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# Nonthermal 60 Hz sinusoidal magnetic-field exposure enhances $^{45}Ca^{2+}$ uptake in rat thymocytes: dependence on mitogen activation

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The effect of a 60 Hz sinusoidal magnetic field of nonthermal intensity on $Ca^{2+}$ metabolism in rat thymic lymphocytes (thymocytes) was assessed in resting cells and in cells activated with the mitogen Concanavalin A (Con A). A 60 min exposure at 37°C to an induced electric field of 1.0 mV/cm produced an average 2.7-fold increase in Con A-dependent $^{45}Ca^{2+}$-uptake compared to non-exposed, isothermal control cells. In contrast, $^{45}Ca^{2+}$ uptake remained unaltered during exposure of resting thymocytes. It was also found that thymocytes with a diminished ability to mobilize $Ca^{2+}$ in response to Con A were most sensitive to the 60 Hz magnetic field. Although the precise mechanism of field interaction is at present unknown, modulation of $Ca^{2+}$ metabolism during cell activation may represent a common pathway for field coupling to cellular systems.

Calcium metabolism; Signal transduction; Extremely-low-frequency electromagnetic field; Mitogen activation; (Rat thymic lymphocyte)

## 1. INTRODUCTION

Cellular studies have recently demonstrated that weak, extremely-low-frequency (ELF) electromagnetic fields can influence processes, such as DNA, RNA or protein synthesis in various cell types, including lymphocytes [1–10]. The cellular and molecular mechanisms by which these fields trigger biological responses are still unknown; mechanisms implying purely thermal effects can be ruled out because the field strengths employed are not associated with thermal loading (<0.01°C). In our laboratory the cell membrane, which plays a key role in mediating signal-transduction events, has been shown to be an important interaction site for electromagnetic field coupling in both natural and synthetic cell membranes [11–13]. Other researchers have also stressed the importance of the cell membrane in field coupling interactions [14,15]. These findings suggest that membrane-mediated signal transduction processes such as those involving calcium transport might be candidates for ELF field interactions. Reported here is evidence that 60 min exposure to nonthermal levels of a 60 Hz sinusoidal magnetic field (1 mV/cm, induced electric field) enhances $^{45}Ca^{2+}$ uptake in rat thymocytes but only if the cells are activated by mitogen. The biological implications of ELF field-induced changes in cellular $Ca^{2+}$ metabolism will be discussed with regard to cellular activation by mitogens and to subsequent $Ca^{2+}$-dependent events which modulate DNA, RNA and protein synthesis. The biological status of the thymocyte cell preparations plays an important role in defining an ELF field response in these studies.

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## 2. EXPERIMENTAL PROCEDURES

### 2.1. *Preparation of rat thymocytes*

Thymocytes were harvested from male Sprague–Dawley rats (250–450 g) and resuspended in assay buffer at 4°C (mM: 145 NaCl, 5 KCl, 1 $CaCl_2$, 5 glucose, 10 Na-Hepes; pH 7.4; mOsm 285). Cell preparations were >98% free of erythrocytes with >94% viability determined by nigrosine dye exclusion. Cells were maintained at 4°C in assay buffer and used within 3 h of preparation.

### 2.2. *Calcium transport assay*

$Ca^{2+}$ transport across the cell membrane was assayed by using $^{45}Ca^{2+}$. Cells ($5.0 \times 10^6$/ml) at 37°C were incubated with $^{45}CaCl_2$ (New England Nuclear, USA) at 5 μCi/ml for 10 min prior to exposure. When Con A-activated thymocytes were used, Con A (Sigma Chemical Corp., USA) at 20 μg/ml was added to the cells 5 min after addition of isotope, and incubation was continued for another 5 min at 37°C. From this cell suspension 4-ml aliquots were transferred into two identical annular ring Petri dishes (Fig. 1b). One dish was positioned inside the solenoid exposure system (Fig. 1a), the other in an isothermal waterbath. After 60 min, three 1-ml aliquots were taken simultaneously from the temperature-matched control sample and the 60 Hz-exposed sample. Viability and cell number were determined using the remaining cells. The six 1-ml aliquots were processed simultaneously for isotope counting: samples were centrifuged at 12000 rpm for 15 s (Eppendorf Mod. 5414); supernatants were discarded and the pellets thoroughly resuspended in assay buffer at 4°C; washing was repeated twice and thereafter the cells were analyzed by liquid scintillation spectroscopy. Cell viability, as determined within 30 min post-exposure, was always >90%, and did not differ

