Pulsed magnetic fields alter the cell surface

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Pulsed magnetic fields (PMFS) are routinely used in the medical community to facilitate bone repair in clinical cases of non-union or pseudarthoses [(1984) Orth. Clin. No. Am. 15, 61–87]. Although this therapeutic regimen appears to be reasonably effective, the mechanism of action between specific PMFs and the target tissue remains unknown. Adding urgency to the need to understand the mechanism are a wide number of reports that have appeared which demonstrate that PMFs similar to those in clinical use can alter many basic physiological functions. We report that a 24 h exposure to PMFs alters the cell surface of *Physarum polycephalum* amoebae. Further, using the technique of aqueous two-phase partitioning, we present evidence for individual magnetic and electric field, cell surface effects.

Cell surface *Pulsed magnetic field* *Cell partitioning*

1. INTRODUCTION

It becomes evident upon reading the literature on the effects of pulsed magnetic fields (PMFs) that their bioeffects are quite diverse. For example, Goodman et al. [2] have shown that PMFs can alter RNA transcription and Liboff et al. [3] conclude that DNA synthesis itself can be affected by PMFs. The release of neurotransmitter in a nerve cell line is reduced upon exposure to PMFs [4], and other calcium-dependent processes are also similarly affected [5]. Recently, Dihel et al. [6] reported an increase in the mitotic indices of *Paramecium* exposed to PMFs that disappeared upon addition of the calcium channel blocker verapamil.

These and other reports of PMF effects led us to seek some common ground upon which one may develop a unified view of how such varied effects might all be produced by weak PMFs. Over the years many authors have singled out the cell membrane as an appropriate site for action. The reasoning is usually that the membrane offers a fairly protective barrier to electric fields and induced currents because of its non-conductive, lipid composition. Thus, if effects are produced inside a cell, there must be some transduction mechanism at the cell surface. It should be noted, however, that the cell membrane does not offer a barrier to low-frequency magnetic fields. This last caveat notwithstanding, we have focussed our efforts on examining PMF effects at the cell surface.

A major problem we faced was to find a method for detecting subtle surface changes induced by exposure to weak fields. The approach we selected makes use of a novel technique sometimes termed 'cell surface chromatography' which is an aqueous two-phase partitioning of cells, conducted on a thin-layer countercurrent distribution (TLCCD) apparatus [7]. Using this technique it is possible to detect both small differences in surface lipid and protein content (hydrophobicity) of intact cells as well as changes in surface charge. Since it is a non-disruptive procedure, the cells can be recovered after analysis for additional experimentation.

Solutions of the two water-soluble polymers

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dextran and polyethylene glycol (PEG) form two immiscible phases if the concentration of each polymer exceeds the critical point [8]. The upper phase is PEG-rich and is relatively more hydrophobic than the lower dextran-rich phase. When cells are mixed with the phases, they partition between the interface and one of the bulk phases, usually the top phase. The amount of partitioning or affinity for the top phase is dependent on the cell's surface properties. In essence, differences in the cell's surface properties are reflected as differences in their partitioning. To amplify these differences, repetitive partitioning steps are carried out in a countercurrent fashion.

The principle of countercurrent distribution is illustrated in fig.1. Countercurrent separation is achieved by stepwise movement of the top phase while the interface and bottom phase remain stationary. The partition steps are performed automatically in 60-chamber circular plates [7]. A good analogy is to think of this process as liquid-liquid chromatography for cells.

