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CELLULAR AND MOLECULAR PATHWAYS OF EXTREMELY-
LOW-FREQUENCY ELECTROMAGNETIC FIELD
INTERACTIONS WITH LIVING SYSTEMS

T. S. Tenforde

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Pacific Northwest Laboratory
Richland, Washington 99352

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T.S. Tenforde

**Pacific Northwest Laboratory
Richland, Washington**

Address correspondence to:

T.S. Tenforde
Life Sciences Center (K1-50)
Battelle, Pacific Northwest Laboratory
Richland, Washington 99352 (U.S.A.)

Tel.: (509) 375-3738

FAX: (509) 375-3686

ABSTRACT

There is growing evidence that environmental electric and magnetic fields in the extremely-low-frequency (ELF) band below 300 Hz can influence biological functions by mechanisms that are only poorly understood at the present time. The primary objectives of this paper are to review the physical properties of ELF fields, their interactions with living systems at the tissue, cellular, and subcellular levels, and the key role of cell membranes in the transduction of signals from imposed ELF fields. Topics of discussion include signal-to-noise ratios for single cells and cell aggregates, resonance phenomena involving a combination of static and ELF magnetic fields, and the possible influence of ELF fields on molecular signaling pathways that involve membrane receptors and cytoplasmic second messengers.

INTRODUCTION

The continuing controversy over the possible health effects of environmental levels of ELF fields has underscored the need to gain a fundamental understanding of the pathways by which these fields interact with living systems. The weak 50/60 Hz electric and magnetic fields to which humans are commonly exposed in the home or workplace, which typically range from 10 to 50 V/m and 0.1 to 0.3 μ T, induce electric fields within the body that are on the order of 5 μ V/m or less. These field levels are several orders of magnitude below the field intensities that generally result in reproducible biological effects in a laboratory setting. A major challenge in both laboratory research and biophysical modeling is to elucidate the mechanisms by which living cells and organized tissues could exhibit a response to weak ELF fields at signal levels comparable to or below the intrinsic physical and biological electrical noise present in living systems.

PHYSICAL PROPERTIES OF ELF FIELDS

ELF fields in tissue have a long wavelength (~ 1000 m at 60 Hz) and skin depth (~ 150 m at 60 Hz), as a result of which these fields behave as though they are composed of independent, quasistatic electric and magnetic field components.¹ As a consequence, the radiating properties of ELF fields can be neglected in their interactions with tissue. Another important property of ELF fields is their extremely small energy. For example, the energy of a 60-Hz photon is 2.5×10^{-13} eV, which is 11 orders of magnitude smaller than the Boltzmann thermal energy, kT ($= 2.7 \times 10^{-2}$ eV at 310 K), and 14 orders of magnitude less than the energy required to break a chemical bond. Substantial laboratory evidence supports the

physical expectation that ELF fields do not disrupt the chemical bonds in DNA, proteins or other biological molecules. Direct genotoxic effects of ELF fields leading to cell death, gene mutation, or neoplastic transformation would therefore not be expected, and in fact, have not been observed.

A third important feature of ELF fields applied to living organisms through air is the nonthermal nature of their interactions. The highest field in tissue that can be induced by an ELF field applied through air is about 1 V/m, which leads to a specific energy absorption rate of 10^{-4} W/kg. This rate of energy deposition is four orders of magnitude less than the body's basal metabolic rate and produces a negligible rate of temperature rise (about 3×10^{-8} C/s). The interactions of ELF fields applied to the body through air are therefore of a nonthermal nature.

