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MAP Kinase Activation in Cells Exposed to a 60 Hz Electromagnetic Field

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Abstract This research provides evidence that mitogen-activated protein kinase or extracellular signal-regulated kinase (MAPK/ERK) is activated in HL-60 human leukemia cells, MCF-7 human breast cancer cells, and rat fibroblast 3Y1 cells exposed to a 60 Hertz (Hz), 1 Gauss (G) electromagnetic field (EMF). The effects of EMF exposure were compared to those observed using 12-*O*-tetradecanoylphorbal-13-acetate (TPA) treatment. The level of MAPK activation in cells exposed to EMF was approximately equivalent to that in cells treated with 0.1–0.5 ng/ml of TPA. A role for protein kinase C (PKC) in the process leading to MAPK activation in EMF exposed cells is also suggested by the results. MAPK activation is negated by an inhibitor to PKCα, but not PKCδ inhibitors, in cells subjected to EMF exposure or TPA treatment. Thus, similarities between the effects of EMF exposure and TPA treatment are supported by this investigation. This provides a possible method for revealing other participants in EMF–cell interaction, since the TPA induction pathway is well documented. J. Cell. Biochem. 90: 1197–1206, 2003. © 2003 Wiley-Liss, Inc.

Key words: electromagnetic fields; signal transduction; TPA; PKC

The possibility of adverse health effects associated with exposure to extremely low-frequency (ELF) electromagnetic fields (EMF) has been a public concern since an epidemiological study first suggested a possible relationship between residential high-voltage power lines and childhood leukemia [Wertheimer and Leeper, 1979]. This was an unexpected correlation since, unlike ionizing radiation and other environmental carcinogens, ELF EM fields do not cause direct DNA damage. It is assumed that if EMF exposure plays a role in tumorigenesis, or induces any other malignant changes, it is accomplished either by promoting a process that is already initiated and/or by modifying cell regulatory processes.

Laboratory studies in the last two decades, however, show that other biological effects can be attributed to EMF exposure. The mechanism of the interaction between EMF and cellular systems is still unclear, but hypothetically changes can be initiated at the cell surface, affecting surface constituents such as membrane-receptor complexes and ion-transporting channels. The effect of EMF is hypothesized to be propagated and amplified by signal transduction pathways, ultimately leading to modification of cell behavior. Considering that many of the signal transducers are implicated in the multistage process of tumorigenesis, it is plausible that the effect of chronic EMF exposure may enhance a propensity toward malignancy in cells.

Previous studies in our lab showed that differentiation of HL-60 cells from a promyelocytic form (hemapoietic progenitor cells) to phagocytic macrophages can be induced by exposure to 60 Hertz (Hz), 1 Gauss (G) field, as

Abbreviations used: ELF, extremely low frequency; EMF, electromagnetic fields; G, Gauss; Hz, Hertz; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; MAPK, mitogen-activated protein kinase; PKC, protein kinase C.

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well as by treatment with tumor promoting phorbol ester 12-*O*-tetradecanoylphorbal-13-acetate (TPA) [Tao and Henderson, 1999]. This finding provided a launching point for the investigation of the signal procession involved in EMF elicited differentiation, since the transduction sequence in TPA-induced differentiation pathways has been studied in depth [Nishizuka, 1986; Kharbanda et al., 1994; Marquardt et al., 1994; El-Shemerly et al., 1997]. By comparing the two pathways, we hope to provide more information about the relationship between cell signaling and EMF exposure.

Signal transduction pathways were implicated in EMF induced bioeffects when researchers found that changes in calcium flux were elicited in response to EMF exposure [Adey et al., 1982]. As a ubiquitous response to many stimuli initiated at the cell membrane, Ca^{2+} flux leads to activation of several important signal transducers such as protein kinase C (PKC). Later studies suggested that EMF could use Ca^{2+} phospholipid-dependent PKC induction in a fashion similar to that of TPA or other mitogens [Walleczek, 1992; Goodman et al., 1993]. The Src proto-oncogene family protein-tyrosine kinase (PTK) LYN and its downstream substrate SYK are stimulated in B-lineage lymphoid cells by low-energy EMF leading to activation of phospholipase C-γ2 (PLC-γ2) and PKC [Uckun et al., 1995; Dibirdik et al., 1998]. These findings directed our interest to MAP kinase cascade, another important component of the signaling pathway induced by extracellular mitogens such as TPA, which acts downstream of PTK and PKC. Through this cascade, signals are integrated, amplified, and transduced into nuclear signals.

In the present study, we focus on the effect of EMF on the activation of MAPK in diverse cell lines (HL-60, MCF-7, and 3Y1). The MAP kinase cascade includes three serine/threonine kinases: Raf-1, MAP/ERK kinase (MEK), and a mitogen-activated protein kinase or extracellular signal-regulated kinase (MAPK/ERK). It plays a pivotal role in correlating growth factor receptor activation at the cell membrane resulting in transcriptional modification in the nucleus and ultimately in cell proliferation and differentiation. The phosphorylation cascade is set into motion when Raf-1 is activated through interaction with GTP-binding Ras (active form), as well as phosphorylation by PKC [Kolch et al., 1993]. Once activated, Raf-1 activates MEK via phosphorylation, which in turn activates MAPK by phosphorylation. The present studies show that MAPK activation is elevated in all the cell lines exposed to 60 Hz, 1 G EMF.

