Interaction Mechanisms of Low-Level Electromagnetic Fields in Living Systems

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Interaction of low-level combined static and extremely low-frequency magnetic fields with calcium ion transport in normal and transformed human lymphocytes and rat thymic cells

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Introduction

The alteration of release of calcium ions from biological tissue by electromagnetic stimulation has been reported by several authors (Kaczmarec and Adey 1974; Bawin *et al.* 1975, 1978; Bawin and Adey 1976; Blackman *et al.* 1979, 1980*a*,*b*; Joines and Blackman 1980, 1981; Joines *et al.* 1980). Bawin *et al.* (1975) reported that 147 MHz radiofrequency (RF) electromagnetic radiation altered the release of calcium ions from chicken brains *in vitro* only at specific modulation frequencies between 6 and 20 Hz, with a peak at 16 Hz. Similar findings have also been reported for human and other neuroblastoma cells (Dutta *et al.* 1984, 1989). Further examination of this phenomenon demonstrated the presence of power-density windows at different carrier frequencies (Blackman *et al.* 1979, 1980*a*, 1985*a*,*b*; Sheppard *et al.* 1979).

Blackman *et al.* (1985*b*) found that static magnetic fields in the order of the geomagnetic field density interacted with the applied low-frequency dynamic electromagnetic fields. Although there are several reports of experimental results, there is no model that can fully explain the mechanism of interaction involved at the very low field strengths in question (Blackman *et al.* 1988).

Liboff (1985) proposed a model which tried to explain the interaction of undulating electromagnetic fields with ionic species at geomagnetic flux densities. A charged ion moving in a plane normal to the Earth's magnetic field will experience a radial force (Lorentz force):

$$qvB = \frac{mv^2}{R}$$

where q is the charge of the ion; m the mass of the ion; v its velocity, R the radius of the curvature of the path.

Because of this force, the ion will execute a circular or a helical path. The velocity can be simply expressed as the product of the frequency of rotation f, and the path-length, leading to a unique frequency corresponding to the geomagnetic field $\boldsymbol{B}$:,

$$f = \frac{qB}{2\pi m}.$$

This is the same condition for accelerating charged particles in a cyclotron, and the phenomenon is therefore named 'ion cyclotron resonance'.

The Earth's geomagnetic field varies from about 70 μT at the poles to 25 μT at the geomagnetic equator, and averages about 50 μT at mid-latitude. For such fields, frequencies in the range of 10–100 Hz correspond approximately to charge/mass ratios of 0.01–0.1 electronic charge per atomic mass unit, indicating that biologically important ions, heavier than protons but lighter than enzymes and proteins, appear to have geomagnetic cyclotron resonance frequencies.

The 'ion cyclotron resonance hypothesis' was explored by Liboff *et al.* (1987) in an experiment involving incorporation of calcium-45 ($^{45}Ca^{2+}$) in mixed human lymphocytes. The geomagnetic horizontal field component was adjusted to 21 μT. The experiment was first performed at an amplitude of the applied oscillating field of 150 μT and a sharp *minimum* was obtained at the frequency of 14.3 Hz, which corresponds to the ion cyclotron frequency at 21 μT. The experiment was then repeated at an amplitude of 21 μT and now a sharp, narrow *maximum* occurred at 14.3 Hz.

Although there appears to be experimental evidence for resonance phenomena, the cyclotron resonance hypothesis is shown to violate the laws of classical mechanics (Halle 1988). Halle (1988) also demonstrated that the magnetic effect on single-ion dynamics is insignificant, due to dynamic friction in fluid media, and argues that the experimental response should rather be a collective phenomena.

Lednev (1990) and Hart (1990) treat quantum mechanically the interaction of a low-frequency field with an ion bound loosely to a membrane surface in the presence of a static magnetic field, and claim evidence for resonance phenomena.

In the present paper we have tried to demonstrate the presence of resonance phenomena for influx and efflux of radioactive calcium-45 ions in human normal and transformed lymphocytes and in rat thymocytes.

Material and methods

Magnet coils

The apparatus used for exposure consisted of two pairs of Helmholtz coils placed orthogonally to each other. The axis of the vertical coils was oriented in the north–south direction and the axis of the other pair in the horizontal plane. The diameter of the coils was 230 mm wound with 100 turns of 1.5 mm diameter enamelled copper wire. The horizontal coils were coupled in series at a distance of 230 mm and used for compensating the vertical component of the Earth's geomagnetic field. A flux-gate magnetometer was used as an indicator and was balanced to zero field in the vertical direction. The vertical component of the geomagnetic field was balanced to 21.0 μT using the bias voltage from the pulse generator. A sinusoidal time-varying field with adjustable frequency and amplitude was also applied to the vertical coils. The frequency was monitored by using a frequency meter. The amplitude of the undulating field was checked at the centre of the coils using a pick-up coil. The induced electro-motoric force was recorded on the oscilloscope.