BIOLOGICAL EFFECTS AND MEMBRANE INTERACTIONS

Despite the minimal perturbations of molecular structure by environmental levels of ELF fields, there is abundant evidence for responses to these fields at the tissue and cellular levels.²⁻⁵ For example, small functional changes in excitable tissues and neuroendocrine alterations have been reported in response to ELF fields that induce tissue voltage gradients ≤ 10 mV/m. These alterations include changes in evoked brain potentials,⁶ heart rate,⁶ and nocturnal synthesis of pineal melatonin.^{7,8} In addition, a large number of cellular phenomena, including alterations in growth rate, gene expression and macromolecular synthesis, have been reported to occur in response to fields of moderate to weak intensity.⁵ These effects include alterations in biosynthesis of specific messenger RNA and proteins at field levels below 1 mV/m.⁹ Finally, there is a rapidly growing body of information that implicates the cell membrane as a primary site of ELF field interactions.^{2,5,10,11} A wide variety of cell membrane structural and functional properties have been reported to be altered in response to ELF fields, including changes in Ca^{++} binding to anionic fixed charges at the cell surface (e.g., sialic acid residues of membrane glycoproteins), changes in the transport of ions such as Ca^{++} and the secretion of small solutes such as insulin, and alterations in ligand-receptor interactions that trigger changes in the biosynthetic and functional states of cells. The threshold tissue field levels that lead to such effects appear to vary widely depending upon the end point studied, but in nearly all cases are ≤ 0.1 V/m. In the specific case of field-induced Ca^{++} desorption from fixed-charge sites on the membrane surface, the effective field level has been reported to be as low as 1-10 $\mu\text{V/m}$.^{12,13}

The phospholipid bilayer that forms the structural matrix in membranes of

living cells is an electrical insulator, and the membrane electrical conductivity is about 5 orders of magnitude less than that of the extracellular medium or the cytoplasm. As a result, the membrane of a living cell forms an excellent electrical barrier, as well as a superb chemical barrier, that mediates cellular interactions with the external environment. For this reason, it is generally believed that cellular responses to weak ELF fields are initiated by membrane interactions that serve as the primary mechanism of field transduction. Under typical exposure conditions with induced ELF fields in the extracellular medium of ≤ 1 V/m, the "leakage" field in the cell cytoplasm is less than $1 \mu\text{V/m}$. This conclusion also holds for the circulating electric fields induced directly in the cell cytoplasm by magnetic induction. For example, a sinusoidal 60-Hz, 0.1 mT field induces a maximum electric field of $0.2 \mu\text{V/m}$ in the cytoplasm of a cell with a $10 \mu\text{m}$ radius. These considerations reinforce the importance of the cell membrane in ELF signal reception and transduction.

Another point to be made regarding the role of cell membranes in ELF signal transduction is the amplification of the extracellular field that occurs across the membrane. By solving Maxwell's equations for the specific case of a dielectric shell (membrane) surrounding a spherical conductor (cytoplasm), it can be easily shown that the electric field across the membrane is greater than that in the extracellular medium by a factor $1.5 R/d$, where R is the cell radius and d is the membrane thickness. For a spherical cell with a radius of $10 \mu\text{m}$ and a membrane thickness of 5 nm , the field across the membrane is therefore predicted to be 3000 times greater than that in the extracellular medium. A weak environmental field that induces a voltage gradient of $5 \mu\text{V/m}$ in the extracellular fluid thus produces a field of about 15 mV/m across the cell membrane.

ELECTRICAL NOISE IN BIOMEMBRANES

Several physical and biological sources of electrical noise within the cell membrane may impose a lower limit on the strength of an ELF field that can be recognized as a coherent signal.¹⁴ The four major sources of electrical noise in biological membranes include: (1) Johnson-Nyquist thermally-generated electrical noise, which produces a $3 \mu\text{V}$ transmembrane voltage shift at physiological temperatures; (2) $1/f$ noise associated with ion current flows through membrane channels, which typically produces a $10 \mu\text{V}$ transmembrane voltage shift; (3) "shot" noise, which results from the discrete nature of ionic charge carriers and is a minor source of membrane electrical noise; and (4) endogenous biological background fields produced by electrically active organs such as the heart, muscles and the nervous system, which can exceed the contribution of physical noise sources by an order of magnitude or more.

In the presence of these various sources of membrane noise, which produce time-averaged fields greater than 10 V/m across the cell membrane, it is therefore of interest to explore the minimum strength of induced electric fields in tissue that can achieve a signal-to-noise ratio greater than one within the cell membrane. For example, Weaver and Astumian¹⁵ arrived at an estimate of approximately 0.1 V/m as the smallest applied electric field that exceeds the Johnson-Nyquist noise signal in the membrane of a single cell with an elongated cylindrical geometry (such as a fibroblast, neuron, or muscle cell). Larger threshold field levels, greater than 1 V/m, were predicted for small spherical cells such as lymphocytes. Further increases in the threshold field level are expected if other sources of electrical noise within the cell membrane are taken into account.

