Effects of Electromagnetic Field Exposure on Gene Transcription

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Abstract Exposure of whole animals, isolated tissues, and cells to electromagnetic fields of various characteristics has resulted in a substantial literature detailing a wide range of effects at the morphological, physiological, biochemical, and molecular levels. In recent years, considerable effort has been devoted to defining a mechanism by which electromagnetic fields can couple to biological systems and generate this plethora of effects. As a consequence, there has been a growing interest in electromagnetic field–induced alterations in gene expression. Key studies are discussed which indicate that exposure of several cell types to electromagnetic fields that differ in waveform, amplitude, and frequency induced general changes in gene transcription. Moreover, exposure of T-lymphoblastoid cells to a 60 Hz sinusoidal magnetic field altered the transcription of genes encoding c-*fos*, c-*jun*, c-*myc*, and protein kinase C. Future studies in this area should focus on independent replication of key studies and identification of which events in the signal transduction pathways leading to gene transcription are altered by electromagnetic field exposure.

Key words: gene transcription, electromagnetic fields, magnetic fields, physical agents

Electric and magnetic fields, known collectively as electromagnetic fields (EMF), are ubiquitous physical agents that have been reported both as clinically efficacious tools and as a source of concern for human health and development. On the one hand, EMF exposure to asymmetric fields pulsed at various frequencies in the extremely low frequency (ELF) range (0–300 Hz) has been reported as beneficial in the treatment of recalcitrant fractures and pseudoarthroses [Bassett, 1990]. On the other hand, a large number of occupational and residential epidemiological studies have consistently described an association between long-term exposure to low-level ELF EMFs (sinusoidal EMFs at frequencies of 50 and 60 Hz) and an increased incidence of adult and childhood cancers [Pool, 1990]. However, what is lacking at the basic science end of these issues are robust cellular effects which have been replicated in different laboratories and a basic understanding of the mechanism by which EMFs couple to biological systems, influence and alter key biochemical processes, and ultimately generate a beneficial or deleterious outcome.

Research in the area of bioelectromagnetics has seen a necessary and encouraging turn in recent years. There has been a decrease in reports which simply catalog effects of EMF exposure and an increase in studies which are concerned with defining the underlying basis of observed effects. There is, for instance, an increased interest in the effects of EMF exposure on gene transcription, and I believe this has occurred for three reasons. First, there is a recognition that many EMF-induced alterations in cell function (e.g., long-term modifications of tumor cell properties [Phillips et al., 1986] cannot have developed without alterations in gene transcription. Second, knowledge of those genes whose transcription is altered by EMF exposure will provide a means of assessing both short- and long-term consequences of such exposure. Third, there is the opportunity, using a specific change in gene transcription as the end point, to move "upstream" one step at a time, defining EMF-induced effects at each step leading to gene transcription. Such an approach could lead ultimately to the identification of some essential initial event in the interaction of an EMF with a biological system and help define the elusive entity called *mechanism*. Consequently, it is the

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purpose of this Prospect to provide first a critical assessment of key studies dealing with EMF-induced effects on gene transcription, and then to look to what the future in this area might hold.

EMF EXPOSURE AND GENE TRANSCRIPTION

In 1983, Goodman et al. reported that two different EM signals, a 72 Hz single pulse and a 15 Hz pulse train, each increased gene transcription in salivary gland cells of the dipteran, *Sciara coprophila,* compared to unexposed control cells, as assessed by transcription autoradiography and cytological nick translation. These techniques allow a qualitative assessment of gene transcription based on the incorporation of ^{3}H-uridine into nascent RNA chains attached to chromosome regions. This group also separated pulse-labeled total cellular RNA by size class using sucrose density gradient centrifugation. Their results indicated increased precursor incorporation into RNA of a size class consistent with that expected for mRNA (6–10S). Interestingly, no label was detected in fractions corresponding to 18S and 28S rRNA species.

In a later report by Phillips and McChesney [1991], exposure of CCRF-CEM T-lymphoblastoid cells to the same 72 Hz single pulse signal employed by Goodman et al. [1983] was found to increase incorporation of ^{3}H-uridine into both total cellular RNA and mRNA. In this study, precursor incorporation into total cellular RNA was increased nearly twofold after 30 min exposure as compared to levels in unexposed control cells, achieving its greatest stimulation of 3.2 times greater than control after 2 h EMF exposure. Over the next 22 h exposure, ^{3}H-uridine incorporation remained at levels 2–2.5 times control values. On the other hand, ^{3}H-uridine incorporation into mRNA isolated from EMF-exposed cells showed no increase compared to controls after 30 min exposure, peaked after 2 h exposure (2.9 times control values), and thereafter declined to near control levels over the next 14 h exposure. Importantly, EMF exposure had no effect on cellular uptake of ^{3}H-uridine at any time of exposure. These data are consistent with an EMF effect on increased mRNA and rRNA production.