MATERIALS AND METHODS

Cell Lines

The HL-60 cell line was derived from a patient with acute promyelocytic leukemia. The subline we use was obtained from Dr. I. Weinstein, Columbia University Health Sciences, New York. The cells were maintained in RPMI 1640 (Gibco BRL Life Technology, Carlsbad, CA) with 10% fetal bovine serum (FBS, Sigma, St. Louis, MO), 1% antibiotic–antimycotic (Gibco). The medium was changed every other day to maintain rapid exponential growth of the cells. The viability of cells was determined by trypan blue dye exclusion; cell density was determined by hemocytometer.

The human breast cancer cell line MCF-7 was obtained from Dr. R. Goodman, Columbia University Health Sciences, New York. Rat fibroblast 3Y1 cells, 3Y1 cells overexpressing c-Src pro-oncogene, and 3Y1 cells overexpressing v-Src oncogene were obtained from Dr. D. Foster, Hunter College-CUNY, New York. These cell lines were maintained in Dulbecco's Modified Eagle Medium (DMEM), purchased from Gibco, containing 10% FBS (Sigma), and 1% antibiotic–antimycotic (Gibco).

Exposure Conditions

Cells were exposed to a 60 Hz, 1 G EM field in a Helmholtz Coil Exposure System designed by Electric Research and Management, Inc. (ERM, State College, PA). The exposure conditions were selected as those typical of other experiments that have detected the effects of EMF on signaling pathways. The ERM exposure system provided a graded series of field settings that were maximized by a function generator. A sine-wave generator (Wavetek 11-MHz function generator, model 21) with variable frequency control was used. The exposure coils consisted of two double-wound coils in an approximate Helmholtz configuration. The coils were supported by an acrylic frame in which the test samples were placed. The coils were placed in 2 μ metal cans, each inside a separated compartment of the same double-door incubator. The magnetic fields in this incubator have been measured over a period of 5 years at about 2 mG.

Flasks were placed on a plexiglass stand in the horizontal plane in an area shown to have a uniform magnetic field and maximum field strength inside the coil. Field characteristics were measured using a Tektronix 2245A oscilloscope and a Metex Digital Multimeter.

All experiments were conducted in T-25 culture flasks. Before each experiment, logarithmically growing HL-60 cells ($0.8-1.2 \times 10^6$ cells/ml) were starved in medium containing 0.5% FBS for 16–20 h [Lu et al., 1997]. The cell concentration was then adjusted to 1×10^6 cells/ml. Fifteen milliliter cells were aliquoted into T-25 flasks (control and experimental) 1 h before the experiments. MCF-7 and 3Y1 cells were grown in T-25 flasks to near confluence (3Y1 and C-Src 3Y1) or until about 70% of the flask surface was covered (MCF-7 and v-Src 3Y1). They were then starved in low-serum medium (0.5%) for 16–20 h prior to each experiment [Lu et al., 1997]. The cells in control and experimental flasks were subcultures of the cells from one single flask to minimize discrepancies between the cultures.

The coils were turned on at least 30 min before exposure, and remained in that state while the flasks were being removed. The cells were subjected to: (a) no treatment, (b) EMF exposure, (c) treatment with TPA at different concentrations, and (d) EMF exposure imposed upon TPA treatment. All experiments were under the same environmental conditions. TPA was dissolved in DMSO. An equal amount of DMSO was added to the control flasks. The final concentration of DMSO in the medium was 0.1%.

PKC Inhibitors

PKC inhibitors Staurosporine, Gö 6976, and Rottlerin (Calbiochem, San Diego, CA) were added to T-25 flasks at least 30 min before experiments at concentrations recommended by the manufacturer (0.2, 1, and 5 μM, respectively, dissolved in DMSO) to test their effects on EMF induced MAPK activation.

Protein Extraction and Quantification

The following procedures were adapted from "Current Protocols in Molecular Biology." Flasks were put into an ice slurry immediately after being subjected to EMF exposure or TPA treatment for 10, 15, 20, 30 min and remained there for 10 min. HL-60 cells were collected by centrifugation at 1,500 rpm for 5 min. MCF-7 and 3Y1 cells were released from the bottom of the flasks by a cell scraper in the presence of 1× TBS with 0.2% of EDTA, and then spun down at 1,500 rpm for 5 min. After washing in 1× TBS once, cells were lysed in "lysis" buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1.5% Triton X-100, 1 mM EDTA, 0.2 mM Na_3VO_4, 0.2 mM PMSF, 1× protease inhibitor cocktail set I [500 μM AEBSF, 1 μM E-64,500 μM EDTA, 1 μM Leupetin, 1 μg/ml Aprotinin] purchased from Calbiochem). Lysates were centrifuged at 14,000 rpm for 30 min at 4°C and the supernatants were saved for Western blotting analysis. Protein concentration of the supernatants was determined by Standard Protein DC Assay (Bio-Rad, Hercules, CA).