Calcium tracer and radioactivity measurements

Radioactive calcium-45 with a radioactivity concentration of 370 MBq ml^{-1} and low stable calcium concentration was used. About 0.2 μl was added to the stock solution of mixed media used in each experimental series. The cells were collected on a filter (Whatman GFA) and washed seven times with inactive media of the same composition as the one in which the cells were exposed. The filters were mounted on the glasses of slide-frames and could slide in a reproducible way in position under an end-window GM-tube counter.

Cells and media

These were specially prepared as described for each experiment.

Experiments and results

Uptake of ^{45}Ca in normal human lymphocytes

Normal human lymphocytes were prepared to 6×10^6 cells per ml in calcium-free buffer solution (Hank). Calcium-45 tracer solution was prepared in 0.02 mM Ca to an activity concentration of 40 kBq ml^{-1}. Triplicate control and experimental round-bottomed microtitre plates were

prepared immediately prior to magnetic field exposure by combining 50 μl ^{45}Ca-tracer solution and 50 μl cell suspension. The horizontal magnetic field was adjusted to zero and the vertical field to 21–22 μT. The applied oscillating vertical field had an amplitude of 29.7 μT peak to peak. The frequency of the vertical magnetic field was 14.27 Hz. The experiment was first performed at 60 min exposure time and then repeated several months later at 15 and 60 min exposure times.

The ratio of activity measurements of exposed and control cells was 1.5 ± 0.4 (SD) in the first experiment after 60 min exposure. In the second experiment the corresponding ratio at 15 min was 1.5 ± 0.6 and at 60 min 0.6 ± 0.2. Thus there seems to be no significant difference in calcium uptake between exposed and control cells in those two experiments.

Study of the effect of ELF frequency on the uptake of ^{45}Ca in human lymphocytes and transformed lymphoma cells

Normal human lymphocytes and transformed lymphoma (YAG) cells were adjusted to 2×10^6 cells per ml in calcium-free buffer solution (Hank). Calcium-45 tracer solution was prepared in 0.23 mM Ca to an activity concentration of 74 kBq ml^{-1}. Triplicate control and experimental round-bottomed microtitre plates were prepared immediately prior to magnetic field exposure by combining 50 μl ^{45}Ca-tracer solution and 50 μl cell suspension. The horizontal magnetic field was adjusted to zero and the vertical field

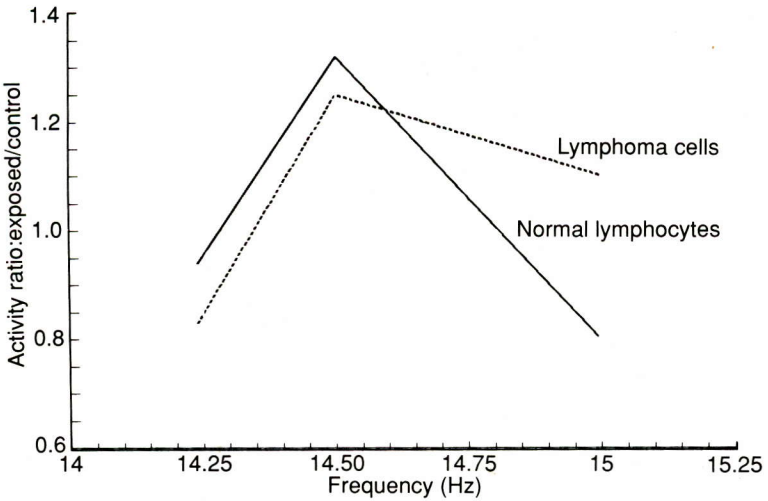

Fig. 11.1 The ratio of ^{45}Ca activity in exposed and non-exposed (control) normal lymphocytes and lymphoma (YAG) cells.

to 21–22 μT. The applied oscillating vertical field had an amplitude of 29.7 μT peak to peak and the exposure was performed at the three different frequencies: 14.27, 14.50, and 15.00 Hz.

The quotient of calcium-45 activity was measured after 60 min in exposed cells and control cells. The results are given in Fig. 11.1. The frequency was varied at a constant vertical magnetic flux density of 21 μT.

