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Pulsed magnetic field effects on calcium signaling in lymphocytes: dependence on cell status and field intensity

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The effect of 3-Hz, monopolar, quasi-rectangular magnetic field pulses on $^{45}Ca^{2+}$ uptake in resting and mitogen-treated rat thymic lymphocytes was evaluated. A 30-min, non-thermal exposure to the pulsed magnetic field (B_{peak} = 6.5 mT, E_{max} = 0.69 mV/cm, J_{max} = 2.6 μA/cm^2) reduced Concanavalin A-induced $^{45}Ca^{2+}$ uptake by 45%. It was observed that (i) the induction of the 3-Hz field response dependend on Ca^{2+} signal transduction activation; (ii) the response direction (stimulation or inhibition) depended on the level of lymphocyte mitogen responsiveness, and (iii) the field response magnitude increased with increasing magnetic field flux densities (B_{peak} = 0, 1.6, 6.5 and 28 mT). Our results demonstrate field effects at B_{max} nearly 10^4 greater than that of the average human environment for low-frequency magnetic fields and they are consistent with the independent results from other 3-Hz pulsed magnetic field studies with lymphocytes.

Calcium; Immune system; Signal Transduction; ELF Electromagnetic Field; Radical Pair Mechanism; Rat thymic lymphocyte

1. INTRODUCTION

Electromagnetic cellular interactions which are not thermally mediated but which apparently occur through an as yet unidentified *non-thermal* mechanism have been reported for different cellular systems in recent years [1–5]. Investigations of potential electromagnetic field effects on electrically *non-excitable* cells such as the cells of the immune system are of particular interest. The modification of immune cell activity by an EMF[2] would be of general physiological significance since the immune system is essential in protecting the organism against invasion by pathogens and is involved in tumor growth control [6]. To date, at least ten different laboratories, including our own, have reported ELF magnetic field influences on lymphoid cells, and stimulatory as well as inhibitory field effects on parameters related to calcium metabolism or RNA- and DNA-synthesis have been observed [6]. The field magnitude at which stimulatory or inhibitory effects have been observed is higher than that found in the average human environment (< 1 μT). Our previous study characterized the non-thermal effects of 60-Hz sinusoidal magnetic field exposures on Ca^{2+} uptake in resting rat thymic lymphocytes as compared to Con A-activated cells [7]. We then established that the activation of Ca^{2+} signaling pathways is a prerequisite for triggering field effects on Ca^{2+} uptake in rat thymic lymphocytes [7]. The present study investigated the ability of 3-Hz magnetic field pulses to affect Con A-induced Ca^{2+} signaling in the same cell model system. Here, we report the dependence of the 3-Hz field response on the cellular activation status and the applied field magnitude. Part of this study has previously been presented in abstract form [8]. Our results agree with earlier, independent 3-Hz, pulsed magnetic field studies with mitogen-treated lymphocytes [9–13].

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Abbreviations: EMF, electromagnetic field; ELF, extremely-low-frequency; Con A, concanavalin A; B, magnetic flux density, E, electric field intensity; J, current density; $[Ca^{2+}]_i$, cytoplasmic free calcium concentration; AC, alternating current; DC, direct current.

2. MATERIALS AND METHODS

2.1. *Lymphocyte Preparation and $^{45}Ca^{2+}$ Uptake Assay*

Rat thymic lymphocyte preparation, the quantitation of cellular $^{45}Ca^{2+}$ uptake and the calculation of the percentage field effect on Con A-induced Ca^{2+} uptake was done as already described [7], except cell transfer steps from one container (e.g. Petri dish) to the 1.5-ml tubes during cell processing was avoided. From the start of the 30-min field exposure until the end of the cell sample processing for scintillation counting, the samples remained in the same 1.5-ml eppendorf tubes. Thus, normalization of the isotope counts to the number of the recovered cells was not necessary. In a typical experiment, 5 μCi/ml $^{45}Ca^{2+}$ were added to 7 ml of cell suspension (5.0×10^6 cells/ml) and incubated for 5 min at 37°C. Thereafter, in the case of the activation experiments, Con A (2 or 20 μg/ml) was added to the 7-ml suspension, the cells were mixed thoroughly, and 1 ml-aliquots were transferred into each of six 1.5-ml tubes. Immediately, three of the tubes were placed in the water bath in the exposure system and the other three tubes in the isothermal control water bath (37 ± 0.1°C). At the end of the 30-min exposure, the cells contained in the six tubes (exposed and control samples) were sedimented simultaneously by centrifugation, and washed and analyzed by scintillation spectroscopy as described [7]. Statistical analyses were done by the paired or, where indicated, by the unpaired *t*-test. As an additional control, experimental runs

following exactly the above protocol were done, except that the exposure system was *non-energized* (for relevant results see Section 3.5).