Western Blotting Analysis

The activation of MAPK was detected by an antibody that recognized only the activated form of MAPK dually phosphorylated on Thr(183) and Tyr(185) residues (Sigma). Western blots were conducted according to instructions provided by "ECL Western Blotting Analysis System" (Amersham Life Sciences, Piscataway, NJ). Twenty micrograms of total protein in 20 μl was loaded into the wells of the SDS–polyacrylamide gel (stacking gel: 4%; separating gel: 8%) and run at 10 mA for 2–3 h. The proteins were then transferred to 0.45 μM nitrocellulose membrane (Bio-Rad) at 200 mA overnight in a 4°C refrigerator. After soaking in blocking buffer (5% non-fat milk powder in 1× TBS) for an hour at room temperature, the membrane was then incubated with the primary antibody (mouse monoclonal anti-MAPK, activated (diphosphorylated ERK-1 and -2) clone MAPK-YT, diluted 1:1,000 in blocking buffer) at 4°C overnight. The nitrocellulose membrane was rinsed in washing buffer (1× TBS + 0.1% Tween-20) three times before incubating with horseradish peroxidase-conjugated goat anti mouse IgG diluted (1:2,000) in blocking buffer for an hour at room temperature. After three rinses in washing buffer, the membrane was covered with ECL chemiluminescence (Amersham Life Sciences) Western blotting detection reagents and allowed to react for 5 min. The protein bands were detected using X-ray film exposed to the membrane. The density of the bands were measured with Personal Densitometer (Molecular Dynamics, Amersham Pharmacia Biotech,

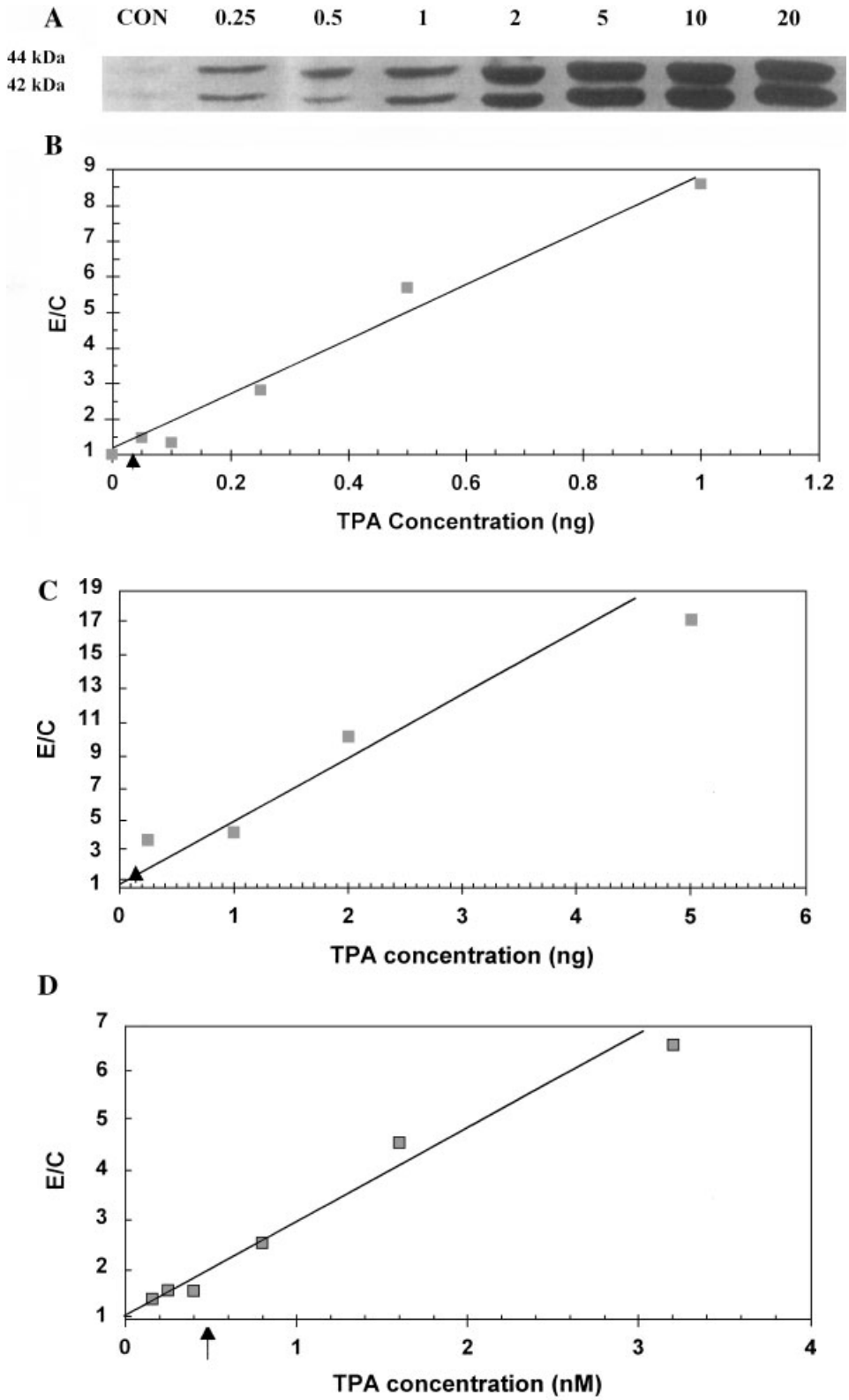

A
CON
0.25
0.5
1
2
5
10
20
44 kDa
42 kDa
B
E/C
TPA Concentration (ng)
C
E/C
TPA concentration (ng)
D
E/C
TPA concentration (nM)

Fig. 1.