These calculations of signal-to-noise ratio consider only the transmembrane electric potential shift introduced by an extracellular field. It has been argued rather convincingly that the field and noise sources that are most relevant to biological signal transduction are those that reside within the highly charged electrical double layer that exists at the cell surface. This double layer, frequently referred to as the Helmholtz-Stern layer, is comprised of fixed anionic charges on the outer membrane surface and diffusible cations in the surrounding medium. As described originally by Debye and Hückel, the average thickness of the diffuse electrical double layer at cell surfaces is approximately 0.8 nm in a physiological medium.¹⁶ By considering Johnson-Nyquist noise and other sources of electrical noise within the double layer, it can be concluded that the minimum electric field required in the extracellular medium to achieve a signal-to-noise ratio greater than one is about 10 mV/m.

Another important factor to be considered in calculating the threshold field level that exceeds intrinsic electrical noise in biological membranes is the junctional coupling that occurs between cells in organized tissues. For an aggregate of N cells with electrically coupled membranes, the threshold field level to achieve a signal-to-noise ratio of one is lower than the threshold for single cells by approximately $N^{-5/6}$ as a result of two factors: (1) field amplification due to the larger size of the aggregate, and (2) reduction of membrane voltage noise as a result of the larger capacitance of the aggregate.¹⁷ For example, if an aggregate of 10^6 electrically coupled cells is considered, the threshold field level to achieve a signal-to-noise ratio of one is predicted to be lower by 100,000 than the threshold for a single cell. Because of the junctional coupling exhibited by most biological tissues, the threshold field to achieve membrane and cellular responses may therefore be on the order of 1 μ V/m as contrasted to the value of about 0.1 V/m or higher predicted for single cells. A similar

conclusion has been reached by Pilla¹⁸ from an electrical network model of cells that communicate electrically via junctional coupling.

BIOLOGICAL SIGNAL TRANSDUCTION PATHWAYS

The most singly important issue in understanding the pathways by which weak ELF signals could influence membrane and cellular functions is the elucidation of mechanisms by which these fields are transduced within the cell membrane. One approach that has been taken in addressing this question is to consider the intricate biochemical pathways that have evolved as a mechanism by which a living cell communicates with its extracellular environment. As illustrated in Fig. 1, the binding of a single molecule of a mitogenic substance to a specific receptor within the membrane triggers a cascade of events that involve conformational shifts in membrane-associated proteins. These events, in turn, lead to signal transduction and amplification via the production of cytoplasmic second messengers and internal effectors such as free Ca^{++} and protein phosphorylases (kinases) that regulate DNA transcription and protein biosynthesis.^{19,20} The end result of a single mitogen binding event at the membrane surface is thus a cytoplasmic signal that is amplified to a level that can produce robust effects on macromolecular synthesis and cellular responses involving significant changes in functional and proliferative states.

The interaction of ELF fields with biological membranes could, in principle, lead to alterations in each component of this elegant signaling process that occurs in living cells. A useful working hypothesis, illustrated in Fig. 2, is that the pericellular fields and currents induced by an applied ELF field initiate electrochemical events within the cell membrane that are important elements of the primary signal transduction and amplification process. These biochemically-mediated events then produce cytoplasmic second messenger responses that trigger changes in the biosynthesis of macromolecules and alterations in cellular growth, differentiation, and functional properties.

During the past decade, a growing body of experimental evidence has been acquired that supports this general picture of the sequence of events leading to ELF signal transduction and amplification at the cellular level. It has been demonstrated, for example, that pulsed electromagnetic fields (PEMF) with ELF repetition frequencies inhibit the production of cAMP by bone cells in response to the binding of parathyroid hormone (PTH) to surface receptors.²¹ Further studies have shown that the PEMF action leads to inability of the PTH-receptor complex to activate the alpha subunit of G protein, thereby interfering with the sequence of events that

leads to activation of adenylate cyclase at the cytoplasmic membrane interface.²² Other studies using human lymphocytes have shown that exposure to microwave fields with amplitude modulation at ELF frequencies leads to the inhibition of non-cAMP-dependent histone kinases.²³