2.2. *The Magnetic Field Exposure System*

A water-cooled solenoidal exposure system, similar to the one used previously [7], was built and a custom-made glass chamber for holding three 1.5-ml tubes immersed in water (at 37.0°C) in a vertical position was fixed in an acrylic tube and positioned inside the solenoid (Fig. 1). A water bath of identical design was used as the isothermal control bath and was positioned 4 m away from the exposure system for the parallel control experiments. Temperature variations were monitored with an ELF EMF-non-interacting thermistor probe (Vitek, Inc.) directly immersed in the cell suspension and did not exceed 0.1°C from 37.0°C in the exposed and control samples. Monopolar, quasi-rectangular magnetic field pulses were generated at a rate of three per second (50% duty cyclic) by feeding appropriate current signals from a custom-made, computer-controlled power supply capable of producing currents up to 200 Ampères through the solenoid. B_{peak} was 0, 1.6, 6.5 or 28 mT. Induced E_{max} inside the cell suspension was calculated to be 0, 0.04, 0.16 and 0.69 mV/cm, respectively. J_{max} was 0, 0.6, 2.6 and 11.2 μA/cm^2 according to $J_{max} = \sigma E_{max}$ (conductivity, σ, of the cell suspension was 1.6 S/m). The environment ELF magnetic field strength at the control site was < 1 μT_{rms}.

3. RESULTS

3.1. *Inhibitory 3-Hz pulsed magnetic field effects on $^{45}Ca^{2+}$ uptake in Con A-treated lymphocytes*

When lymphocytes were incubated with the T-cell mitogen Con A, an activator of Ca^{2+} transmembrane signaling processes in thymic lymphocytes, Con A-induced $^{45}Ca^{2+}$ uptake was enhanced by 53% after 30 min when no field was applied (Table I). In the presence of the 3-Hz, 6.5-mT pulsed magnetic field, however, this Con A-induced Ca^{2+} uptake was reduced by 45% in comparison to identically Con A-treated, but non-exposed, control cells (see group A in Table I). The detailed results from one experimental series are shown in Fig. 2, wherein is shown a significant inhibitory effect of magnetic field pulses on $^{45}Ca^{2+}$ uptake of a lymphocyte preparation which is responsive to Con A-stimulation. Incubation of the lymphocytes with Con A at a concentration of 2 μg/ml caused a comparatively smaller increase in $^{45}Ca^{2+}$ uptake, which again appeared to be inhibited by the 3-Hz field. Only field effects at the higher, more effective Con A-concentration (20 μg/ml) were further characterized in the experiments described here (Table I).

3.2. *Stimulatory 3-Hz pulsed magnetic field effects on $^{45}Ca^{2+}$ uptake in Con A-non-responsive lymphocytes*

One third of the lymphocyte preparations studied did not respond (or responded very poorly) to Con A stimulation as measured by $^{45}Ca^{2+}$ uptake. Con A-responsive cell preparations are designated 'A' in Table I. Non-responsive cells (group B in Table I) incubated with Con A in the presence of the 3-Hz, 6.5-mT pulsed magnetic field, showed a significant increase of $^{45}Ca^{2+}$ uptake by an average of 39% as compared to matched control cells. Shown in Fig. 3 is a 90% field-induced