Piscataway, NJ) and analyzed by Image-Quant program.

Statistical Analysis

Data were analyzed statistically by Microsoft Excel Description Analysis Program. Student's *t*-tests were used to determine if the differences between two samples (the population exposed to EMF vs. those not exposed) were significant. A *P* value equal to or less than 0.05 was used as the criteria of significance.

RESULTS

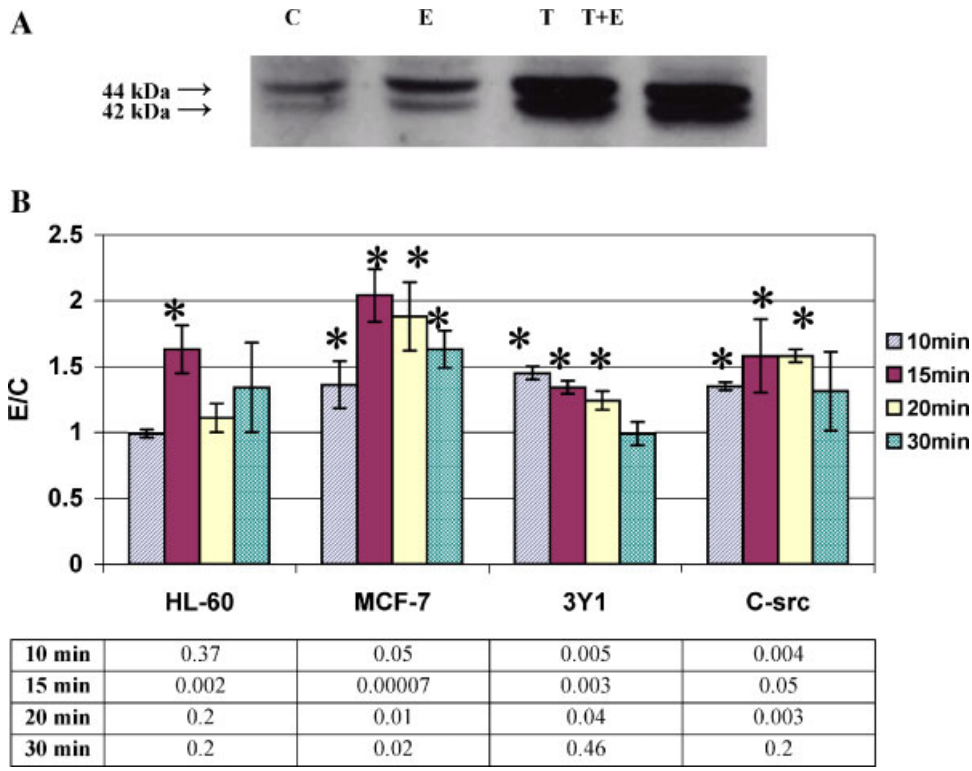
Previous studies [Kharbanda et al., 1994] reported that MAPK was activated in HL-60 cells treated with TPA. The present research confirmed this observation. In addition, it showed that MAPK was induced by TPA in a dosage dependent way in HL-60, MCF-7, 3Y1, and c-Src transfected 3Y1 cells. v-Src transfected 3Y1 has a level of MAPK activation that is extremely high with or without TPA, indicating that this pathway is turned on permanently in these cells due to the introduction of *v-Src* gene. Figure 1 presents the results of TPA treatment in 3Y1, c-Src, MCF-7, and HL-60 cells. Different time points were tested and robust responses were seen in cells treated with TPA as early as 10 min (Fig. 3).

An increased level of MAPK activation was also observed in HL-60, MCF-7, 3Y1, and c-Src expressing 3Y1 (c-Src) cells exposed to 60 Hz, 1 G EMF for 10–30 min with some variations between different cell lines (see Fig. 2). MCF-7 cells showed the strongest response to EMF. Significant increases were seen after 10, 15, 20, and 30 min of exposure and reached a peak at 15 min that was over twofold the levels seen in control cells. The increase was significant at 15 min in HL-60 cells; while in 3Y1 and c-Src cells, significant increases were found after 10, 15, and 20 min of EMF exposure. The effect peaked at 10 min for 3Y1 cells and 15 and 20 min for c-Src. The magnitude of the increase in MAPK activation was comparable to that observed in cells treated with a low concentration of TPA. While MCF-7 was the cell line most sensitive to EMF, its response to TPA was relatively weaker than others. In MCF-7 cells, EMF exposure was equivalent to treatment with 0.5–0.6 ng/ml of TPA. In HL-60, 3Y1 and c-Src cells, EMF exposure has the same impact as that produced by 0.05–0.1 ng/ml of TPA (Fig. 1B–E). In previous experiments, TPA treatment was shown to block estrogen receptor activity in MCF-7 cells and led to growth arrest and altered cell morphology [Tzukerman et al., 1991; Martin et al., 1995].

