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Low frequency electric and magnetic fields have different effects on the cell surface

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There is a considerable controversy over the nature of weak electromagnetic-field effects in living organisms. Part of the controversy can be traced to a lack of understanding of whether electric or magnetic fields are involved in producing bioeffects. We find that both 60 Hz electric and magnetic fields alter the cell surface of *Physarum polycephalum*. Exposure to electric fields increases the negative charge on the cell surface while magnetic-field exposure decreases the hydrophobic character of the surface. These effects appear to be additive and independent of the waveform of the applied fields.

Cell surface; Cell partitioning; Electromagnetic field; Aqueous partition chromatography

1. INTRODUCTION

Although it has been known for some time that weak alternating electromagnetic fields (EMFs) can directly alter cellular function [1–3], the nature of the interactions producing such effects is not understood. Indeed it is difficult to imagine a plausible interaction mechanism when the energy associated with the fields is often ten or more orders of magnitude smaller than the thermal energy of random motion. Changes in metabolic rates, respiration rates, levels of ATP, DNA, and various proteins, and nutrient transport rates have all been observed, but most of these have been reported at a single set of exposure conditions and field characteristics. To provide rigorous tests of proposed mechanisms it will be necessary to determine how various biological responses depend upon EMF characteristics and, in particular, how the individual electric and magnetic fields influence these responses.

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We report here on measurements of cell surface characteristics in cells exposed to alternating weak electric and magnetic fields using a chromatographic technique called two-phase aqueous polymer thin layer countercurrent distribution (TLCCD) [4,5]. These measurements lead to the conclusion that both electric and magnetic fields alter the cell surface, independently, and in different ways. It is also possible to detect changes in the cell surface using a simple single-tube partition technique that can be performed in 20 min with standard laboratory equipment.

2. MATERIALS AND METHODS

Physarum polycephalum amoebae were exposed for 24 h to either or both 60 Hz sinusoidal electric and magnetic fields, applied at levels of 0.1 mT and 1.0 V/m. Field intensity values are rms values measured in the growth medium which has a resistivity of 2.0 $\Omega \cdot m^{-1}$. When both electric and magnetic fields were applied, they were applied at right angles in a horizontal plane and in phase with one another.

Electric fields were established by applying a voltage directly to the stainless steel sides of a rectangular growth flask. The electrodes were in contact with the growth medium; we have previously reported studies that ruled out electrolysis products

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as a source of biological effects [6]. Magnetic fields were applied by energizing large Helmholtz coils that surround the growth flask.

The exposure vessels are rectangular shake flasks 5 cm by 5.2 cm, filled to a depth of approx. 1 cm with growth medium. The magnetic field passes through the flasks in such a way that the maximum cross section of conductive growth medium seen by the field is 5 cm^2. The electric field induced by the magnetic field has a maximum value of 200 μV/m at the periphery of the medium within the flask; this should be compared to an applied electric field of 1 V/m. The power absorbed by the medium from the applied fields is negligible: 10 μW from the electric field and at least seven orders of magnitude less than this from the magnetic field.

After exposure the cells were harvested and analyzed using TLCCD. The TLCCD apparatus was manufactured by Bioshef (Biochemistry Department, University of Sheffield, England) and has been described elsewhere [7]. Briefly, it is comprised of two rotating disks each containing 60 chambers that match up and form a single well when the disks are placed flush against one another. The interface between the aqueous phases is just below the interface between the two disks. The upper polyethylene-glycol-rich phase (PEG) is moved forward one chamber at a time in a stepwise manner by rotating the upper plate 6°. The interface between the phases and bottom phase in each chamber remains stationary in the lower plate chambers. After rotation and mixing, cells are allowed to equilibrate and partition between the top phase and the interface before the upper plate is rotated again.

This process produces a stepwise liquid-liquid chromatography for cells. In the experiments reported here half the chambers (30) were devoted to control cells and the other 30 were used for exposed cells. Two different two-phase systems were used simultaneously, using separate pairs of disks, to examine different aspects of the cell surface following EMF exposure.

The first system is prepared by mixing 150 g of 20.06% (w/w) dextran (T-500, Pharmacia Biochemicals), 75 g of 40% (w/w) PEG (PEG-8000), and 165 g of 0.20 M phosphate buffer (pH 7.0) and diluting to 600 g with water. The buffer is prepared by adding 39 ml of 0.20 M KH_2PO_4 to 69 ml of 0.20 M K_2HPO_4. This mixture creates an electrostatic potential difference between the two phases; the top phase is positive with respect to the bottom phase. We refer to this as a 'charged' system. Cells subjected to this system will partition primarily on the basis of surface-charge-associated properties [8].

The second system was prepared by mixing 146 g of 20.25% (w/w) dextran, 60 g of 40% (w/w) PEG, 30 g of 0.20 M phosphate buffer (pH 7.0), 30 g of 1.00 M NaCl and 334 g of water. This system has no appreciable potential difference between the phases, so we refer to it as an 'uncharged' system or the zero-potential (zp) system. In this system the cells partition on the basis of non-charge-associated properties referred to here collectively as hydrophobicity.

