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Environmental Magnetic Fields Inhibit the Antiproliferative Action of Tamoxifen and Melatonin in a Human Breast Cancer Cell Line

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We have previously reported that environmental-level magnetic fields (1.2 μT [12 milligauss], 60 Hz) block the growth inhibition of the hormone melatonin (10^{-9} M) on MCF-7 human breast cancer cells in vitro. We now report that the same 1.2 μT, 60 Hz magnetic fields significantly block the growth inhibitory action of pharmacological levels of tamoxifen (10^{-7} M). In biophysical studies we have taken advantage of Faraday's Law of Current Induction and tested whether the 1.2 μT magnetic field or the associated induced electric field is responsible for this field effect on melatonin and tamoxifen. We observe that the magnetic field component is associated with the field blocking effect on melatonin and tamoxifen function. To our knowledge the tamoxifen studies represent the first experimental evidence for an environmental-level magnetic field modification of drug interaction with human breast cancer cells. Together, these findings provide support to the theory that environmental-level magnetic fields can act to modify the action of a drug or hormone on regulation of cell proliferation. Melatonin and tamoxifen may act through different biological pathways to down-regulate cell growth, and further studies are required to identify a specific biological site of interaction for the 1.2 μT magnetic field. *Bioelectromagnetics 18:555–562, 1997.* © 1997 Wiley-Liss, Inc.[†]

Key words: MCF-7; tamoxifen; melatonin; magnetic fields; cell proliferation; human breast cancer cells

INTRODUCTION

One biological effect of low-frequency, time-varying electric and magnetic fields that has been reported by several investigators is the depression of secretion of the hormone melatonin from the pineal gland into the blood stream. This effect, first reported by B. Wilson in rats [Wilson et al., 1981, 1983, 1986], has since been observed in cultured pinealocytes [Welker et al., 1983] and hamsters [Yellon, 1994] and has been reported in abstract form in some human volunteers exposed to 200 milligauss (mG), 60 Hz magnetic fields at night [Graham et al., 1993, 1994]. These observations, in conjunction with the finding that melatonin can provide protection against breast cancer in animal models [Subramanian and Kothari, 1991] has led Dr. Richard Stevens to propose that magnetic fields may increase risk of breast cancer [Stevens et al., 1992].

Melatonin is known to have a spectrum of biological functions including immune function enhancement and oncostatic properties [Yu and Reiter, 1993; Brzezinski, 1997]. Of particular interest is melatonin's inhibition of dimethylbenz(a)anthracene (DMBA)-induced rat mammary gland carcinogenesis [Subramanian and Kothari, 1991]. Consistent with an in vivo model of magnetic field interaction involving suppression of melatonin secretion from the pineal gland, recent animal studies by Drs. W. Loscher and M. Mevissen have reported that magnetic fields can enhance DMBA-induced breast cancer cell growth in rats in a dose-dependent manner [Loscher et al., 1993; Mevissen et al., 1993]. In in vitro studies Dr. D. Blask has demonstrated that melatonin at physiological levels inhibits MCF-7

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human breast cancer cell growth [Hill and Blask, 1988; Cos and Blask, 1990; Cos et al., 1991], further supporting the oncostatic properties of melatonin. Using MCF-7 cells obtained from Dr. Blask, we have confirmed Blask's original observation that melatonin inhibits MCF-7 cell growth, and we have reported experimental evidence for a magnetic field interaction with MCF-7 cells: Continuous exposure to environmental-level 1.2 μT, 60 Hz magnetic fields block melatonin's growth-inhibitory action on MCF-7 cells, while having no significant effect on untreated cells [Liburdy et al., 1993c,d]. Three laboratories have independently reported results in abstract form that are consistent with this magnetic field effect on melatonin [Blask et al., 1993a,b; Blackman et al., 1996; Luben et al., 1996].

To investigate a possible biological mechanism for such a magnetic field effect, we have used the antiestrogen tamoxifen to ask whether such fields decrease its growth inhibitory action. Tamoxifen, the most widely used antiestrogen therapy for the control of breast cancer, induces an alternate conformational change in the estrogen receptor (ER) upon binding [Martin et al., 1988], which allows the receptor complex to bind to its DNA-binding regions but not transcribe its ER response genes. In biophysical studies, we have addressed the question of whether the magnetic field itself or the induced electric field associated with the magnetic field exposure, is critical for the field effect involving melatonin or tamoxifen. To carry out these studies and test for a magnetic or electric field dependence, we have used a cell culture exposure system utilizing a mu-metal shielding chamber that generates a uniform magnetic field [Liburdy, 1994, 1995] and we have oriented (rotated by 90°) the magnetic field vector so that the induced electric field is significantly reduced according to Faraday's Law of Current Induction.