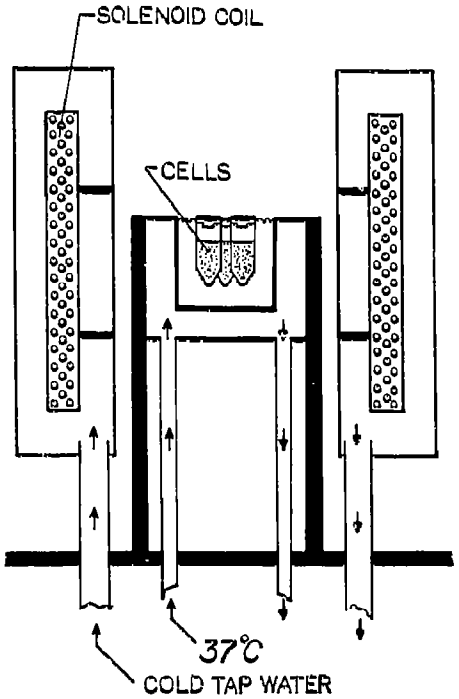

Fig. 1. Cross-section view of the water-cooled, solenoidal magnetic field exposure system (components drawn to scale). Design specifications of solenoid coil: r = 0.064 m; l = 0.125 m, N = 175, copper magnet wire No. 10; R = 0.32 Ω. For details see Section 2.

increase in $^{45}Ca^{2+}$ uptake for a cell preparation which was not responsive to Con A (at 20 μg/ml).

3.3. *No effect of the 3-Hz pulsed magnetic field exposure on $^{45}Ca^{2+}$ uptake in resting lymphocytes*

In the absence of Con A (i.e., with non-activated, G_0-state lymphocytes), there was no effect of the 3-Hz,

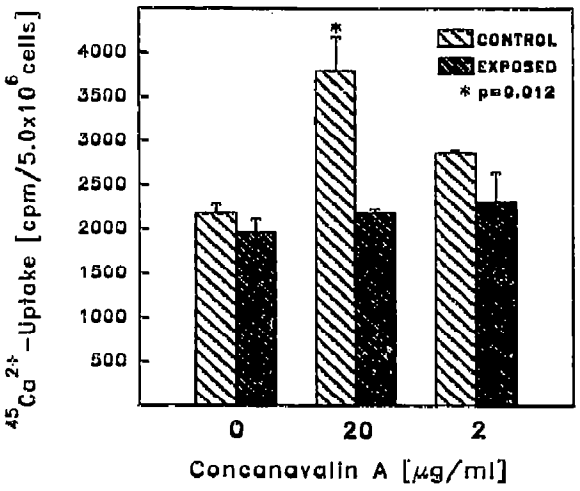

Fig. 2. Effects of a 3-Hz pulsed magnetic field on Con A-responsive rat lymphocytes. See group A in Table I. Results are expressed as means ± S.E.M. of triplicate determinations. Statistical significance was assessed with the unpaired t-test.

Table 1

Relationship between Con A-dependent $^{45}Ca^{2+}$ uptake and the direction of the field response at B_{peak} = 6.5 mT.

Group	Con A-dependent $^{45}Ca^{2+}$ uptake	3-Hz magnetic field effect on Con A-dependent $^{45}Ca^{2+}$ uptake
A[a] (n = 10)	+ 53.1 ± 5%[b] $P < 0.01$	− 45.0 ± 17% $P < 0.025$
B (n = 6)	+ 5.3 ± 5% $P > 0.05$	+ 39.2 ± 12% $P < 0.025$

[a]A, Con A-responsive lymphocyte preparation; B, Con A-non-responsive lymphocyte preparation. [b]values are means ± S.E.M.

6.5-mT pulsed magnetic field on cellular Ca^{2+} transport in eight different experiments. The results from two such experiments are shown in Figs. 2 and 3 (0 μg Con A/ml).

3.4. *Correlation of the biphasic magnetic field response with the cellular activation status*

The pulsed field effects illustrated in Figs. 2 and 3 represent extreme cases of the two observed responses (i.e. inhibition and stimulation) to the 3-Hz, 6.5-mT magnetic pulsed fields. However, only in these cases did we observe such remarkably large effects. In most other experiments, effects typically in the order of 30–60% were measured, and in five out of 16 experiments no effects were observed for reasons still unknown. The results from the 16 separate experiments, including the experiments with the negative findings, are summarized in Fig. 4. This graph illustrates that the response of the different lymphocyte preparations towards Con A as well as the magnitude and the direction of the 3-Hz field