It is possible to reproduce the findings reported here by means of examining the partitioning of cells in a single-test tube experiment. A single-test tube experiment permits measurement of the partition coefficient for cells in a particular phase system. The partition coefficient is defined as the number of cells in the upper phase divided by the number of cells at the interface. The partition coefficient measured this way is an average partition coefficient if more than one population of cells exist in the sample. TLCCD measurements have the advantage over single-tube experiments of being able to detect subpopulations of cells with different partition coefficients. For the data shown in fig.1, E + H/chg, the partition coefficient for the exposed cells is approx. 1.5 compared to 1.0 for the control cells. Such a large difference can be easily seen in a single-tube experiment.

For a single-test tube partitioning experiment the charged and uncharged solutions must be made up slightly differently to achieve optimum performance. A charged system containing 5.5% (w/w) dextran and 6.0% (w/w) PEG in the final test solution is made by mixing 27.2 g of 20.25% (w/w) dextran and 15.0 g of 40.0% (w/w) PEG solutions with 27.5 g of 0.200 M potassium phosphate buffer at pH 7.0 and diluting to 90.0 g with water. An uncharged system containing 5.0% (w/w) dextran and 4.6% (w/w) PEG is made by mixing 24.7 g of 20.25% (w/w) dextran and 11.5 g of 40.0% (w/w) PEG solutions with 5.00 g of a 0.200 M potassium phosphate buffer at pH 7.0 and 5.00 g of 1.00 M NaCl and diluting to 90.0 g with water.

The system solutions should be prepared immediately before use and kept mixed with a magnetic stirring bar. Pipette 9.0 ml of the desired system solution into a volume-calibrated tube and allow solutions to equilibrate for 20 min in a water bath at 25.5°C. One ml of a dilute cell suspension containing a known number of cells is then added to the tube and the tube is inverted 30 times. Tubes are replaced in the water bath and allowed to partition for 20 min before noting the volume of the top phase. Select an aliquot of the upper phase for counting to determine the total number of cells in the upper phase and divide by the total number of cells added to the system to obtain a partition coefficient. A similar procedure was used by us in a previous report [9].

3. RESULTS AND DISCUSSION

TLCCD separations were performed in both the charged and uncharged phase systems; the data are shown in fig.1. For cells exposed simultaneously to electric and magnetic fields (labelled E + H in the

Fig.1. TLCCD distributions plotted as cell count versus tube number. Cells exposed to fields (——); control cell population (– – –). Field condition is indicated in each plot with E + H indicating the presence of both electric and magnetic fields. Panels are paired to display TLCCD distributions in both charged (chg) and uncharged (zp) partitioning systems (see text). Distributions have been normalized so that total cell count summed over all chambers is constant. Note that a shift in a distribution peak from the center chamber, no.15, by one chamber to no.16, corresponds to a 14% increase in partition coefficient from 1.00 to 1.14, where the partition coefficient is defined as the ratio of cells in the upper phase to those at the interface. Repeated analyses of control cells against control cells produced superimposable distributions similar to those shown in the bottom righthand panel labelled 'E-only/zp'.