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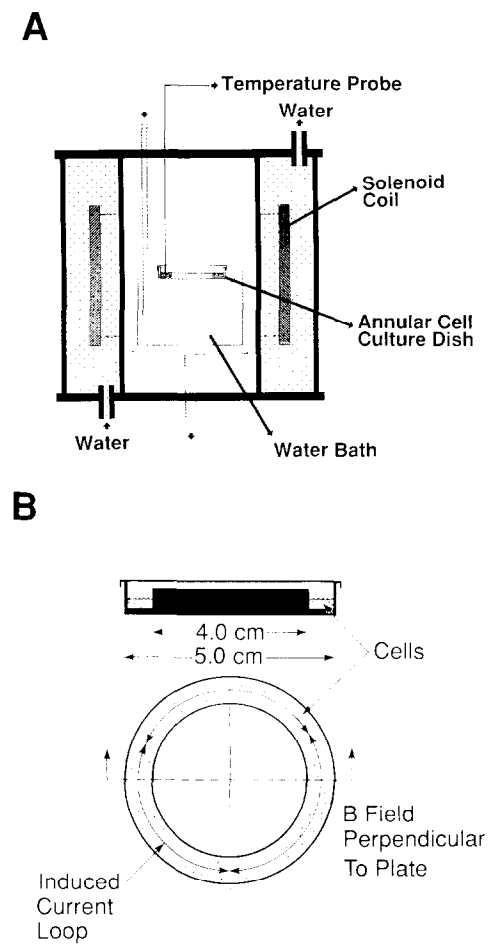


Fig. 1. Schematic representation of the magnetic field exposure system. (A) Cross-section view of the solenoidal, water-cooled exposure system with components drawn to scale. (B) Annular ring Petri dish. See section 2 for details regarding dosimetry, temperature control, ambient field environment, and the rationale for employing an annular ring Petri dish.

by more than ±2% between exposed and corresponding isothermal control samples. Osmolarity and pH of the cell suspension before and after field and control exposure agreed to within ± 0.5%.

2.3. *Magnetic field exposure system and temperature control*

A 60 Hz power frequency current was fed through the solenoid in the exposure system depicted in Fig. 1; heat was effectively removed by a water-cooling system. The resultant magnetic field established in the bore of the solenoid was mapped using a Gaussmeter (Bell, Mod. 620) with a calibrated axial Hall-effect probe. Magnetic flux density in the active exposure volume was 22.0 $T_{rms}$ (field uniformity was within 3%). A glass container was placed in the center of the solenoid as a secondary waterbath for the Petri dish; this system was physically separated from the solenoid in order to prevent transmission of any mechanical vibrations. A plastic annular Petri dish, fabricated from Falcon dish No. 3001 (lid) and No. 3002 (bottom) using acrylic cement (GC Electronics, USA; Fig. 1b), was placed inside the secondary waterbath (Fig. 1a). Temperature was routinely checked before and after all field and control exposures; selected temperature monitoring during exposures was also performed. Temperature variations did not exceed ±0.1°C from 37.0°C as detected by an ELF field noninteracting thermistor probe (Vitek, Inc., USA) with an accuracy of ±0.01°C. For comparison, the temperature rise induced by the 60 Hz ELF field due to Joule heating in a nonthermostatted sample over a 1 h period is calculated to be less than $10^{-6}$°C. The geometry of the cell suspension volume exposed to the magnetic field is very important: a cell suspension in a standard Petri dish has a series of induced current loops that degenerate to zero at the center of the dish. As a result exposures in an ordinary dish are spatially nonuniform with regard to induced electric field intensity ($E$). Therefore, we employed an annular dish to expose cells to a spatially defined induced $E$, where $E_{max} = \pi \cdot f \cdot r \cdot B_0$, $f$ is the frequency in Hz, $r$ the maximal annulus radius in m, and $B_0$ the magnetic flux density in tesla [16]. $E_{max}$ refers to the maximum value of the sinusoidally oscillating induced $E$. In our experiments a 5.0 cm diameter circular path for induced $E_{max}$ corresponds to 1 mV/cm. This is associated with a maximum induced current density, $J_{max}$, of 16 $\mu$A/cm$^2$ where $J_{max} = \sigma \cdot E_{max}$; conductivity ($\sigma$) of the cell suspension was measured to be 1.6 S/m (YSI, Mod. 35, USA). The local geomagnetic field intensity in the nonenergized exposure system parallel to the applied 60 Hz magnetic field was 44 $\mu$T (Bell, Mod. 620). The 60 Hz magnetic field environment in the control waterbath was less than 1 $\mu T_{rms}$.