MATERIALS AND METHODS

Cell Culture Techniques

MCF-7 cells [Soule et al., 1973] at passage 18 were a generous gift of Dr. David Blask of the Mary Imogene Bassett Hospital Research Institute, Cooperstown, NY. Cells were maintained in a monolayer and passed as described [Liburdy et al., 1993c]; fetal bovine serum (product 101, lot 10786) was obtained from Tissue Culture Biologicals, Tulare, CA. For tamoxifen sensitivity assays, MCF-7 cells (passages 25–37) were harvested in 0.2% EDTA phosphate buffer (2 g/l Na_2-EDTA, 8 g/l NaCl, 0.2 g/l KH_2PO_6, 1.15 g/l Na_2HPO_6), dispersed by passing three times through a 25-gauge needle, and seeded at 0.1×10^5 cells/35 mm dish in 1.5 ml of media. After cell attachment, media was changed, with or without chemical treatment. Tamoxifen (Sigma Product T9262, Sigma Chemical Co., St. Louis, MO) and melatonin (n-acetyl-5-methoxytryptamine; Sigma Product M5250) solutions were prepared in minimum ethanol, followed by serial dilution in media (final ethanol concentrations are approximately 0.001% and 0.00001% for tamoxifen and melatonin, respectively). On counting days, triplicate plates were harvested with trypsin solution at 37 °C (0.50 g/l trypsin, 0.5 g/l EDTA, 1.0 g/l glucose, and 0.58 g/l $NaHCO_3$) and counted by hemocytometer.

Magnetic Field Exposure System

Cells were exposed continuously during growth curves using the cell culture exposure system shown in Figure 1 [Liburdy, 1994, 1995]. We used several such exposure systems so that simultaneous experiments could be conducted on the same cells but at different field strengths. Special features are (1) a perforated Plexiglas platform table, (2) a four-coil Merritt exposure system (plastic frame wound with double-wrap, bifilar cable, turn ratio of 26/11/11/26) [Merritt et al., 1983; Kirschvink, 1992a], (3) a ventilated mu-metal chamber (Co-Nectic AA shielding (1 mm), Magnetic Shield Corporation, Perfection Mica Co., Bensenville, IL) to eliminate extraneous magnetic fields, (4) a water-jacketed incubator (Queue Systems, Inc., Parkersburg, WV, Model 2710), maintained at 37 ± 0.5 °C, and (5) the ability to rotate the Merritt coil 90° so that the magnetic field vector is rotated from a standard vertical orientation to a horizontal orientation. This rotation significantly reduces the induced electric field without altering the magnetic field flux density experienced by the cells.

Field dosimetry was performed as described [Liburdy et al., 1993c; Liburdy, 1995]. Our protocol requires that field readings are taken before and after experiments; values were within approximately 5%. Static (DC) magnetic fields were reduced to approximately 0.1 μT by the mu-metal chambers. Temperature inside the mu-metal chambers was monitored with thermistor probes (YSI, Inc., Yellow Springs, OH) placed adjacent to cell culture plates. Measurement of CO_2 levels inside of the mu-metal chambers where cells are cultured have been performed using (a) a remote infrared sensing probe and (b) a remote thermocouple sensing probe, and both indicate that CO_2 levels inside the chambers are maintained at 5% CO_2 (Incubator Services, Barnesville, OH).

Statistical Analyses

Data were tested for statistical significance using the SigmaPlot Student's *t* test (Jandel Corporation, Corte Madera, CA). All error bars in the figures represent the standard error of the mean.

Fig. 1. Cell culture exposure system showing the combination of the 4-square Merritt coil and the mu-metal chamber, which are both placed inside of a commercial cell culture incubator. Also shown is a thermistor temperature probe which is threaded through one ventilation hole into the mu-metal chamber and placed inside the chamber at the position where cell culture plates are typically located. The 4-square Merritt coil can be rotated 90° to reorient the magnetic field vector from the standard vertical position to horizontal.