The studies by Goodman et al. [1983] and Phillips and McChesney [1991], while pointing to general changes in gene transcription in EMF-exposed cells, do not address the more important issue of which specific genes were affected. Additionally, other key questions become apparent. Which signal characteristics (e.g., frequency, waveform, amplitude) are important determinants to cell responsiveness? Do different signals affect the same or different genes? Do different cell types respond similarly to a given signal? Are there genes whose transcription is decreased by EMF exposure? By what mechanism does EMF exposure alter gene transcription?

In a series of papers appearing in the bioelectromagnetics literature in 1986 and 1987, Goodman and colleagues described results from exposure of salivary gland cells from *Sciara coprophila* to a large variety of EM signals in an attempt to identify specific signal characteristics responsible for EMF-induced changes in general gene transcription [Goodman and Henderson, 1986a,b, 1987; Goodman et al., 1987]. In all studies, transcription autoradiography was used to assess transcriptional activity, and in 3 of 4 of the studies, sucrose density gradient centrifugation was also employed to fractionate RNA by size class. Despite the use of 15 different symmetric and asymmetric EM signals that varied not only in waveform, but also in frequency, amplitude, and other characteristics, no clear picture emerged that would allow one to assign special significance to any parameter. These investigators do conclude, however, based on transcription autoradiography results, that EMF exposure increases transcription at chromosomal loci that are already active at the time of experimentation. Additionally, ^{3}H-uridine incorporation was increased in RNA species of 6–10S and 20–25S. The data from these studies must be viewed with caution for several reasons. First, there is inconsistent treatment of control cell cultures. In one of the studies [Goodman and Henderson, 1986a], all control cells were shielded in a mu-metal box, while in the other three studies, control cells were sham-exposed without mention of placement in a mu-metal container. This is no trivial matter, since mu-metal would shield the cells against the local static magnetic field as well as stray fields from the exposure apparatus. In this vein, Blackman [Blackman et al., 1990] has discussed the necessity to consider interactions between an applied oscillating EMF and the local static field. Therefore, it is significant that Goodman and colleagues report no data to confirm that the transcriptional response of dipteran salivary gland cells is the same in mu-metal–shielded and unshielded cells. Additionally, in no study is ^{3}H-

uridine associated with RNA of a size class expected for rRNA. Finally, although many experiments are reported to have been done, only data from single experiments is presented, making it impossible to judge inter-experimental variation and truly evaluate any real differences in the response to the various EM signals.

Several research groups have measured changes in the levels of specific mRNAs in EMF-exposed and control cell populations. This has been done both to define the importance of EM signal characteristics to cellular response and to provide understanding of previously observed EMF-induced alterations in biological function. For instance, Cadossi et al. [1989] exposed mice to a pulsed EMF after total body X-ray irradiation. They reasoned that, since pulsed EMFs have been used for many years to promote healing of nonunions in bone [Bassett, 1990] and since exposure of human lymphocytes to pulsed EMFs increased responsiveness to mitogen stimulation [Cantini et al., 1986], perhaps pulsed EMF exposure could have clinical utility for modulating bone marrow cell proliferation. Using Northern blot analysis, they found increased histone H3 and nuclear protein p53 mRNA levels in spleens of animals exposed to both X-ray irradiation and pulsed EMF exposure. They interpret their results as indicating that pulsed EMF exposure increased the entry of spleen cells into the cell cycle prior to the repair of X-ray–induced damage. Therefore, while pulsed EMF exposure may have utility for increasing cell proliferation, it may also potentiate the damaging effects of other agents.

Fitzsimmons et al. [1986] found increased mitogen release to the medium in cultures of chick calverial cells exposed to a low-amplitude (10^{-7} V/cm) low frequency (10–16 Hz) capacatively coupled electric field. In order to study the mechanism by which an induced electric field may increase bone cell proliferation, these investigators exposed human osteosarcoma TE-85 cells to a similar electric field [Fitzsimmons et al., 1992]. They found that electric field stimulation was associated with increased accumulation of mRNA for the growth factor, insulin-like growth factor II (IGF-II). They acknowledge that accumulation of a transcript can occur from either transcriptional or posttranscriptional-mediated processes, and are presently determining the mechanism underlying their observed effect.