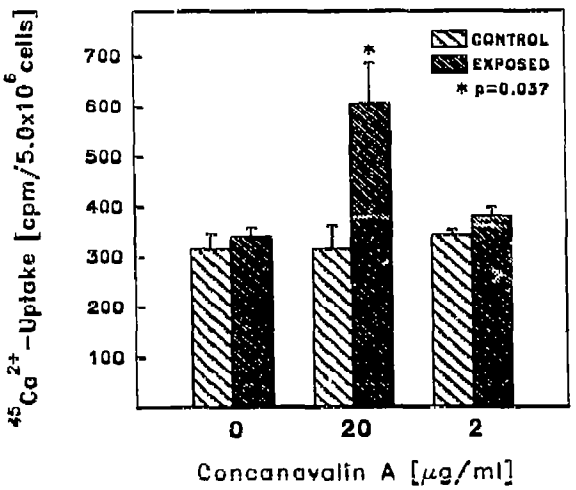

Fig. 3. Effects of a 3-Hz pulsed magnetic field on rat lymphocytes which are not responsive to Con A. See group B in Table I and legend to Fig. 2.

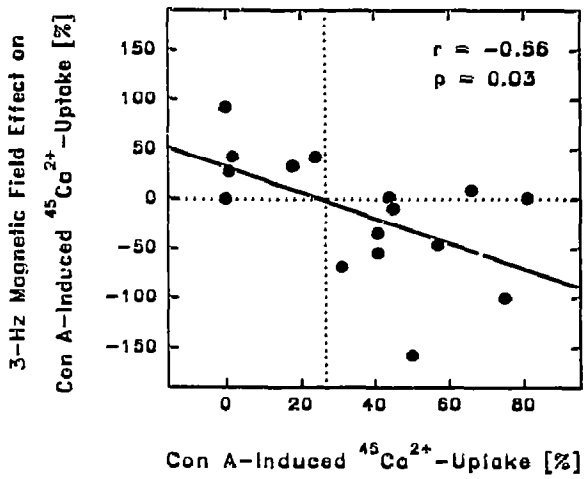

Fig. 4. Correlation of the 3-Hz pulsed magnetic field effect with Con A-responsiveness. The solid line represents the least-squares fit to the 3-Hz field effect on Con A-induced $^{45}Ca^{2+}$ uptake vs. the level of Con A-responsiveness. The dotted vertical line marks 25% Con A-induced $^{45}Ca^{2+}$ uptake. For details see text.

effect varies greatly. Nevertheless, it is clear that Ca^{2+} uptake by cells with no or poor (< 25%) Con A-responsiveness was stimulated by the pulsed field, whereas in cell preparations with better than 25% Con A-responsiveness, Ca^{2+} uptake was significantly reduced (see also Table I). A regression analysis of the magnitude of the field stimulation effect on Con A-induced $^{45}Ca^{2+}$ uptake (y-axis) versus the level of Con A-responsiveness of the 16 tested lymphocyte samples (x-axis) reveals a significant negative correlation between these two parameters ($r = -0.56$, $P = 0.03$; see Fig. 4).

3.5. *Field intensity dependence of the 3-Hz pulsed magnetic field effect*

In addition to the experiments at B_{peak} = 6.5 mT, 30-min exposure experiments at B_{peak} = 0, 1.6 and 28 mT were also performed. For this part of the study only lymphocyte preparations with a Con A-responsiveness greater than 25% were exposed, with the exception of exposures at the highest field strength (B_{peak} = 28 mT). At this intensity Con A-induced $^{45}Ca^{2+}$ uptake was always inhibited both in weakly as well as normally Con A-responsive lymphocytes (not shown). Experiments done when no current signal was fed through the solenoid (sham-exposure runs at B = 0 mT), as expected, did not significantly alter Con A-triggered $^{45}Ca^{2+}$ uptake (3.2 ± 13.4%, $P > 0.5$, n = 4; see Fig. 5). Furhter, 30-min exposures to the 1.6-mT, 3-Hz field decreased $^{45}Ca^{2+}$ uptake by 29.8 ± 21.2%, although this field response still was statistically nonsignificant ($P > 0.05$, n = 4). In contrast, the 6.5 and 28-mT magnetic field pulses significantly reduced Con A-induced $^{45}Ca^{2+}$ uptake by 45.7 ± 17% ($P < 0.025$) and 95.6 ± 5.3% ($P < 0.01$), respectively (see Fig. 5).