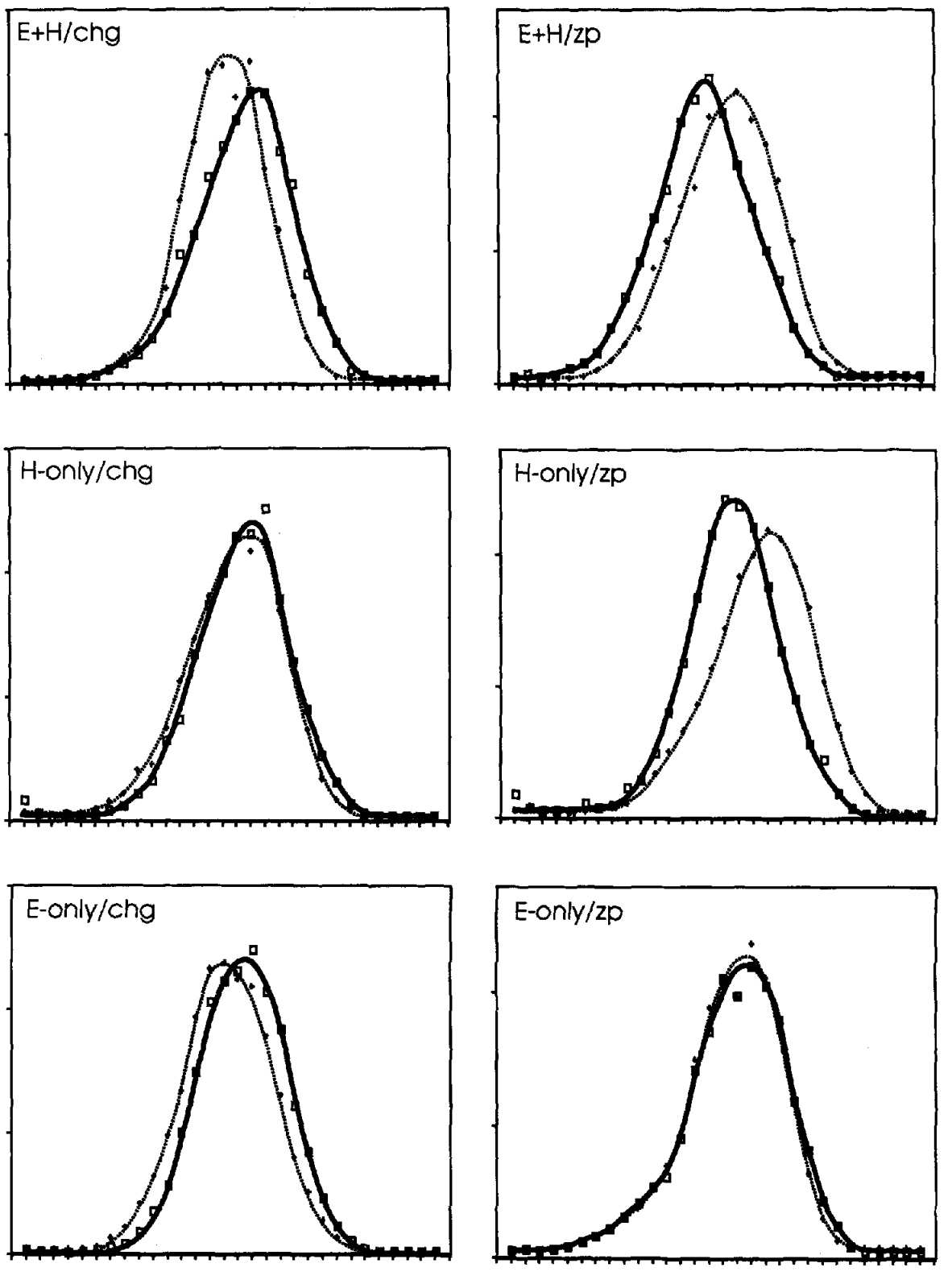
E+H/chg
E+H/zp
H-only/chg
H-only/zp
E-only/chg
E-only/zp

figure) the TLCCD profile is shifted to the right relative to the control population in the charged system and to the left in the uncharged system. These data imply that EMF exposure has both increased the negative surface charge on the cells, demonstrated by their greater affinity for the upper phase, and decreased the cells' hydrophobicity, producing a reduced attraction for the PEG-rich upper phase in the uncharged phase system.

Cells exposed to magnetic fields alone (H-only) display a TLCCD profile that is unchanged from controls in the charged system but shifted to the left in the uncharged system. This means that the cells have a diminished affinity for the upper phase which corresponds to a decrease in hydrophobic character of the amoebae. Cells exposed only to electric fields (E-only) produce complementary results: they shift to the right with respect to controls in the charged system but are unchanged in the uncharged system. This means that electric field exposure produces an increase in the net negative surface charge of the amoebae. Collectively these data may be interpreted as evidence that electric and magnetic fields each induce effects on the cell surface that can be detected by TLCCD.

These findings corroborate findings from two earlier studies. In 1979 we first reported that application of either weak sinusoidal electric or magnetic fields produced similar responses in cells [10]. More recently we used the TLCCD technique to examine cultures of *Physarum* to which pulsed magnetic fields, identical to those in use in the clinic to facilitate bone repair, had been applied [11]. Pulsed magnetic fields produce a concomitant electric field and cells exposed to pulsed magnetic fields displayed a response to TLCCD similar to the E+H sinusoidal fields studied here.

When a pulsed electric field was applied to emulate the electric field pulse produced by the magnetic field without the presence of a magnetic field, cells responded to TLCCD in a manner identical to cells exposed to sinusoidal electric fields seen in this study. The clear conclusion from both this study and [11] is that the field effects are independent of waveform, at least insofar as cell surface effects are concerned.

Another feature of these data worthy of note is the time it takes to produce the observed changes in the membrane surface. All data reported here were taken on cultures exposed for 24 h. Exposures of 18 h produced smaller effects; longer exposures did not appear to further increase the magnitude of the effects. In contrast, physiological changes such as reduced ATP and O_2 levels often require several months of exposure to fields before becoming manifest [12].

The exposure system used in this study should be relatively straightforward to replicate using readily available equipment. The field waveform, a 60 Hz sinewave, is uncomplicated and far simpler to generate than those we have employed in previous studies [11]. Our findings, and the findings of similar future studies, should be useful in setting constraints for proposed mechanism of action of electromagnetic fields, and for providing new insights into development of such models.

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