Goodman and colleagues have also reported transcript level measurements, assessed by dot-blot analysis, in HL-60 human myelogenous leukemia cells exposed to a variety of EM signals. They found that transcripts with homology to β-actin, histone H2B, and v-*myc* increased (maximal increase, 2–3 times control levels) in response to a 20 min exposure to 4 different EM signals, as follows: 60 Hz sine waves ≥ 72 Hz sine wave ≥ 72 Hz single pulse = 15 Hz pulse train > 1.5 Hz pulse train > control levels [Goodman et al., 1989]. In a subsequent paper, they extended their studies to, among other parameters, the effect of signal amplitude on putative transcript level, this time reporting increases in transcripts with homology to v-*myc*, β-actin, histone H2B, β-tubulin, and v-*src* after exposures of 10, 20, and 40 min [Goodman and Shirley-Henderson, 1991]. Specifically, they report that (1) for cells exposed to a 60 Hz sine wave magnetic field at 570 mgauss amplitude, increased transcript levels occurred as early as 3–4 min; (2) the patterns for each transcript measured were identical; (3) the level of each transcript peaked at 4 times control values when cells were exposed to an EM signal at 57 mgauss for 20 min. The significance of these data, if any, is difficult to evaluate. The five genes of interest to this group have not been shown to respond to any stimulus with the same time course. For example, treatment of HL-60 cells with granulocyte-macrophage colony stimulating factor (GM-CSF) has been reported to produce a twofold increase in c-*myc* mRNA, which remained elevated for at least 5 h with continuous stimulation; no change in actin mRNA was detected [Schwartz et al., 1991]. Curran et al. [1985] treated HL-60 cells with the phorbol ester, TPA, and detected no alteration in the level of c-*myc* mRNA until 8 h after treatment, when expression fell by approximately twentyfold. Additionally, when HL-60 cells are induced to differentiate, c-*src* family mRNAs achieve maximal levels at 3–4 days, at which time differentiation is complete [Notario et al., 1989]. Histone H2B expression is tightly coupled to DNA synthesis [Shakoori et al., 1992]. Goodman and colleagues argue that their data imply a general response of the cell to the applied EM signal, or that a subset of genes involved in growth and differentiation are influenced by EMF exposure. These arguments, however, are inconsistent with the vast literature detailing cell responsiveness in general and HL-60 cell responsiveness specifically.

Additionally, there are two other complications when evaluating these studies. First, in one study control cells were "physically isolated" from extraneous EMFs [Goodman et al., 1989], while in a second study [Wei et al., 1990] both exposed and control cells were placed in mu-metal containers. In a third study, it appears that only exposed cells were placed in a mu-metal container [Goodman and Shirley-Henderson, 1991]. The problems associated with this have already been discussed. Second, Goodman et al. obtain the results presented above only if cellular RNA is isolated by one method, and that method is never described adequately in their reports other than to state that it involves phenol extraction [Goodman and Henderson, 1987]. Two independent groups have been unable to replicate these data [Krause et al., 1991; Czerska et al., 1991].

Phillips et al. [1992a] have addressed the question of EMF effects on specific gene transcription using the nuclear run-on assay. This technique allows mRNA strands that are already initiated in vivo to be completed in vitro, thus providing an accurate assessment of gene transcription at the time of cell lysis. This group reported that exposure of CEM-CM3 T-lymphoblastoid cells, cultured at cell densities of 5×10^5 and 1×10^6 cells/ml, to a 1 gauss sinusoidal magnetic field at 60 Hz for times of 15–120 min altered the transcription of the genes encoding c-*fos*, c-*jun*, c-*myc*, and protein kinase C (β-form). Specifically, c-*fos* transcription exhibited a mean maximal 2.5-fold increase in transcription after 30 min exposure, and this effect was independent of cell density. Transcription of the c-*fos* gene returned to control levels by 60 min exposure and remained at that level for the remainder of the experiment. On the other hand, c-*jun* transcription decreased by 70% after 30 min exposure in cells cultured at 5×10^5 cells/ml. Cells cultured at the higher density of 1×10^6 cell/ml, however, exhibited increased transcription of c-*jun*, achieving a mean maximal 2.2-fold increase after 60 min exposure. Transcription of c-*myc* also increased at both cell densities, reaching a plateau at about 2 times control levels. In subsequent experiments [J.L. Phillips, unpublished data], we have found that c-*myc* transcription in exposed cells remained at 2–3 times control levels for at least 8 h, but less than 24 h. Transcription of protein kinase C (β-form) gene was altered at both cell densities, although the data is not as easy to evaluate quantitatively. In several experiments at the higher cell density, protein kinase C transcription was detected in exposed cultures while no transcription was detected in control cultures. Nonetheless, magnetic field exposure increased transcription of the protein kinase C gene after 15–30 min, and this is then followed by a decrease in transcription to levels below those observed in control cells. Transcript levels were also measured for these 4 genes, and the results paralleled those from the run-on experiments. Interestingly, transcription of 4 other genes (metallothionein, transferrin, insulin receptor, and ornithine decarboxylase) was unaffected.