## 3. RESULTS

### 3.1. *Effect of 60 Hz magnetic field exposure on Con A-activated thymocytes*

The effect of a sinusoidal 60 Hz magnetic field on transmembrane $Ca^{2+}$ transport in rat thymocytes that were activated by the T-cell mitogen Con A was characterized. Fig. 2 shows the values for net $^{45}Ca^{2+}$ uptake observed during a 60 min exposure period ($E_{max}$ = 1.0 mV/cm) and for nonexposed but identically and simultaneously treated control cells. Incubation of thymocytes with Con A for 1 h at 37°C in the absence of the 60 Hz field resulted in a 2.4-fold increase in $^{45}Ca^{2+}$-uptake relative to nonactivated, resting cells. In contrast, in the presence of the field an average 3.9-fold increase in $^{45}Ca^{2+}$ uptake was observed in Con A-activated thymocytes compared to resting cells. Analysis by two-tailed paired $t$-test shows that this field effect on $Ca^{2+}$-uptake is statistically significant ($n$ = 5, $P$ = 0.007).

### 3.2. *Effect of 60 Hz magnetic field exposure on resting thymocytes*

Unlike Con A-activated cells, resting thymocytes exposed to the ELF field for 1 h under identical exposure conditions showed no significant alteration of $^{45}Ca^{2+}$ uptake relative to nonexposed control cells (Fig. 3).

### 3.3. *Quantitation of the 60 Hz magnetic field effect and correlation with mitogen activation of thymocytes*

To quantitate the 60 Hz magnetic field-induced increase in Con A-dependent $^{45}Ca^{2+}$ uptake, the contribution from spontaneous $^{45}Ca^{2+}$ uptake into thymocytes was subtracted from both the exposed and matched control samples which received Con A (Fig. 2). This eliminated the contribution from Con A-independent, spontaneous $^{45}Ca^{2+}$ uptake which was not affected by the ELF field as was shown in Fig. 3. Comparison of Con A-dependent $^{45}Ca^{2+}$ uptake after

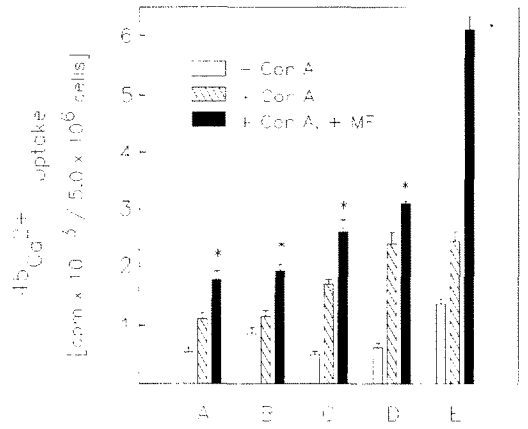


Fig. 2. Effect of a 60 Hz magnetic field of nonthermal intensity on $^{45}Ca^{2+}$ uptake in Con A activated rat thymocytes. −Con A, thymocytes in the absence of Con A; +Con A, thymocytes in the presence of Con A; +MF, thymocytes exposed to the 60 Hz magnetic field for 60 min. A–E denote thymocyte preparations taken from five different rats. Results are expressed as means ± SD of triplicate determinations. Data from individual experiments were statistically analyzed by the unpaired $t$-test: *, $P < 0.03$. Analysis of the pooled data by the paired $t$-test ($n = 5$): $P = 0.007$.

ELF exposure with the corresponding uptake in matched control samples revealed an average 2.7-fold increase in Con A-dependent $^{45}Ca^{2+}$ uptake due to the ELF exposure. The field responses among the cells from the five different rats were observed to vary from 1.4- to 4.5-fold. Review of the data in Fig. 2, however, revealed a correlation: thymocyte preparations with a diminished ability to mobilize $Ca^{2+}$ in response to Con A were more responsive to the ELF field (e.g. compare experiment D with E in Fig. 2). A statistical analysis of these data revealed that the percentage increase of Con A-dependent $^{45}Ca^{2+}$ uptake due to the field exposure was negatively correlated, in a log-linear fashion, with the percentage increase in mitogen activation of thymocytes to Con A alone ($r = -0.94$, $P =$