Uptake of and efflux of ^{45}Ca in rat thymus lymphocytes

Lymphocytes from thymus of Wistar W/FU rats were prepared either in RPMI (10 per cent fetal calf serum from Flow, Edinburgh, Scotland) or in buffer solution to 5×10^6 cells per ml with a calcium concentration of 2 mM. Calcium-45 tracer solution was prepared either in normal medium or in buffer solution with a calcium concentration of 2 mM and with the activity concentration of 150 kBq ml^{-1}. Triplicate control and experimental vials with 0.5 ml cell suspension and 0.5 ml calcium-45 solution were prepared immediately prior to magnetic field exposure. The vials were exposed in the centre of the combined pairs of coils, where the horizontal magnetic field was adjusted to zero and the vertical field to 21–22 μT. The applied oscillating vertical field had an amplitude of 29.7 μT peak to peak. The frequency of the vertical magnetic field was 14.27 Hz. Aliquots were taken from the vials every 15 min. The cells were immediately separated and measured for radioactivity. After 1 h in the magnet the cells were spun down

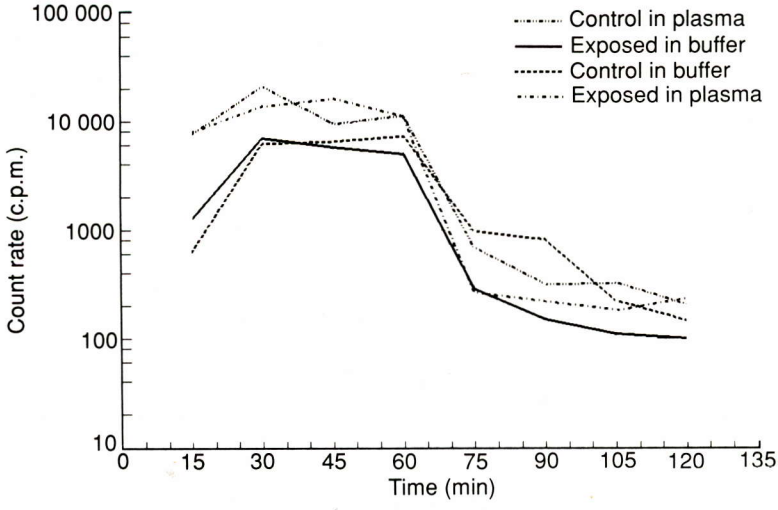

Fig. 11.2 The count rate of calcium-45 measured in both exposed and non-exposed (control) cells of rat thymus in plasma and buffer solution, respectively.

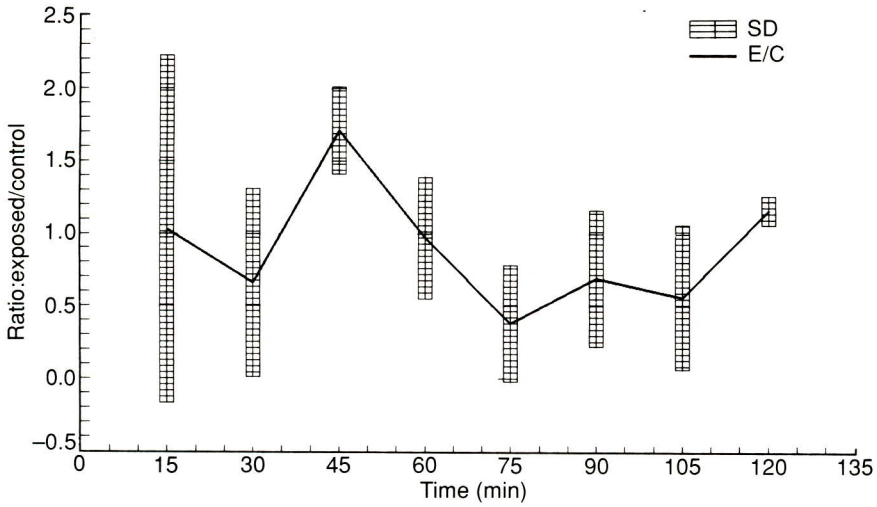

Fig. 11.3 The ratio of ^{45}Ca activity between exposed and non-exposed (control) cells incubated in plasma.

and washed with activity-free medium seven times. The same procedure was performed with the control. Then the exposure was continued in the magnet for another hour and samples were taken every 15 min.