A similar response seen in cells exposed to EMF or TPA again raises the question of whether the response to each shares the same signal transduction pathway. An experimental series comparing the effects of EMF and a low concentration of TPA (0.25 ng/ml) was conducted to determine whether they follow similar time frames in MAPK induction. The data suggest that cells were affected by either treatment, but they seem to sustain their responsiveness for a longer period to TPA than to EMF (Fig. 3). In these experiments, the cells were also simultaneously exposed to both TPA and EMF to test whether they act synergically or whether their effects are additive on MAPK activation. The results indicate that EMF and TPA could have an additive effect on this pathway in 3Y1 and c-Src cells, but the results were not significant in HL-60 and MCF-7 cells.

The involvement of PKC, an upstream activator of MAPK cascade in the cellular response to EMF exposure, has been proposed [Walleczek, 1992; Goodman et al., 1993]. In present experiments, the effects of three PKC inhibitors on EMF or TPA induced MAPK activation in HL-60 and MCF-7 were tested. The results show that Staurosporine (inhibits several isoforms of PKC including α and δ) as well as Gö 6976 (inhibits PKC-α only)

Fig. 1. 12-*O*-tetradecanoylphorbal-13-acetate (TPA)-induced activation of mitogen-activated protein kinase (MAPK) in 3Y1, c-Src, MCF-7, and HL-60 cells. **A**: 3Y1 cells were treated with 0.25, 0.5, 1, 2, 5, 10, or 20 ng/ml of TPA for 15 min (CON, control, without TPA). Cell lysates were subjected to Western blotting analysis against monoclonal anti-MAP kinase, activated (diphosphorylated MAPK/extracellular signal-regulated kinase (ERK)-1 and 2, 42, and 44 kDa). The density of the bands indicates the activity of the kinase. **B**: The graph summarizes a series of experiments as showed in (A). The density of the control (C) or experimental (E) bands (42 and 44 kDa) were determined by densitometry. The ratio, E/C, allows a comparison of level of MAPK activation in 3Y1 cells treated with different concentrations of TPA. **C–E**: Levels of MAPK activation in c-Src, MCF-7, and HL-60 cells in response to TPA treatment at different concentration for 15 min. The arrows point to the TPA concentration that elicited the same level of activation of MAPK in 3Y1 cells as that induced by exposure to electromagnetic field (EMF, 60 Hertz (Hz), 1 Gauss (G)).



10 min	0.37	0.05	0.005	0.004
15 min	0.002	0.00007	0.003	0.05
20 min	0.2	0.01	0.04	0.003
30 min	0.2	0.02	0.46	0.2

Fig. 2. Effects of EMF exposure on MAPK activation in different cell lines. **A**: MCF-7 cells were exposed to 60 Hz, 1 G EMF (E), treated with 1 ng/ml of TPA (T) or subjected to both EMF and TPA (T + E) treatment for 20 min (CON, control). The levels of the activated form of MAPK in the cell lysates were determined by Western blots as described in Figure 1. **B**: The graph presents the results of exposures of HL-60, MCF-7, 3Y1, and c-Src cells to 60 Hz, 1 G EMF for 10, 15, 20, and 30 min. The values of the bars (E/C) are the ratios of MAP activation levels in experimental samples (E) over those of the controls (C) as determined by Western blot (error bars represent SE). *, Indicates significant difference from control; Student's *t*-test, $P \leq 0.05$. (*P* values are presented in the table beneath the chart.) [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

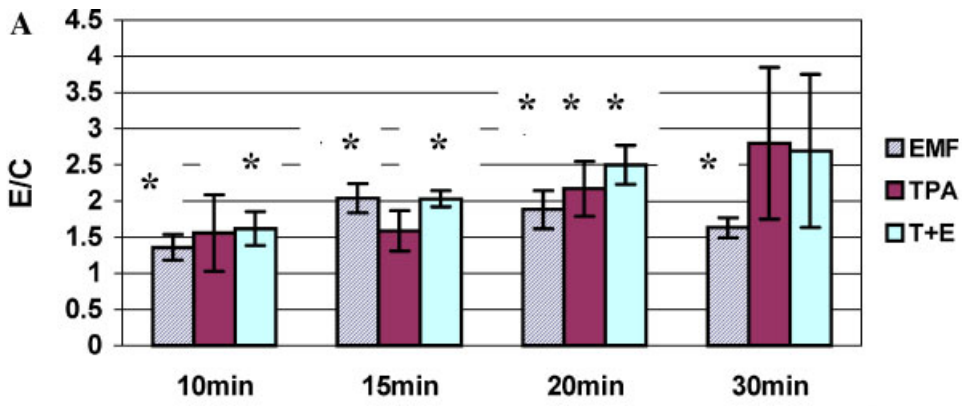
significantly reduces the level of MAPK activation induced by EMF exposure in both cell lines. Rottlerin, at the concentration used in these experiments inhibits PKC-δ, seems to have no influence on the outcome (Fig. 4A). The effects of PKC inhibitors on MAPK activation induced by EMF exposure or TPA treatment are compared in Figure 4B,C. Staurosporine and Gö 6976 have basically the same effect on the results of TPA treatment as on EMF exposure, suggesting that PKC-α plays a role in the cells' response to EMF. The effects of Rottlerin were not statistically significant.