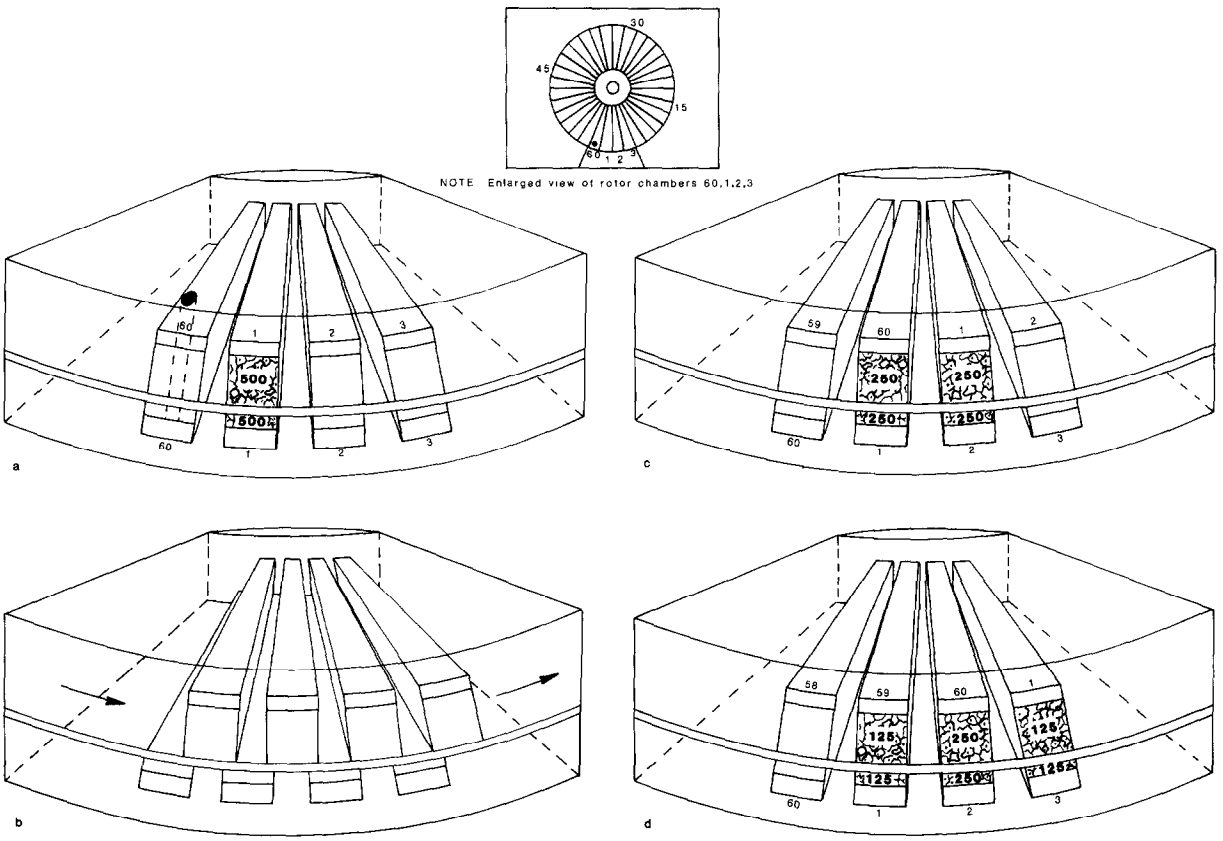

Fig.1. Schematic representation of 3 steps of a thin-layer countercurrent distribution procedure. Inset at the top is an overview of a 60-chamber rotor. (a) An expanded view of 4 thin-layer chambers at the beginning of an experiment: chamber 1 contains the original cell load, chambers 60, 2 and 3 contain only upper and lower phase solutions. For purposes of illustration, it is assumed that 1000 cells are added (the real number is about 10^6) and that they distribute themselves equally between the interface and upper phase, placing 500 cells in each, as shown. (b) Following a 30 s shaking period and a 10 min separation period, the top rotor is rotated in a counterclockwise direction by one chamber producing the situation depicted in (c) where the 500 cells in the upper phase have been moved to the second chamber and new top phase has been brought into chamber 1. The rotor is shaken again and the phases are allowed to repartition, leaving 250 cells in each partition of both chambers. (d) The top plate is moved counterclockwise by one chamber resulting in the further redistribution of cells, as shown.