The ^{45}Ca activity was measured in each triplicate preparation and the mean values were calculated. Figure 11.2 shows the count rate of measured activity in both exposed and non-exposed (control) cells of rat thymus in plasma and buffer solution, respectively. Figure 11.3 gives the ratio of ^{45}Ca activity between exposed and non-exposed (control) cells incubated in plasma, and Fig. 11.4 gives the same ratio for cells incubated in buffer.

Uptake of ^{45}Ca in human lymphocytes and leukaemia cells

Premyelocytic leukaemia cells (U 937 from Uppsala) and fresh human lymphocytes were prepared in RPMI (10 per cent fetal calf serum). The cells were spun down at 12000 r.p.m. for 5 min and washed twice in calcium-free trypsin buffer solution, PBS (phosphate-buffered saline) without Ca and Mg. Fifty microlitres of the cell suspension (2×10^5 cells) were mixed with 50 μl ^{45}Ca ('carrier free') in microtitre plates (NUNC Odense Denmark).

Triplicate control cells and experimental cells were prepared. The plates were exposed in the centre of the combined Helmholtz coil arrangement. The vertical component of the geomagnetic field was adjusted to zero and the horizontal to 21–20 μT. The applied oscillating field had an amplitude of 29.7 μT peak to peak, and a frequency of 14.27 Hz. Exposure times of

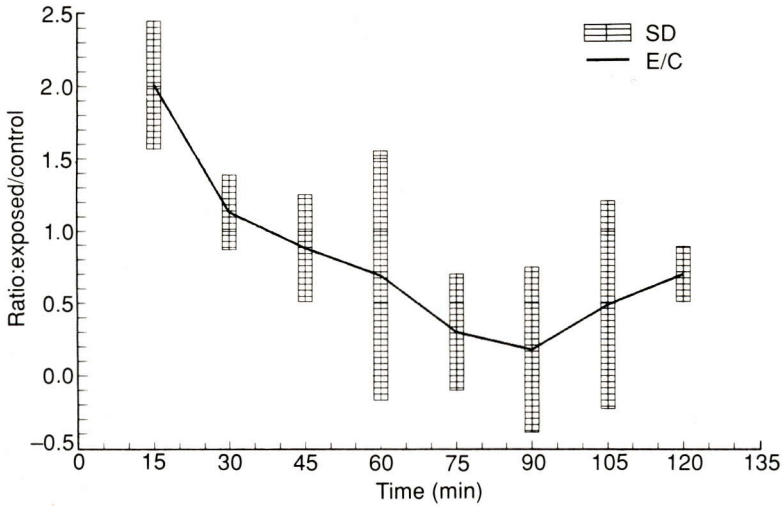

Fig. 11.4 The ratio of ^{45}Ca activity between exposed and non-exposed (control) cells incubated in buffer.

leukaemia cells were 5, 10, 15, 30, and 60 min; and for human lymphocytes, 15 and 60 min. After exposure the exposed cells and their controls were separated from the buffer and collected on Millipore filters (type AP-20 (Cat. No. AP 200 2200), Millipore, Ireland). One fraction of the cells exposed for 60 min was washed, cooled on ice to 4°C, and washed in ice-cooled calcium-free buffer. It was then exposed for another 15 min in the magnet.

The radioactivity was recorded in each triplicate preparation of leukaemia cells and the mean value was calculated. The quotient between exposed and control samples, as given in Fig. 11.5 varied from 0.89 to 3.24. There was a significant difference at 15 min, with a quotient of 3.24 ± 1.00 (SD). For human lymphocytes the quotients between magnet-exposed cells and controls were 0.83 ± 0.45 at 15 min and 0.54 ± 0.62 at 60 min. We observed the tendency of a higher quotient at 15 min although the values are lower than for leukaemia cells where the ratios were 3.2 at 15 min and 1.32 + 60 min.

Efflux of ^{45}Ca in leukaemia cells

Premyelocytic leukaemia cells (U 937) were prepared in RPMI (10 per cent fetal calf serum). The cells were incubated for 1 h in RPMI medium with ^{45}Ca added. The cells were washed twice in cooled (4 °C) calcium-free medium and exposed in the coil arrangement, as described previously, for