DISCUSSION

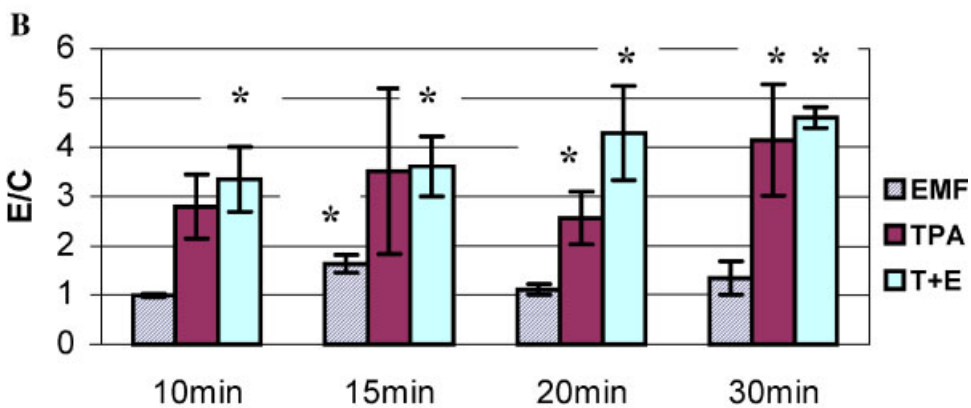
This research was based on our previous observation that differentiation was induced in HL-60 cells exposed to a 60 Hz, 1 G EM field. The effect of EMF exposure on HL60 cell differentiation mimicked treatment with low concentrations of TPA [Tao and Henderson, 1999]. As cell signaling systems have been perceived as a plausible mean of mediating the bioeffects of EMF exposure, we wanted to know whether MAPK kinase, an important signal transducer stimulated by TPA, as well as other extracellular mitogens, is also affected in cells exposed to the field. In addition to HL-60 cells, human breast cancer cells MCF-7 and rat fibroblast 3Y1 cells were also tested. Unlike HL-60 cells, which grow in suspension, epithelial MCF-7 cells, and fibroblastic 3Y1 cells grow on an inert surface. Thus, intercellular communication plays a role in their response to extracellular stimulation. MCF-7 cells were chosen because epidemiological studies indicated a correlation between breast cancer and EMF exposure [Demers et al., 1991]. There are also reports show that EMF exposure inhibits the antiproliferative effects of both melatonin and tamoxifen in MCF-7 cells [Harland et al., 1999; Blackman et al., 2001]. For 3Y1 cells (parental and parental cells overexpressing either c-Src or v-Src), it was important to determine whether they exhibit different reactions to EMF, as TPA has been

previously reported as being able to induce a transformed phenotype in 3Y1 cells overexpressing c-Src proto-oncogene. TPA, however, did not have this effect on untransformed parental cells [Lu et al., 1997]. We have shown in this study that MAPK was activated temporarily in HL-60, MCF-7, and 3Y1 (parental and c-Src transfected parental) cells exposed to low-energy EMF (60 Hz, 1 G) and to the same extent seen in cells subjected to treatment of low concentration of TPA (0.05–0.6 ng/ml). Considering the critical role MAPK cascade played in connecting extracellular stimulation with transcriptional modification in the nucleus, the involvement of MAPK in a wide range of EMF elicited bioeffects is implicated.

The present findings provide further evidence that alternations initiated at the cell membrane by EMF might be propagated through signal transduction pathways. Important members of signaling systems such as protein tyrosine kinases [Uckun et al., 1995; Dibirdik et al., 1998; Kristupaitis et al., 1998], PLC-γ2 [Dibirdik et al., 1998; Kristupaitis et al., 1998], PKC [Monti et al., 1991; Tuinstra et al., 1998], and calcium [Walleczek, 1992; Karabakhtsian et al., 1994; Kim et al., 1998] have been shown to be affected by EMF in previous investigations.