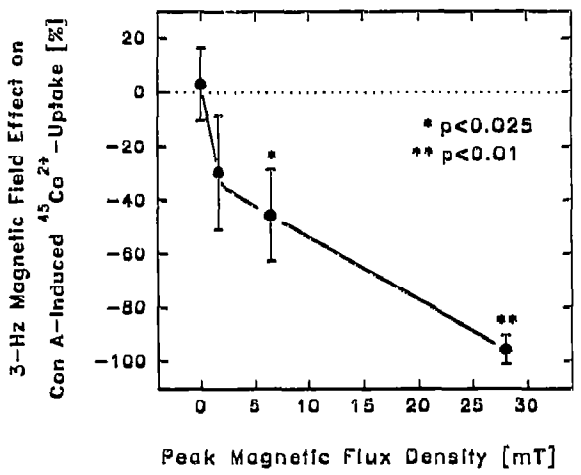

Fig. 5. Intensity dependence of the 3-Hz pulsed magnetic field effect. Bars are ± S.E.M. The number (n) of individual experiments at each peak magnetic flux density value were: 0 mT (n = 4), 1.6 mT (n = 4), 6.5 mT (n = 10) and 28.0 mT (n = 3). For details see Section 3.5.

4. DISCUSSION

We report here that non-thermal intensities of pulsed magnetic fields can alter $^{45}Ca^{2+}$ uptake in Con A-treated rat thymic lymphocytes. We confirmed our previous finding that Ca^{2+} uptake in resting cells was not influenced by the field [7], and found that the direction of the observed field responses (stimulation or inhibition) was dependent on the cellular activation status (see Fig. 4 and Table I). Further, the magnitude of the field response increased with increasing magnetic flux densities (Fig. 5). In our initial study with 60-Hz sinusoidal magnetic fields (B_{rms} = 22 mT) we found that $^{45}Ca^{2+}$ uptake in the same experimental model system was *enhanced*, not reduced [7]. We now find that 3-Hz pulses, in a dose-dependent manner, may *reduce* Con A-triggered $^{45}Ca^{2+}$ signaling. This shows that qualitatively different field responses can be triggered by applying different EMF signals. In combination, these results suggest that variations in both physical field exposure characteristics as well as biological parameters (e.g. the state of the cell) can determine the outcome of cellular EMF exposure experiments. Inhibition, stimulation or no field effect can be observed in direct dependence on physical and biological boundary conditions.

Interlaboratory reproducibility of basic experimental findings is a major concern in investigations of potential biological EMF effects. It is, therefore, of interest to compare our findings with the independent results reported by Conti et al. In 1985 concerning 3-Hz pulsed magnetic field effects on mitogen-dependent $^{45}Ca^{2+}$ uptake in human lymphocytes [10,11]. Although Conti et al. [10,11] did not specify the maximum dB/dT value used in their study, it is nevertheless clear that the exposure conditions used by these authors were very similar to ours (i.e., f = 3 Hz monopolar pulses, quasi-rectangular wave form, B_{peak} = 6 mT). Both their study and ours find that $^{45}Ca^{2+}$ uptake in mitogen-treated lymphocytes is reduced significantly by the 3-Hz field (see Table II). Additionally, both studies find that Ca^{2+} uptake in resting, G_0-state lymphocytes is not affected by the pulsed field. In contrast to our study, Conti et al. did not report stimulatory field responses. This is not surprising, however, since the lymphocyte preparations used in their experiments were clearly responsive to the mitogen treatment [10, 11]. Thus, their findings do not contradict our own observation of the stimulatory field effect, because we show that Ca^{2+} uptake can be enhanced only in cell preparations with no or poor mitogen-responsiveness (see Table I and Fig. 4). Within this context, it should also be pointed out that ELF pulsed magnetic fields not only may alter Ca^{2+} uptake but also may increase $[Ca^{2+}]_i$ as was shown with spectrofluorimetry of Indo-1-loaded HL-60 cells by Carson et al. [14].