RESULTS

Inhibition of Tamoxifen Action by a 1.2 μT, 60Hz Magnetic Field

Figure 2 presents experimental data showing the effect of 60 Hz, 0.2 or 1.2 μT magnetic fields on tamoxifen's growth inhibition of MCF-7 cells over a range of doses (from 10^{-6} to 10^{-8} M). This range includes tamoxifen's pharmacological dose of 150 ng/ml, corresponding to 6×10^{-7} M [Swain and Lippman, 1990]. Cell growth on day 7 is shown normalized to 100% for untreated MCF-7 cells. At 0.2 μT, tamoxifen inhibits its cell growth in a dose-dependent manner: exhibiting 68% inhibition at 10^{-6} M tamoxifen, decreasing to 40% and 1% at 10^{-7} and 10^{-8} M, respectively. These data agree well with previous reports of tamoxifen's in vitro growth inhibitory activity on MCF-7 cells [Lippman et al., 1976]. In a 1.2 μT magnetic field, the growth inhibitory action of 10^{-7} M tamoxifen is reduced significantly, from 40 to 17% ($P < .0001$). Of these 12 experiments, one experiment involved a protocol in which the experimenter was blind as to the chemical treatment of cells with similar results obtained. Regarding the reproducibility of this effect in our hands, in 11 of the 12 experiments in Figure 2 in which we have seen tamoxifen (10^{-7} M) inhibit MCF-7 cell growth, we have observed a significant ($P < .05$) blocking effect by the 1.2 μT magnetic field. Interestingly, the 1.2 μT field was observed to have no significant effect on 10^{-6} M tamoxifen (68 vs. 66%). There may exist a tamoxifen dose-threshold response that depends on the level of toxicity displayed by tamoxifen on MCF-7 cells at higher doses.

Because it could be argued that the results presented in Figure 2 might represent a field effect that is observed only on day 7 of cell growth, we conducted experiments in which cell growth was followed for 9 days. These data are shown in Figure 3 (a) (0.2 μT data) and 3 (b) (1.2 μT data). The 1.2 μT blocking effect was seen on both days of tamoxifen sensitivity during MCF-7 exponential growth [compare Figure 3 (a) and (b)].

The Magnetic Field Is Associated with the Field Blocking Effect

We next asked whether the magnetic field itself or the induced electric field component is responsible

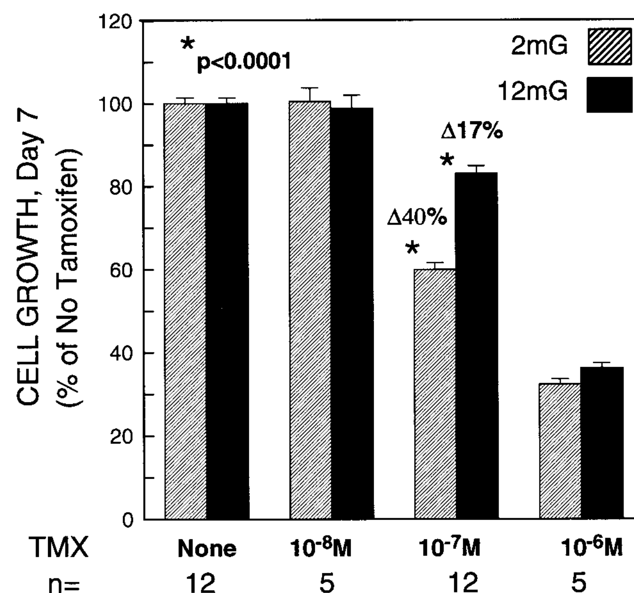

Fig. 2. Effect of a 1.2 μT (12 mG) vs. a 0.2 μT (2 mG) magnetic field on inhibition of MCF-7 cell growth on day 7 by 10^{-8}M to 10^{-6}M tamoxifen (TMX). In all experiments, cells were grown within mu-metal shields. Results are the means of 5 or 12 experiments.