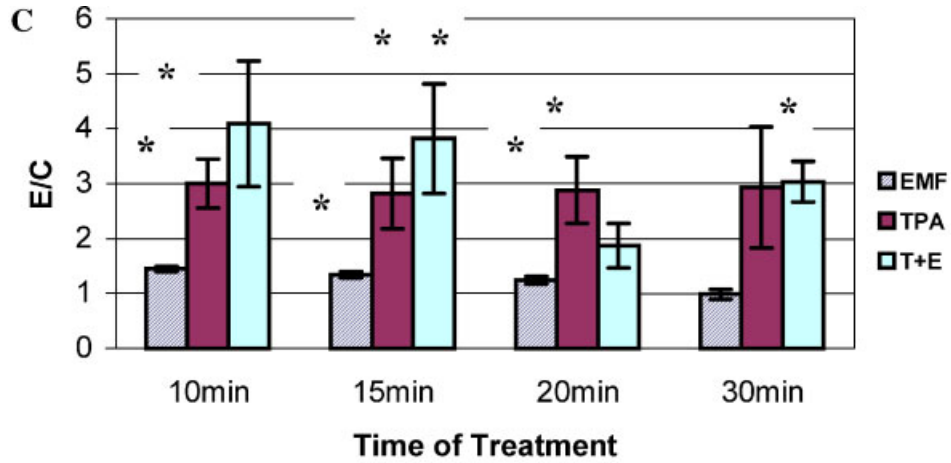


EMF	0.05	0.00007	0.01	0.02
TPA	0.2	0.09	0.05	0.11
T + E	0.04	0.006	0.02	0.13

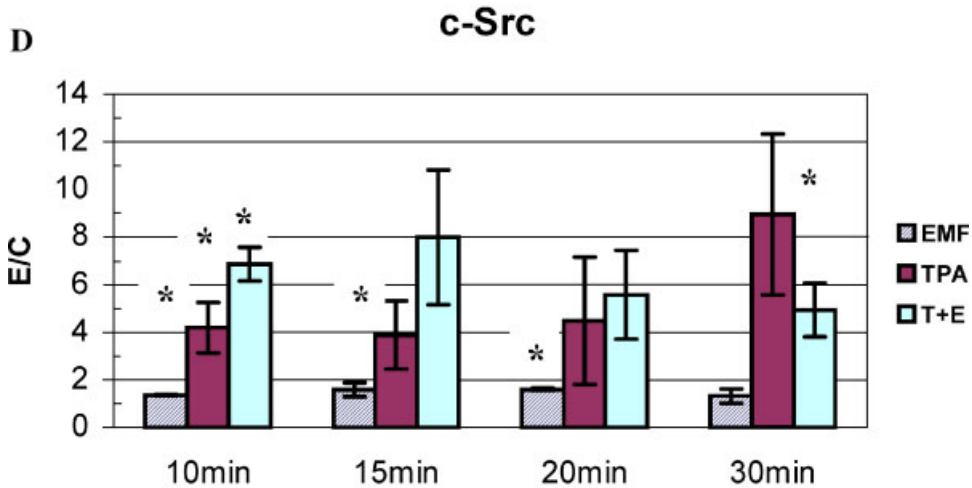


EMF	0.37	0.00007	0.2	0.2
TPA	0.06	0.12	0.05	0.05
T + E	0.04	0.03	0.04	0.002

Fig. 3. Comparison of the effects of EMF, TPA alone, EMF and TPA on MAPK activation in different cell lines over time. MCF-7, HL-60, 3Y1 cells, and c-Src transfected 3Y1 cells were exposed to EMF (60 Hz, 1 G), TPA (0.25 ng/ml), or [EMF + TPA] for 10, 15, 20, and 30 min. The levels of the activated form of MAPK in the cell lysates were determined by Western blots as described in Figure 1. The values of the bars (E/C) are the ratios of MAP activation levels in experimental samples (E) over those of the controls (C). **A**: MCF-7 cells. **B**: HL-60 cells. **C**: 3Y1 cells. **D**: c-Src transfected 3Y1 cells (error bars represent SE). *, Indicates significant difference from control. A *P* value equal to or less than 0.05 was used as the criteria of significance. (The *P* values of the data are presented in the tables beneath the charts.) [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]



EMF	0.005	0.003	0.04	0.46
TPA	0.02	0.05	0.05	0.11
T + E	0.06	0.05	0.08	0.02



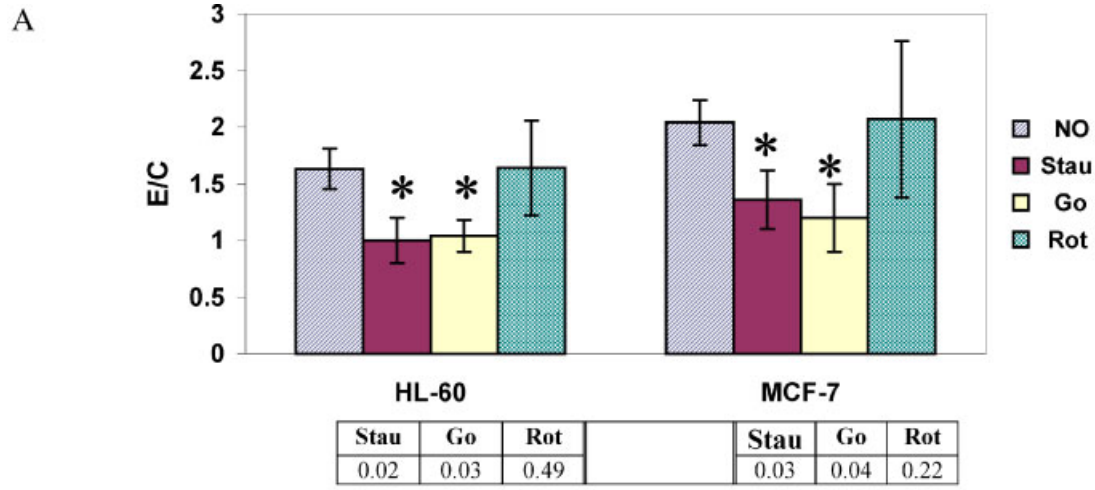
EMF	0.004	0.05	0.003	0.2
TPA	0.05	0.07	0.16	0.07
T + E	0.007	0.07	0.07	0.04