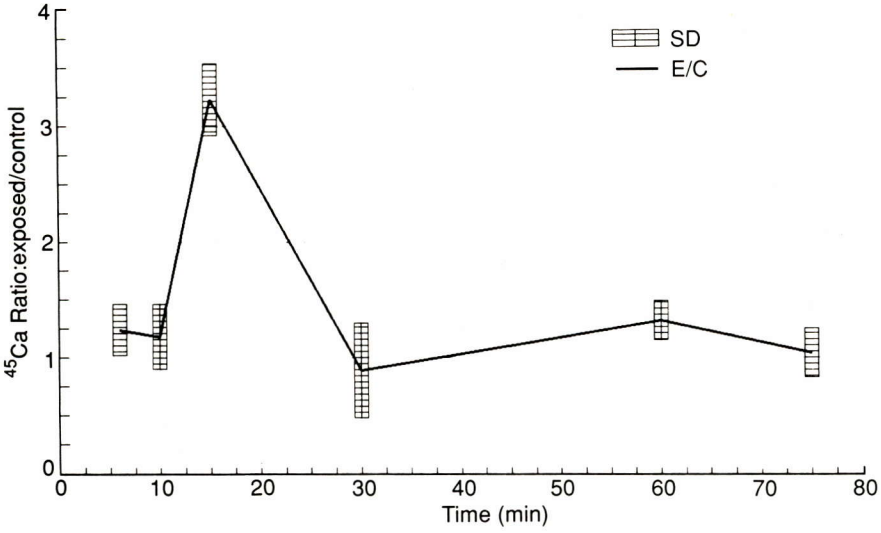

Fig. 11.5 Time variation of the ratio ^{45}Ca activity between exposed and non-exposed (control) premyelocytic leukaemia cells in calcium-free trypsin buffer.

15 min. After exposure the cells were filtered and measured as above. The quotient between exposed and control cells was 1.04 ± 0.21 (SD).

Temperature dependence of uptake of ^{45}Ca in leukaemia cells

Premyelocytic leukaemia cells (U 937) were prepared in RPMI (10 per cent fetal calf serum). The cells were spun down at 12000 r.p.m. for 5 min and washed twice in calcium-free trypsin buffer solution, PBS without Ca and Mg. Fifty microlitres of cell suspension (2×10^5 cells) were mixed with 50 μl ^{45}Ca 'carrier free' solution in small conical vials. The exposure took place for 30 min at the temperatures 4, 21, 37, and 43 °C in a specially constructed water-bath placed within the coil arrangement.

Table 11.1 The ratio of the ^{45}Ca activity at various temperatures

Temperature of exposure (°C)	Cells in calcium-free medium (average ± SD)
4	1.01 ± 0.34
21	0.69 ± 0.22
37	0.90 ± 0.22
43	0.82 ± 0.38

The results shown in Table 11.1 indicate that the quotient between exposed and control cells was less than 1 but with no significance.

Discussion

The uptake of ^{45}Ca in normal and transformed human lymphocytes indicates no significant fluctuation with frequency in the expected 'cyclotron resonance' region.

As shown in Fig. 11.2, the uptake of ^{45}Ca in both exposed and control lymphocyte cells is quite high. Therefore the effect must be quite strong to show any difference. The uncertainty in the exposed/control activity ratios is quite high and no resonance pattern can be seen in the fluctuations (Fig. 11.3).

An interesting observation, however, is the slight delay in the uptake of ^{45}Ca by the exposed cells in comparison to the control cells. This results in a time variation of the exposed/control ratio, as shown in Fig. 11.4. The same observation is true for the premyeolcytic cells, which results in the pattern shown in Fig. 11.5 with a peak at 15 min. The results of the temperature study shown in Table 11.1 indicate no effect at 4 °C but a reversed relationship with a ratio below 1 at higher temperatures.

Our negative findings, and in general the difficulty of reproducing earlier findings by other laboratories, might be caused by the fact that the sensitivity of calcium metabolism of the cell to ELF radiation seems to be highly dependent on the metabolic state of the cell. Walleczek and Liburdy (1990) showed that cells treated with concanavalin A, increasing the cells' mitogenic activity, largely enhances ^{45}Ca uptake. The dependence with time of ^{45}Ca uptake, as indicated by our experiments, might possibly be an expression of changing metabolic activity in the cell culture.

Another reason for the lack of effect in the present experiments might be that the radiation power-density was outside the power-density window that has been shown to exist in other experimental systems (Blackman *et al.* 1979).

The experimental method of studying influx and efflux of ^{45}Ca in lymphocytes should be further improved to study the kinetics of ^{45}Ca in more detail at various temperatures and varying experimental parameters. Therefore, further studies on this subject should be performed on the following premises:

(1) premyelocytic cells should be used;
(2) the temperature should be kept at 37 °C;
(3) the frequency amplitude of the oscillating field should be varied in order to find the resonance conditions;

(4) the calcium-concentration dependence should be carefully verified;
(5) biological transformations should be studied.

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