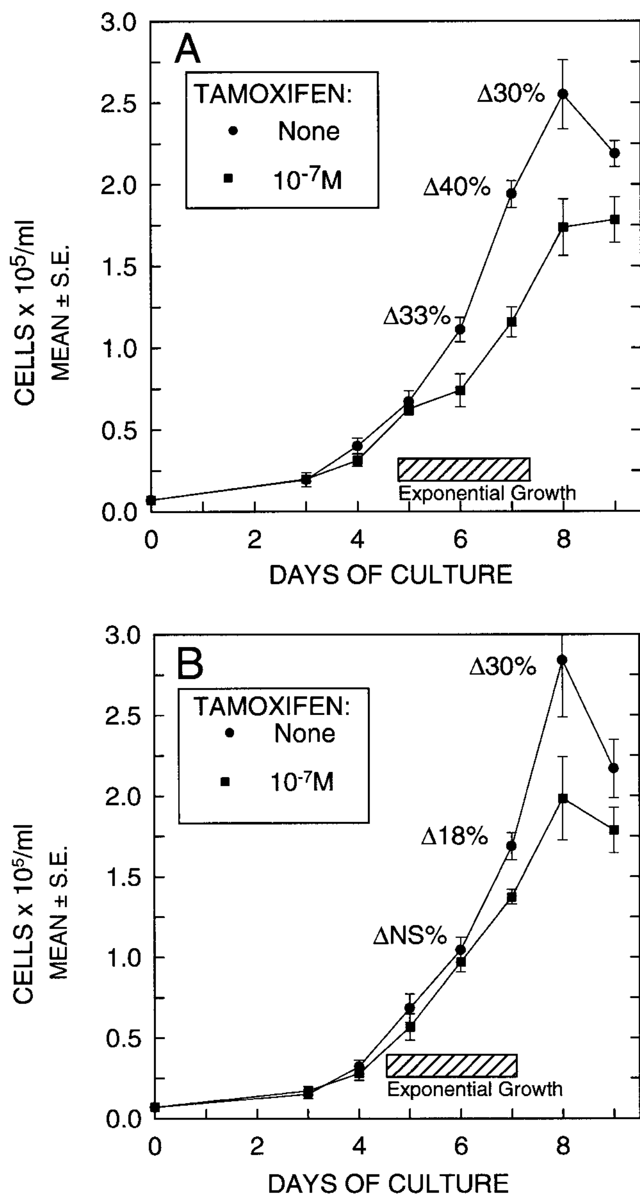

Fig. 3. Growth curve of MCF-7 cells in the presence or absence of 10^{-7}M tamoxifen. Exponential growth occurs on days 5, 6, and 7. (**A**) Growth in a 0.2 μT (2 mG) magnetic field. Tamoxifen yields 33% and 40% inhibition, respectively, on days 6 and 7. (**B**) Growth in a 1.2 μT (12 mG) magnetic field. Tamoxifen exhibits 0% and 18% inhibition on days 6 and 7.

for the blocking effect of tamoxifen reported here, as well as the blocking effect we have previously reported for melatonin [Liburdy et al., 1993c,d]. According to Faraday's Law of Induction, a time varying magnetic field will induce an electric field in an object proportional to the radius of the cross-sectional area of the conducting medium perpendicular to the incident magnetic field [Bassen et al., 1992; Liburdy, 1992a]. Therefore, to differentiate electric field (E field) and magnetic field (B field) effects on cell growth, we simultaneously exposed MCF-7 cells in three matched incubators to either a 0.2 μT magnetic field, a 1.2 μT magnetic field, or a second 1.2 μT magnetic field, rotated 90° (with the field direction parallel to the plate surface). This exposure situation along with magnetic field and induced electric field exposure values are depicted in Figure 4. Rotating the 1.2 μT field 90° reduces the effective cross-section seen by the magnetic field and diminishes the induced E field nearly 5.6-fold (from an average induced E field component of 1.96 to 0.353 μV/m, while maintaining a constant 1.2 μT B field [M. Misakian, personal communication: Stuchly and Xi, 1994]. The electric field induced by the parallel magnetic field is essentially uniform over the entire dish surface (E_{rms} = ~0.353μV/m; rms, root mean square). In the perpendicular magnetic field; however, the induced electric field varies with the radius of the dish via Faraday's law; the average electric field corresponds to radius/2.

Figure 5 shows results for MCF-7 cell growth in the presence of tamoxifen (10^{-7} M) and a 0.2 μT magnetic field; all values are expressed as a percent of the untreated culture cell counts in the same field. MCF-7 cell growth was significantly inhibited on day 7 by an average of 40% across four experiments ($P < .0001$).