Fig. 3. (*Continued*)

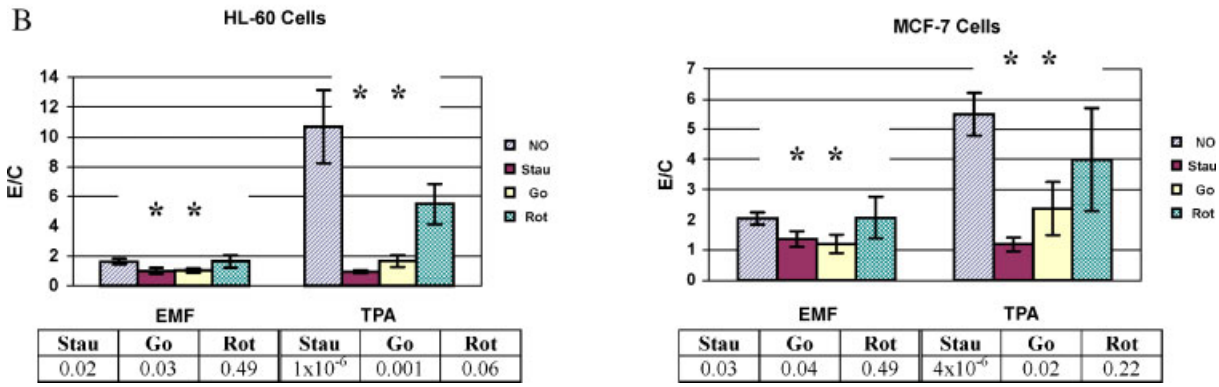
Calcium influx and PKC are upstream of MAPK in TPA induced effects. It is conceivable that through a similar pathway, signals elicited by EMF at the cell surface are relayed to MAPK, which in turn could lead to increased steady state transcript level of early response genes c-*fos* and c-*myc* [Karabakhtsian et al., 1994; Rao and Henderson, 1996].

The involvement of PKC in EMF induced MAPK activation is also indicated in the present research. PKC can be activated by short-term treatment of TPA. One of its 11 isoforms, calcium dependent PKCα, has the ability to phosphorylate and activate c-Raf-1 [Kolch et al., 1993]. MAPK activation in cells exposed to EMF was reduced by inhibitor to PKCα but not calcium independent PKCδ. This is in keeping with previous results that showed that calcium dependent PKC is activated by EMF exposure [Monti et al., 1991]. In our studies, however, how PKC-α per se is activated in HL-60 cells exposed to 60 Hz, 1 G EMF is still inconclusive.

The present study also shows that any change caused by ELF EMF is likely to be small and temporary. Different cell lines or different cells in a population do not all respond to EMF exposure [Tao and Henderson, 1999]. Some cell lines or sublines are more sensitive to



Stau	Go	Rot		Stau	Go	Rot
0.02	0.03	0.49		0.03	0.04	0.22



Stau	Go	Rot	Stau	Go	Rot
0.02	0.03	0.49	$1x10^{-6}$	0.001	0.06

Stau	Go	Rot	Stau	Go	Rot
0.03	0.04	0.49	$4x10^{-6}$	0.02	0.22

Fig. 4. The effects of protein kinase C (PKC) inhibitors on EMF and TPA induced MAPK activation. HL-60 or MCF-7 cells were incubated in medium containing Staurosporine (0.2 μM), Gö 6976 (1 μM), or Rottlerin (5 μM) for at least 30 min before being exposed to 60 Hz, 1 G EMF or 1 ng/ml of TPA for 15 min. The levels of the activated form of MAPK in the cell lysates were determined by Western blots as described in Figure 1. The values of the bars (E/C) are the ratios of MAP activation levels in experimental samples (E) over those of the controls (C). NO: Without PKC inhibitor; Stau: Staurosporine; Go: Gö 6976; Rot: Rottlerin. **A**: The effects of PKC inhibitors on EMF induced MAPK activation. **B**: Comparison of the effects of PKC inhibitors on EMF and TPA induced MAPK activation (error bars represent SE). *, Indicates significant difference from non-inhibition; Student's *t*-test, $P \leq 0.05$. (*P* values are presented in the tables beneath the charts.) [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

EMF than others. The same is true within a population of cells and perhaps only a small number of "initiated" cells are susceptible to EMF induction. This may contribute to the often contradictory results in this area. Thus, sensitive assays must be developed to detect small changes over background noises. The nature of low-energy EMF is that its presence is probably perceived by the cell as small, short-lived disturbance (as compared to TPA). Yet, the possibility of malignancy that resulted from an accumulative effect of chronic exposure can not be totally ignored.

Finally, the present research again shows that TPA induction pathways provide model for study of EMF–cell interaction. The shared characteristics seen so far in these two pathways are encouraging. Using this direction, we expect to learn more about EMF and cell signaling.

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