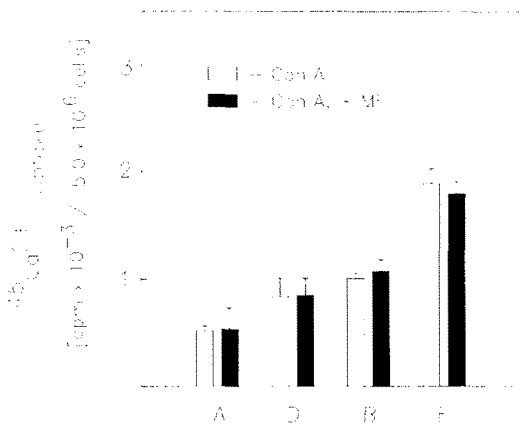


Fig. 3. Effect of a 60 Hz magnetic field of nonthermal intensity on $^{45}Ca^{2+}$ uptake in resting rat thymocytes. See legend to Fig. 2.

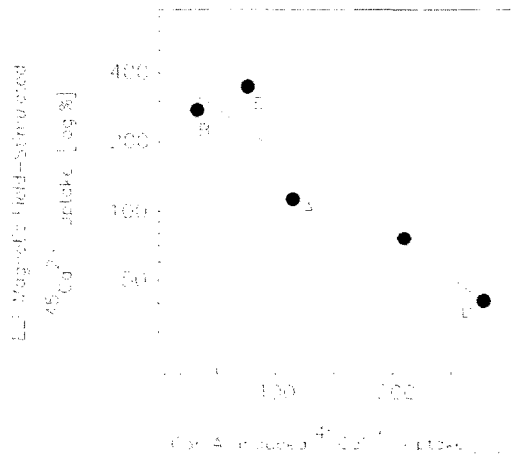

Fig. 4. Correlation of the magnetic field effect with mitogen activation. The line represents a least-squares fit to the log[% ELF field effect on Con A-dependent $^{45}Ca^{2+}$ uptake] vs % of mitogen activation as measured by Con A-induced $^{45}Ca^{2+}$ uptake ($r = -0.94$, $P = 0.016$). For details see text and legend to Fig. 2.

0.016; Fig. 4). Thus, reduced or suboptimally mitogen-activated cells were most responsive to the ELF magnetic field.

## 4. DISCUSSION

The results presented here demonstrate that nonthermal levels of a 60 Hz sinusoidal magnetic field (induced $E_{max} = 1.0$ mV/cm, 60 min) can significantly enhance $^{45}Ca^{2+}$ uptake in Con A-activated rat thymocytes, and, importantly, that the level of mitogen activation determines the intensity of the field response. In contrast, $Ca^{2+}$ transport is not influenced in nonactivated, resting thymocytes under identical exposure conditions. These findings provide insight into the role biological factors play in modulating or synergistically interacting with ELF fields to influence $Ca^{2+}$ metabolism in the thymocyte: (i) mitogen activation is necessary for ELF-induced enhancement of $Ca^{2+}$ uptake and (ii) reduced or suboptimal mitogen activation is associated with the largest field response. These observations are consistent with recent reports of altered DNA synthesis in lymphocytes that were exposed to pulsed magnetic fields for periods up to 72 h: field effects on lymphocyte DNA synthesis occurred in mitogen-activated lymphocytes and not in resting cells [5–8,10], and cells with diminished mitogen activation were most responsive to the fields [10]. Based on the findings reported here on $Ca^{2+}$ metabolism and on the role $Ca^{2+}$ plays in mitogen-induced DNA synthesis [17], it is possible that ELF modulation of early mitogen-triggered transmembrane $Ca^{2+}$-flux may represent a common pathway by which subsequent DNA synthesis is altered in ELF exposed cells. Although the cell membrane and its ability to transport $Ca^{2+}$ during activation are apparently in-

volved in the field effects described here, the precise molecular site of interaction remains to be determined. It is reasonable to hypothesize at this time that ELF modulation of either initial surface receptor-ligand interaction or of mitogen-activated $Ca^{2+}$ influx/efflux pathways are involved in triggering this field response. In addition, further research may provide evidence that this ELF field effect is frequency dependent. Although this study did not investigate effects of ELF fields on free intracellular calcium, $[Ca^{2+}]_i$, it is of interest that $[Ca^{2+}]_i$ has been shown to undergo low-frequency oscillations in thymocytes when these cells are activated by mitogen [17]. The findings presented here, and those mentioned above, underscore the important role that the biological status of the cellular system can play in ELF field effects.

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