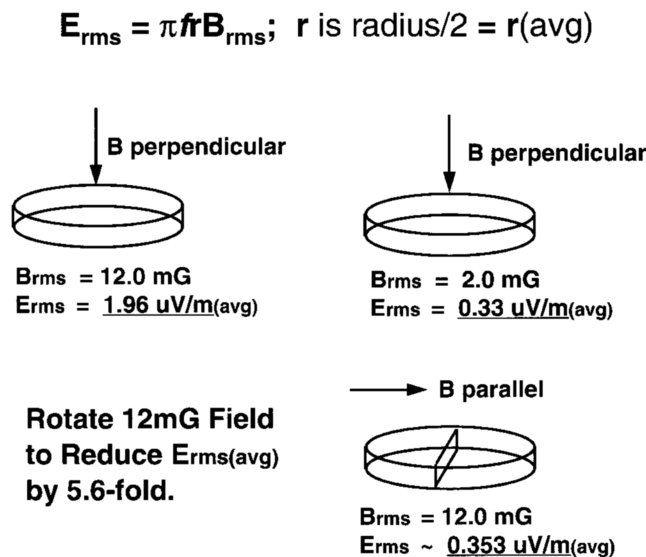

Fig. 4. Estimated values of average induced E (electric) fields in 0.2 μT (2 mG) (perpendicular), 1.2 μT (12 mG) (perpendicular), and 1.2 μT (12 mG) (parallel) magnetic field exposure systems, based on Faraday's Law of Induction. The magnetic B field exposure (B_{rms}) at two different orientations remains the same for the MCF-7 monolayer culture; however, the average induced E field (E_{avg}), which depends on the cross-sectional area of the culture media containing electrolytes seen by the B field, is reduced approximately 5.6-fold when the 1.2 μT field is rotated 90° from the B perpendicular to the B parallel orientation.

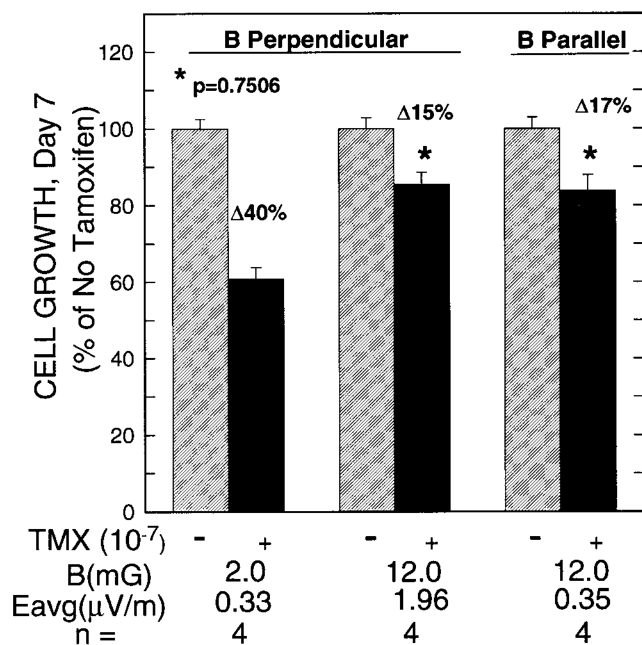

Fig. 5. Effect of 60 Hz magnetic field orientation on tamoxifen (TMX) cytostatic action in MCF-7 cells on day 7. The cells in the 0.2 μT (2 mG) field show an average of 40% inhibition; the 1.2 μT (12 mG) perpendicular and parallel cultures show 15% and 17% inhibition, respectively.

In a 1.2 μT magnetic field, MCF-7 cell growth was also significantly blocked ($P < 0.005$), but inhibition was reduced to 15%. Similar results were seen in the 1.2 μT magnetic field rotated 90°, with an average of 17% inhibition (no significant difference from 1.2 μT perpendicular field; $P > 0.75$). Thus, the 1.2 μT magnetic field component is associated with blocking tamoxifen inhibition of MCF-7 cell growth.