An important question is whether the field effect on lymphocyte Ca^{2+} signaling observed in this study might affect subsequent cellular responses, such as proliferation. Recently, Walleczek (1992) reviewed the relevant

Table II

Pulsed magnetic field studies with mitogen-treated lymphocytes: results from three independent laboratories

Field effect description	Exposure parameters[a]					
	f, Hz	B, mT	dB/dt, T/s	E, mV/cm	t, h	Ref.
55% reduction in [^{3}H]thymidine uptake in human lymphocytes	3.0	6.0	n.r.[b]	n.r.	72	[10,11].
up to 60% reduction in [^{3}H]thymidine uptake in human lymphocytes	3.0	4.5	13.0	0.23	72	[13]
70% reduction in $^{45}Ca^{2+}$ uptake in human lymphocytes	3.0	6.0	n.r.	n.r.	1	[10,11].
45% reduction in $^{45}Ca^{2+}$ uptake in rat thymic lymphocytes	3.0	6.5	6.5	0.16	0.5	[this paper]

[a] f, pulse frequency; B, peak magnetic flux density; dB/dt, time variation of B; E, peak induced electric field strength; t, exposure duration; [b] n.r., not reported.

evidence and proposed that many of the reported ELF EMF effects on immune cells, including lymphocyte proliferation, could, at least in part, be explained by an interference of the applied EMF with Ca^{2+} signaling processes [6]. The findings presented here, together with the independent results from two other laboratories, lend further support to this hypothesis. Conti et al. [9–12] and Mooney et al. [13] reported that 3-Hz pulsed magnetic field exposures of cultured, mitogen-treated human lymphocytes for 6 to 72 h reduced DNA synthetic activity an average of 30 to 60% ($P < 0.01$) compared to non-exposed control cells. Again, in agreement with our observations, the two groups found no significant effects of the 3-Hz field on DNA-synthesis in resting, G_0-state cells [9–13]. For an overview of the consistency of 3-Hz pulsed magnetic field effects see Table II. Apparent successes in reproducing pulsed magnetic field effects in an interlaboratory study, using 50-Hz pulsed magnetic fields and human lymphocytes, have recently been noted by Cadossi et al. [15], although no full account of their data has been made available yet. With regard to possible mechanisms of interaction, it was reported that sinusoidal electric fields alone, although of 4-fold higher intensity than the minimum effective E_{peak} used here, could influence Con A-dependent $^{45}Ca^{2+}$ uptake by rat lymphocytes [16]. Our findings, and other results at $B_{rms} < 1$ mT and very small associated electric field strengths (< 1 μV/cm; [17–20]) can be cited to support a hypothesis of a direct magnetic coupling mechanism.

A physically-plausible magnetic interaction mechanism based on radical pair recombination reactions which are linked to cellular signal transduction and amplification processes has been proposed [5]. Magnetic field intensities similar to the intensities used in most lymphocyte EMF experiments (e.g. 1–30 mT; see [6]) are known from magnetochemistry to be able to influence *non-thermally* the kinetics and product yields from radical pair reactions *in-vitro* [21]. The underlying reaction scheme is well-known and is described by the radical pair mechanism (e.g. [21]). For this mechanism to be applicable to the data reported here, a pathway by which magnetically-sensitive radical-dependent processes could influence mitogen-induced lymphocyte Ca^{2+} signaling must be postulated. There is new evidence that such pathways might exist. For example, Con A-induced Ca^{2+} uptake in rat thymic lymphocytes has been shown to depend on the generation of reactive oxygen radical species (e.g. [22]). There is also evidence from inhibition studies that cytochrome P-450 activity may be involved in Ca^{2+} uptake regulation in rat thymic lymphocytes [23] and it is known that P-450 function proceeds via radical pair recombination steps [24]. Thus, it is plausible to investigate if externally applied magnetic fields (e.g. 3-Hz pulses at $B_{peak} = 6.5$ mT) may interfere with radical pair reactions and, as a consequence, may alter lymphocyte Ca^{2+} regulation. One promising experimental approach to test this hypothesis is the use of differential real-time fluorescence spectroscopy to monitor dynamic changes (e.g. in $[Ca^{2+}]_i$, ionic fluxes or P450 enzyme activity) in magnetic field-exposed and unexposed control samples, simultaneously [27].

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