The possible effects of ELF fields on the kinase-C signaling pathway are of particular interest because this pathway is known to be activated by the binding of tumor promoters such as phorbol esters. Activation of this pathway by the binding of a first messenger leads to a cascade of events that produce activated kinase-C and free cytosolic Ca^{++} ions. Recent studies have demonstrated a significant 70% elevation of kinase-C activity in human HL-60 cells exposed to a 50-Hz pulsed magnetic field.²⁴ The field effect was considerably damped by adding EGTA, a Ca^{++} chelator, to the medium. This observation is consistent with previous findings that kinase-C activation relies on the mobilization of Ca^{++} ions. Another relevant study is the recent finding that stimulation of Ca^{++} uptake into rat thymocytes by the plant lectin, Concanavalin A (Con-A), is significantly augmented by exposure to a sinusoidal 60-Hz magnetic field.²⁵ In lymphoid cells the binding of Con-A to surface receptors triggers cytoplasmic signaling events involved in the kinase-C pathway, including the activation of kinase-C and an elevation of the cytosolic Ca^{++} concentration. Enhancement of the Con-A effect by a 60-Hz magnetic field was shown to be dependent upon the strength of the electric field induced in the extracellular medium.

An *in vivo* study of particular interest in the context of possible ELF field effects on the kinase-C signaling pathway is the recent finding by Stuchly et al. that a 60-Hz, 2-mT magnetic field exerts a copromoting effect on mouse skin carcinogenesis.²⁶ In these experiments skin tumors were initiated by the topical application of 10 nanomole of the carcinogen, 7,12-dimethylbenzanthracene (DMBA), and were then promoted by the application of 4.9 nanomole of the phorbol ester, 12-O-tetradecanoylphorbol-13-acetate (TPA), once per week for a total of 23 weeks. In the group of mice that received DMBA and TPA plus exposure to the 60-Hz field for 6 h/d, 5 d/week throughout the tumor promotion phase, both the percentage of mice with tumors and the number of tumors per mouse increased more rapidly with time than in the group of mice that received DMBA plus TPA alone. For example, at week 18 the percentage of mice with tumors in these two groups were, respectively, 25% and 8% and the mean number of tumors per mouse were 1.90 ± 0.69 (SEM) and 0.65 ± 0.46 . By week 23 the differences between the mice exposed to the magnetic field and the nonexposed group were no

longer statistically significant. Other studies by the same group of investigators have shown that a 60-Hz, 2-mT magnetic field acting alone on DMBA-initiated skin cells does not have a tumor-promoting effect.²⁷ It would be of considerable interest to conduct further experiments in which comparative measurements are made of kinase-C activity in the affected skin cells throughout the tumor promotion phase in the field-exposed and nonexposed groups of mice. Such experiments would provide a test of the hypothesis that ELF fields may exert a copromoting effect on tumor development through an influence on the kinase-C signaling pathway activated by phorbol esters.

Recent studies on rat mammary tumor development following initiation with the carcinogen, nitrosomethylurea (NMU), suggest that ELF fields may also exert direct tumor-promoting activity.²⁸ In these experiments rats were injected intravenously with a 50 mg/kg dose of NMU that produced mammary tumors in 54% of the rats. This percentage increased to 86% in a group of rats initiated with NMU and exposed to a 50-Hz, 0.2 mT magnetic field for 3 h/d for 5 weeks. The latency time to tumor development was also found to decrease significantly in the rats exposed to both NMU and the magnetic field relative to rats that received only NMU. These results are consistent with a tumor-promoting effect of the field, but could also be related to a field-induced suppression of pineal melatonin synthesis and a resultant elevation in breast cancer risk.^{29,30} Further research on the effects of ELF fields on chemically-induced mammary tumors in rodents may provide useful insights into the pathways by which these fields could exert carcinogenic effects.