In analogous studies we tested whether the blocking effect of a 1.2 μT magnetic field on melatonin action [Liburdy et al., 1993c,d], was associated with the magnetic field or the induced electric field. Figure 6 presents the results of these studies. In the absence of melatonin, as we have reported previously, magnetic fields did not affect MCF-7 cell growth significantly. However, across three experiments, growth was significantly inhibited by 10^{-9} M melatonin, for an average of 33% inhibition on day 7 in a 0.2 μT magnetic field ($P < .0001$). When the 1.2 μT field was oriented in the standard position perpendicular to the plane of cells, melatonin's activity was blocked nearly completely ($P = 0.65$). When the 1.2 μT magnetic field was rotated 90° relative to the plane of the plate, melatonin's action was still blocked significantly ($P = 0.11$). These data suggest that the 1.2 μT magnetic field component is associated with blocking melatonin's cytostatic action.

DISCUSSION

In this study, we observe that environmental-level 1.2 μT, 60 Hz magnetic fields partially block tamoxifen's inhibitory action on growth of human mammary tumor (MCF-7) cells in vitro. This finding extends our original observation that a 1.2 μT, 60 Hz magnetic field blocks melatonin's inhibitory action on growth of MCF-7 cells. Unlike many electromagnetic field effects reported in the literature [Liburdy, 1995], we find the magnetic field itself, not the induced electric field, is associated with these field effects.

Induced E fields in the cell culture medium interact initially at the cell membrane, because they cannot penetrate beyond the cell membrane at power-line frequencies. The B field, however, penetrates the cell, increasing the possibilities for a biological site of interaction: signal-transduction molecules (including the estrogen receptor), the nuclear membrane, transcription or translation events necessary for cell growth and division. One simple interpretation of our data is that magnetic fields might inhibit tamoxifen or melatonin entry into the cell; although unlikely, this is a testable hypothesis. Alternatively, a magnetic field might influence one or more of tamoxifen's actions. Tamoxifen is a multiphasic drug and as an antiestrogen it binds

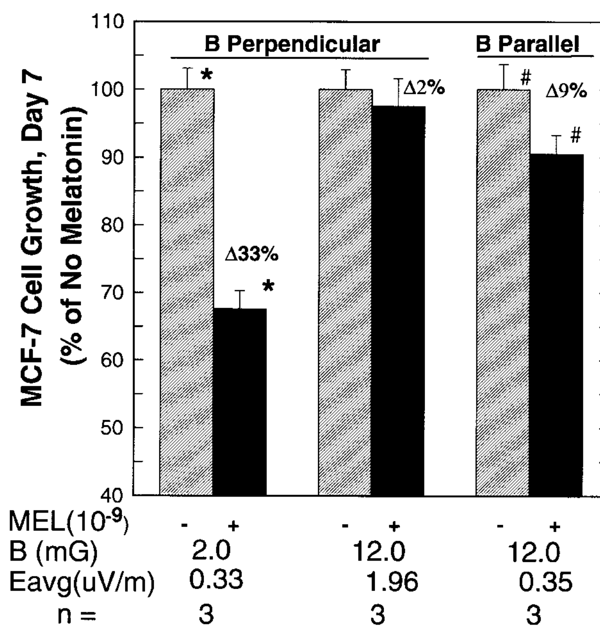

Fig. 6. Effect of the 60Hz magnetic field orientation on melatonin's cytostatic action in MCF-7 cells. Cells were counted after 7 days in culture with or without melatonin (MEL) treatment, in a 0.2 μT (2 mG) perpendicular field, a 1.2 μT (12 mG) perpendicular field, or a 1.2 μT (12 mG) parallel field. The cells in the 0.2 μT field show an average of 33% inhibition by 10^{-9}M melatonin; in the 1.2 μT perpendicular and 1.2 μT parallel fields, inhibition is reduced to 2 and 9%, respectively. *$P < .0001$; # $P = .11$.

to the ER. But it also has other biological effects such as interacting with calmodulin and protein kinase C to inhibit their functions [Taylor et al., 1984]. Such interactions might be influenced by a magnetic field leading to a blockage of tamoxifen's growth inhibitory action. Tamoxifen is also reported to have partial agonist activities [Fujimoto and Katzenellenbogen, 1994], such as a stimulation of uterine tissue growth in animals that might be influenced by magnetic fields.

It is also possible that the magnetic field acts nonspecifically relative to tamoxifen: calcium entry might be enhanced to trigger downstream signal transduction events that overcome tamoxifen's growth inhibitory effects. Calcium is a potentially interesting indicator for future studies because (a) some magnetic field exposures have been reported to elevate intracellular Ca^{+2} levels [Walleczek and Liburdy, 1990; Liburdy, 1992a,b,c, 1995; Liburdy et al., 1993a,b], and (b) intracellular Ca^{+2} concentration is believed to play a role in ER expression in MCF-7 [Ree et al., 1991].