2. MATERIALS AND METHODS

Amoebae from the slime mold *Physarum polycephalum* were maintained in submerged culture using the methods in [10]. We used a generator and coils supplied by Electrobiology, Fairfield, NJ (EBI) to apply a PMF. The magnetic field waveform is a burst of 22 sawtooth peaks having a maximum intensity of about 2.0 mT with a 20 μs rise time and a 200 μs decay; each tooth is separated by 5 μs, with bursts repeated at a rate of 25 Hz. Amoebae maintained in a non-exposed control environment were subcultured into two 125 ml Erlenmeyer flasks containing 30 ml growth medium. The flasks were placed on a reciprocal shaker containing two sets of square 12.7 cm diameter Helmholtz-like coils also furnished by EBI. The coils were positioned to produce a vertical field. One coil was attached to the EBI generator and is referred to as the experimental; the other contained a dummy load and served as the control. Pulse intensity at the control position, due to the current in the experimental coil, was 1/600 that of the applied pulse.

Two polymer systems were used for partitioning. The first was a 'charged system' which partitions cells primarily on the basis of charge-associated surface properties, composed of 5.5% dextran, 5.5% PEG (8000), and 0.05 M/kg potassium phosphate buffer, pH 7.0. This solution system is referred to as charged because there exists a measurable electrostatic difference between the two phases with the top phase positively charged relative to the bottom phase. The second phase system, which we will refer to as an 'uncharged system', was composed of 5.0% dextran, 4.0% PEG (8000), 0.05 M/kg NaCl, and 0.01 M/kg phosphate buffer, pH 7.0.

Following field exposure, control and experimental amoebae were harvested, centrifuged, washed once in the top phase, recentrifuged for 5 min and again resuspended in the top phase. In a typical partitioning run, control amoebae were loaded into chamber 1 and experimental amoebae into chamber 31 of each 60-chamber TLCCD plate; two plates were run simultaneously, one contained the charged polymer system and the other, the uncharged system. Thus, cells subjected to a given exposure regimen were simultaneously analyzed in two different phase systems. To check that both halves of the plates would provide coincident profiles with identical cells, control cells were run periodically on both halves of the plate; coincident profiles were obtained in all cases. To eliminate the possibility of introducing a systematic error, the chambers to which control and experimental cells were loaded were selected in a random manner.

3. RESULTS AND DISCUSSION

The distribution profile obtained from the charged system is shown in fig.2a; the data show the experimental population displaced to the right of the non-exposed controls. The converse occurs in the uncharged system, i.e. the exposed cells are displaced to the left (fig.2b). Since the top phase is positively charged with respect to the bottom phase, we conclude that the PMF-exposed cells have an increased negative surface charge relative to the controls. Displacement of the exposed cell distribution to the left of the controls in the uncharged system reflects a decrease in hydrophobicity.

Although the mechanism by which a PMF might alter the cell membrane is unknown, electromagnetic field theory tells us that the rapidly rising magnetic field component (2.0 mT in 20 μs) will generate a strong transient electric (E) field. To determine whether the induced E-field plays a role in altering the cell surface, amoebae were subjected to a pulsed E-only field resembling the field induced by the EBI system. (The applied pulsed E-field was equal to the maximum electric field induced by the EBI system at the flask's periphery, i.e. 1.1 V/m corresponding to a current density of 50 mA/cm^2.) In these experiments, a set of non-exposed control cells were subcultured into specially constructed boxes containing stainless-steel sides [11]. The boxes were placed on a reciprocal shaker and a pulsed E-field resembling the EBI induced field was applied. The TLCCD profiles obtained from a 24 h E-only exposure are shown in fig.2c,d. It is immediately evident that the E-field profiles analyzed in the charged system are virtually superimposable upon those obtained with the EBI-field generator. In contrast, the uncharged profile is identical to the control.