FUTURE PROSPECTS

Before discussing where research in this area might proceed, it is essential to understand where we are presently. As detailed above, there is good evidence that EMF exposure under certain conditions can alter the transcription of specific genes. However, independent replication of key experiments is crucial if this work is to retain credibility. Furthermore, attention must be paid to the actual conduct of experiments to ensure that results are not generated artifactually (e.g., because of differential treatment of experimental and control systems). In the case of our own studies [Phillips et al., 1992a], it is encouraging that the time dependence of the observed EMF-induced changes in gene transcription is consistent with other reports in the literature for the same genes induced by other agents. Additionally, the EMF-induced increase in c-*fos* transcription is particularly exciting, since agents and pathways reported to alter c-*fos* transcription [Fisch et al., 1987; Gilman, 1988] have also been shown to be sensitive to EMF exposure. For example, EMF exposure of a variety of biological systems has been shown to alter calcium metabolism (e.g., increasing cellular uptake or increasing intracellular concentration of Ca^{2+} [Blackman et al., 1988]. Changes in cyclic AMP levels [Jones, 1984; Farndale and Murray, 1986], in cyclic AMP-dependent and -independent protein kinases [Byus et al., 1984], and in protein kinase C activity [J.L. Phillips, unpublished data] have also been reported to be altered as a result of EMF exposure. Consequently, it is significant that the *fos* promoter contains elements responsive to cyclic AMP, calcium, and protein kinase C–dependent and –independent pathways. It is logical, therefore, to use the EMF-induced in-

crease in c-*fos* transcription as the end-point from which to move "upstream," as mentioned earlier, and identify which response elements appear to be necessary for this transcriptional alteration. In a related study, we have reported recently [Phillips et al., 1992b] that EMF exposure of T-lymphoblastoid cells to a 1 gauss sinusoidal magnetic field at 60 Hz resulted in a 70% decrease in AP-1 binding activity after 30 min exposure. This finding is consistent with both increased c-*fos* transcription and decreased c-*jun* transcription. Additional studies are in progress to characterize EMF-induced changes, if any, in the DNA-binding activity of other c-*fos*–related transcription factors. Also of interest is the effect of EMF exposure on phosphorylation of these transcription factors. Ultimately, what is required is a detailed understanding of how EMF exposure affects signal tranductive pathways that lead to gene transcription, and it is in this area that efforts should be concentrated.

At this point, it is fair to ask whether physical agents, such as EMFs, should be expected to induce biological responses by influencing the same biochemical pathways as chemical agents, and I believe the answer is probably yes. There are, however, major differences between EMFs and chemical agents. The issue of "dose" for chemical agents is a simple concept, ultimately relating time to the number of active molecules. The notion that chemicals interact with discrete, identifiable receptors is also accepted convention. On the other hand, it is not yet known to what "dose" relates for EMF exposure, and considerable effort must be put into identifying what constitutes the exposure metric. Additionally, there would not appear to be an "EMF receptor," at least not in the usual sense. It may be that EMF exposure alters intracellular calcium levels, alters the levels of intracellular free radicals, or in some other way initiates a cascade of events involving signal transduction and cellular response similar to that observed with chemical agents. In this regard, DeGroot et al. [1991] recently detailed the influence of another physical agent, gravity, on c-*fos* expression in epidermal growth factor (EGF)–activated A431 epidermoid carcinoma cells. They found that EGF-induced c-*fos* expression was decreased under simulated hypogravity conditions and increased by hypergravity. Although the mechanism underlying this effect is unknown, these investigators concluded that gravity may alter some step in mitogen-induced signal transduction pathways.

Identifying the mechanism by which EMF exposure alters key cellular processes, such as gene transcription, represents one of the great challenges in biological science today. Utilizing the vast literature base and valuable techniques now available, we have the opportunity to revolutionize our view of how biological systems interact with their physical environment.

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