PHYSICAL MECHANISMS OF ELF FIELD INTERACTIONS

In the search for mechanisms by which extremely weak ELF fields could exert significant biological effects, a number of innovative models have been proposed over the course of the last two decades. One class of models that has received particular attention during the past several years are resonance models that involve the combined action of an ELF field and the static geomagnetic field. For example, ion cyclotron resonance (ICR) was proposed by Liboff in 1985 as a possible mechanism that could facilitate the transport of ions such as Ca^{++} through membrane channels in the presence of the geomagnetic field and a weak ELF field tuned to the ICR frequency.³¹ Although some data on Ca^{++} uptake by lymphocytes³² and diatoms³³ have been cited as lending support to this model, there are also negative experimental findings.^{34,35} In addition, a number of physical arguments can be raised against the ICR model,⁵ the most serious of which is the collisional damping of resonant ion motion that is expected to occur in a

condensed phase.³⁶

Two other resonance models that have been proposed recently are the "quantum beats" model of Lednev,³⁷ in which combined static and ELF fields affect vibrational energy levels and transition probabilities of bound ions (e.g., Ca^{++} ions bound to calmodulin), and the model of Zhadin and Fesenko,³⁸ in which the rotational energy levels of bound ions are pumped by combined static and ELF fields. Shuvalova et al. have presented data on the effect of combined fields on the rate of calmodulin-dependent phosphorylation of myosin that appear to support the predictions of the Lednev model.³⁹ However, a recent study using an optical technique to study Ca^{++} binding to calmodulin and to metallochromic dyes failed to find any effects of combined static and time-varying fields under the resonance conditions predicted by Lednev's model.⁴⁰ Adair has pointed out that the Lednev model of resonant field effects is improbable because of the long lifetime of the excited vibrational states (about 8 sec), during which de-excitation would occur as a result of collisional damping.⁴¹ Similar arguments can be raised against the type of resonant field effects envisioned in the Zhadin and Fesenko model. In addition, the rate of transition of a bound ion to an excited state predicted by this model is so low that a transition would occur only once in several months at typical environmental magnetic field levels.

A large number of nonequilibrium models have been proposed in which field-induced structural and functional perturbations result from membrane interactions that exploit the existence of unstable or metastable states. Examples of such interactions are dissipative instabilities involving cooperative transitions of allosteric membrane proteins in the presence of an applied field. Critical state instabilities in membrane physical properties near the phase transition temperature can also be amplified by an applied electromagnetic field. A host of other models involve coherent field interactions that lead to the excitation of oscillating dipolar modes in membrane proteins or the production of nonlinear oscillations (solitons) that facilitate vibrational energy transfer in macromolecular structures. A number of reviews discussing the physical basis of these nonequilibrium models have been published.^{2,10,42-44}

The discovery of biogenic magnetite particles in the tissues of a large number of organisms, including several mammalian species,^{45,46} has led to speculation that oscillatory magnetomechanical forces and torques on these particles could provide a mechanism for the transduction of signals from weak ELF magnetic fields. Of particular interest is the recent demonstration of magnetite crystals in various anatomic locations within the human brain.⁴⁷ Kirschvink et al. have proposed a model in which oscillatory magnetic forces on magnetite

particles at ELF frequencies are visualized as producing the opening and closing of pressure-sensitive ion channels in membranes.⁴⁸ The 60-Hz field level required to overcome the effects of Brownian motion is predicted from this theoretical model to be on the order of 0.1 mT, which is within the range of local fields at locations close to the surfaces of several types of household appliances and machine tools.

One difficulty with this model is the sparsity of magnetite crystals relative to the number of cells in brain tissue. For example, human brain tissue is reported to contain a few million magnetite crystals per gram, distributed in $5-10 \times 10^5$ discrete clusters.⁴⁷ The number of cells in brain tissue thus exceeds the number of magnetite crystals by approximately a factor of 100. It is therefore difficult to envision how oscillating magnetomechanical interactions of an ELF field with magnetite crystals could affect a significant number of pressure-sensitive ion channels in the brain. However, the effects of such interactions on neural signaling in localized brain regions could possibly result in a biological response, although there is no evidence at present to support this hypothesis. Further studies are clearly needed to reveal the biological role of magnetite and the possible mechanisms through which this mineral could play a role in ELF signal transduction.

CONCLUDING REMARKS

Evidence is mounting for a central role of cell membranes in the reception, transduction and amplification of signals imposed by ELF fields. A major challenge for the future will be the elucidation of specific molecular pathways through which these fields can influence transmembrane signaling events and affect the functional and proliferative states of cells and organized tissues. Further research is also needed to gain an understanding of the ELF signal characteristics that are the most biologically effective, and to define the threshold field parameters above which predictable biological responses occur. Recent laboratory studies have provided a number of clues on the pathways through which ELF fields may operate at the cellular and subcellular levels. However, a great deal of research lies ahead in order to fully characterize the molecular substrates of ELF field interactions and the resultant cascade of electrical and biochemical signals that lead to cellular and tissue responses.