The fact that amoebae exposed to an E-only field showed surface characteristics similar to PMF-exposed cells when partitioned in a charged sytem indicates that the effective negative surface

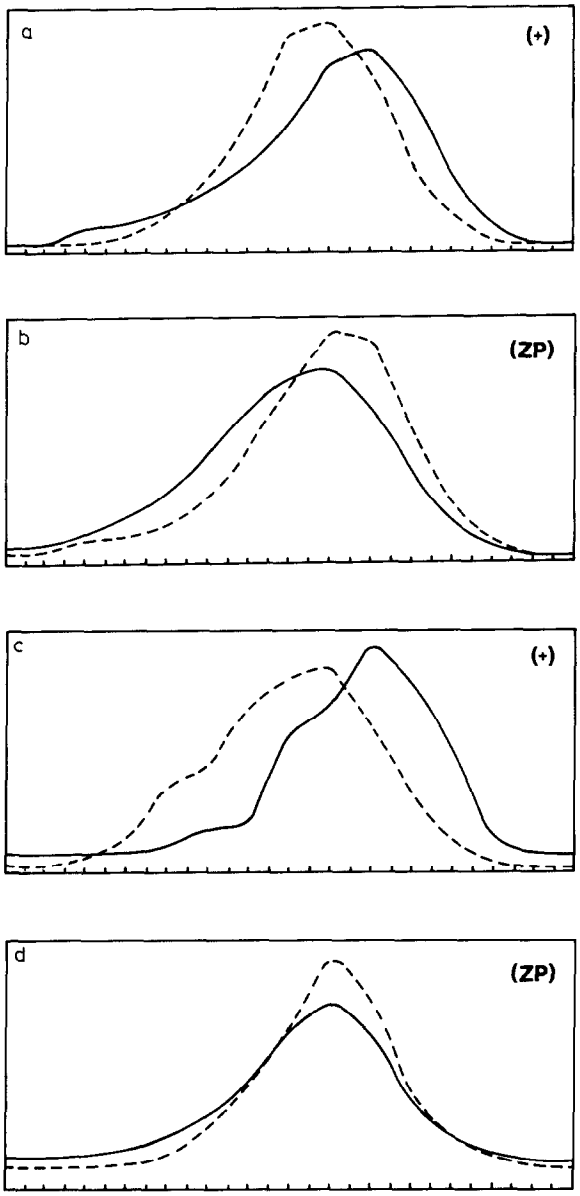

Fig.2. (a,b) TLCCD profile of *Physarum* amoebae exposed for 24 h to a PMF. Normalized cell number is plotted vs plate number. (a) Cell distribution for a phase system in which the upper phase is positively charged (+) relative to the bottom phase. (b) Cell distribution for an uncharged (zp) phase system. (– – –) Control, (——) PMF exposed. (c,d) TLCCD profile of amoebae exposed for 24 h to a pulsed electric field of 1.1 V/m. (c) Cell distribution for a phase system in which the upper phase is positively charged (+) relative to the bottom phase. (d) Cell distribution for an uncharged (zp) phase system. (– – –) Control, (——) pulsed electric field.

charge has increased on these cells. Of equal importance is the fact that E-only cells in the uncharged phase system exhibit a profile different from that of the PMF cells, from which we may conclude that the surface changes detected by the uncharged phase system are only induced when the magnetic field component is present. Collectively, these data suggest that (i) the electric-field component of the PMF waveform is responsible for an increase in negative surface charge, and (ii) the magnetic field itself produces a separate and different effect that is manifested as an increase in the membrane's hydrophobicity.

When amoebae are exposed to a PMF for 24 h, two different changes in cell surface character occur. The first is an increase in the cell's negative surface charge, the other involves a change in non-charge associated surface properties. The E-field induced by the PMF appears to act by increasing the negative surface charge of the cell. The magnetic field itself induces changes in non-charge surface properties that are reflected as a decrease in surface hydrophobicity.

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