In contrast to tamoxifen, the hormone melatonin has been shown to influence human physiological functions including the biological regulation of circadian cycles and sleep; and there is evidence that melatonin may also influence reproduction, tumor growth, and aging [Yu and Reiter, 1993; Brzezinski, 1997]. Studies investigating a mechanism of action have identified two membrane-bound melatonin binding sites: ML1 (high affinity, picomolar) and ML2 (low affinity, nanomolar) [Morgan et al., 1994; Dubocovich, 1995]. ML1 receptors belong to the family of guanosine triphosphate-binding proteins (G protein-coupled receptors) [Acuna-Costroviejo et al., 1994; Ebisawa et al., 1994], and activation of these receptors results in the inhibition of adenylate cyclase activity in target cells. It is believed that these receptors are involved in retinal function, circadian rhythms, and reproduction. ML2 melatonin receptors are coupled to the stimulation of membrane phosphoinositide hydrolysis and signal transduction and may play a role in regulating cell growth; their tissue distribution has not been determined. Melatonin is also reported to bind to target molecules inside the cell. Melatonin can bind calmodulin and may directly affect calcium signaling [Benitez-King and Anton-Tay, 1993]. Melatonin is also reported to bind a family of nuclear retinoid Z receptors, suggesting that melatonin may affect nuclear events during hormone signaling [Becker-Andre et al., 1994]. As yet it is not known whether melatonin receptors are present in MCF-7 cells, and if so, which type.

Investigators have suggested that an induced E field associated with micro Tesla magnetic field strengths may not lead to biological effects, since the induced field is less than the ''thermal noise limit'' within the cells (minimum response threshold of 10^{-6} V/m) [Weaver and Astumian, 1990]. We have shown in this study that inhibition of melatonin's and tamoxifen's action is associated with the magnetic field itself. Several different biophysical models have been hypothesized to describe how a magnetic field might interact with biological systems: the presence of a magnetic sensor(s) such as magnetite within the cell [Kirschvink et al., 1992, 1993; Kirschvink, 1992b; Polk, 1994], free radical magnetochemistry [Reiter et al., 1993; Harkins and Grissom, 1994; Scaiano et al., 1994; Frankel and Liburdy, 1995; Roy et al., 1995], stochastic resonance [Wiesenfeld and Moss, 1994], and biological electron transfer [see abstract, Nair and Liburdy, 1996]. At present a consensus among researchers has not been achieved regarding a specific biophysical interaction to explain ''environmental-level'' magnetic field bioeffects. However, independent evaluation of such bioeffects [Blask et al., 1993a,b; Blackman et al., 1996; Luben et al., 1996] represents one important step in building a solid biological database and in identifying a model system with which biophysical models can be tested.

Recently, experiments were conducted in our laboratory in collaboration with Dr. Stefan Engstrom to test whether the magnetic field inhibition of tamoxifen function, described here, is associated with a relatively fast or a relatively slow interaction timescale [see abstract, Harland et al., 1996]. This question is important in assessing whether the transduction step is an isolated biophysical process or if it is an integral part of a more complex biological structure involving relatively long natural timescales. The findings from these collaborative studies provide support for a relatively slow interaction timescale on the order of milliseconds. This timescale is consistent with a physical transductive step strongly coupled to a biological process, e.g., receptor binding and translocation. Such a timescale is also consistent with certain interaction mechanisms (e.g., parametric resonance [Blackman et al., 1995; Prato et al., 1995; Engstrom, 1996]) but does not support others (e.g., free radical recombination mechanisms).

In the future, studies are needed to identify a possible biologically based interaction site(s), and to assess critical field parameters (e.g., frequency, field intensity threshold, exposure duration dependence). One potentially promising approach to identify receptor involvement is the use of pure antiestrogens, e.g., ICI 182,780, that bind specifically to the estrogen receptor, to test for field effects on ER binding. Use of other specific biochemical agents that bind to calmodulin and PKC to block function may also prove useful. In addition, the use of secondary cell lines derived from MCF-7 parent cells and other human mammary tumor cell lines may provide important information about the biological site of interaction; for example,

there are human mammary epithelial cell types that do not express the estrogen receptor but are tamoxifen sensitive.

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