ARE EXTREMELY LOW FREQUENCY MAGNETIC FIELD EFFECTS ON CYTOSOLIC CALCIUM IN JURKAT CELLS DEPENDENT ON CELL CYCLE?

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ABSTRACT

To determine if extremely low frequency magnetic field (ELF MF) effects on cytosolic calcium concentration ($[Ca^{2+}]_c$) are dependent on the phase of the cell cycle, we synchronised Jurkat E6.1 cells through starvation, thymidine block, and elutriation. Samples with enriched G0-G1, S, or G2-M were exposed to a null static magnetic field or to 60Hz, 100 μT sinusoidal MF plus a 78.1 μT static MF. The $[Ca^{2+}]_c$ was determined using ratiometric fluorescence techniques. A significant decrease in $[Ca^{2+}]_c$ was found when G0-G1 phase was enriched implying that ELF MF effects on $[Ca^{2+}]_c$ are dependent on the phase of the cell cycle.

INTRODUCTION

A number of studies have examined the effect of extremely low frequency magnetic fields (ELF MF) on the kinetics of the cell cycle but we are unaware of any studies that have examined the dependence of an ELF MF effect on the phase of the cell cycle. It is possible that cells may be more sensitive to ELF MF effects during specific phases of the cell cycle, similar to cell killing efficacy of ionizing radiation. Previous results from our laboratory have suggested that the effects of extremely low frequency magnetic field (ELF MF) exposure on cytosolic calcium concentration and calcium signalling are confounded by distribution within the cell cycle [1]. The objective of this study is to determine if ELF MF effects on $[Ca^{2+}]_c$ and calcium signalling in Jurkat E6.1 cells are dependent on the phase of the cell cycle (G0-G1, S, and G2-M).

METHODS

The human lymphocytic cell line, Jurkat E6.1 clone, was synchronized using either serum starvation, double thymidine block, or elutriation. The distribution of the cell culture within the cell cycle was determined using propidium iodide labelling and flow cytometery. ELF MF exposure experiments were performed with enriched G0-G1, S, and G2-M phase cell cultures. We used a custom designed spectrophotometer to measure $[Ca^{2+}]_c$ in cells suspended in conditioned RPMI 1640 medium containing 10% foetal bovine serum. Two magnetic field exposures were compared: zero static MF (Null) and the combination of 60 Hz, 100 μT sinusoidal plus 78 μT static MF (AD+DC). All MF were applied in the vertical direction. Cells were cultured in a mu-metal box to reduce ambient static and ELF MF. The $[Ca^{2+}]_c$ in Jurkat cells loaded with Indo 1-AM was determined using ratiometric techniques. In all experiments, cells were exposed to a null static magnetic field condition (<0.4 μT) for the first 5 min and this was used for normalisation of subsequent data in each experiment. To stimulate a calcium signal, α-CD3 was introduced into the cell samples after 1200s of exposure and the calcium signal was monitored for another 1200s. A number of calcium parameters were compared using multivariate analysis (SPSS) and results were confirmed by a non-parametric test (Mann-Whitney U p-values reported).

RESULTS

When Jurkat cells were synchronized by serum starvation, a significant decrease in normalised $[Ca^{2+}]_c$ between 555-615s was observed when G0-G1 phase enriched cell samples were exposed to the AC+DC (n=16) field in comparison to the Null group (n=14; p = 0.028). When S phase enriched cell samples (n = 5 for both AC+DC and Null groups) were compared, a significant decrease in normalised $[Ca^{2+}]_c$ at t=615s (p=0.047) and t=2100s (p=0.028) was observed. However, no significant differences were detected when the cell samples were G2-M phase enriched. To increase the enrichment of the cell culture, Jurkat cells were synchronized by double thymidine block. No significant differences were detected when the cell samples were S phase enriched.

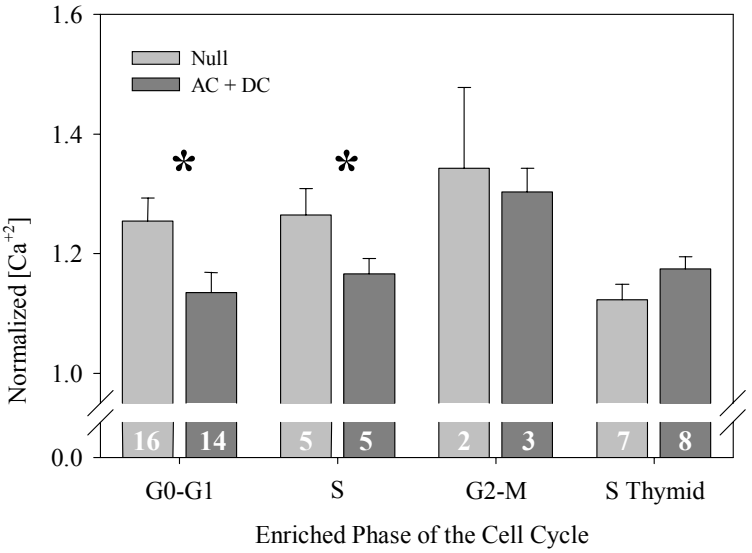

Fig. 1. Preliminary results suggested ELF MF effects on $[Ca^{2+}]_c$ were apparent only when Jurkat cells were enriched with cells in G0-G1 or S phase through serum starvation. The number at the bottom of the bars indicates the sample size and error bars represent the standard error of the mean. The asterix indicates $p<0.05$ when Null and AC+DC groups were compared.

DISCUSSION

We have determined the effect of ELF MF exposure on $[Ca^{2+}]_c$ and calcium signalling in Jurkat cell culture enriched with cells in S phase by two techniques. Despite greater S phase enrichment when the cells were synchronized using thyimidine, ~65% of the cells were in S phase compared to ~42% achieved with serum starvation, no differences in $[Ca^{2+}]_c$ or calcium signalling were detected when thymidine was used. It is possible that the thymidine treatment interfered with the ELF MF effects observed when serum starvation synchronization was used. Future experiments using elutriation techniques to separate cells according to phase within the cell cycle will be completed to determine if ELF magnetic field effects on cytosolic calcium in Jurkat cells dependent on cell cycle.

REFERENCES

[1] C.R. McCreary, A.W. Thomas, and F.S. Prato, "Factors Confounding Cytosolic Calcium Measurements in Jurkat E6.1 Cells during Exposure to ELF Magnetic Fields,". *BEMS*, vol. 23, pp. 315-328, May 2002.

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