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FIGURE LEGENDS

FIG. 1.—Biological signal transduction pathways involving first and second messenger systems.

Abbreviations— cAMP: cyclic adenosine monophosphate; cGMP: cyclic guanosine monophosphate; IP₃: trisphosphoinositol (which releases Ca⁺⁺ from intracellular stores).

FIG. 2.—Hypothesized sequence of membrane-mediated events leading to cellular and tissue responses to applied ELF fields.

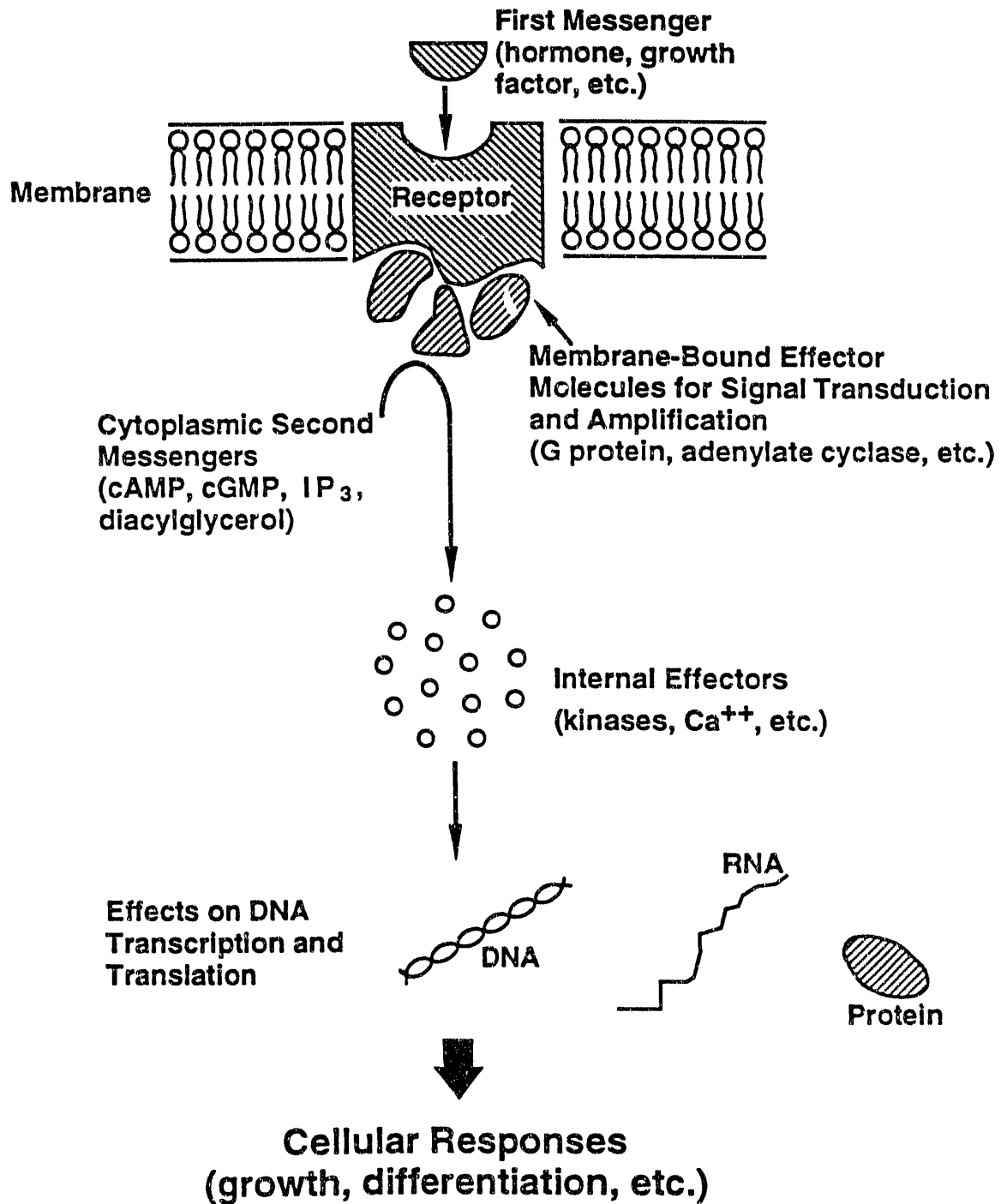


FIGURE 1

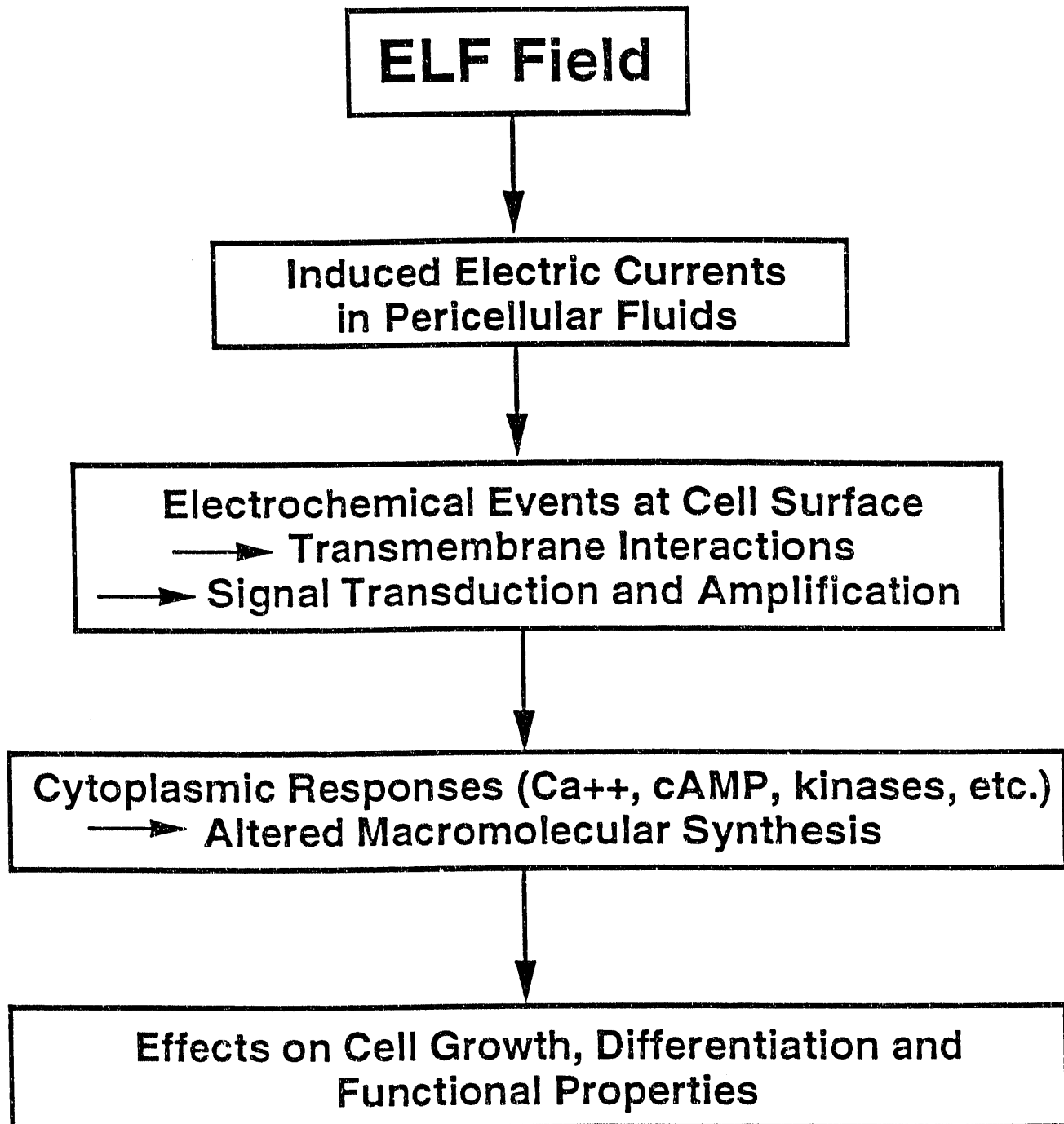


FIGURE 2

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