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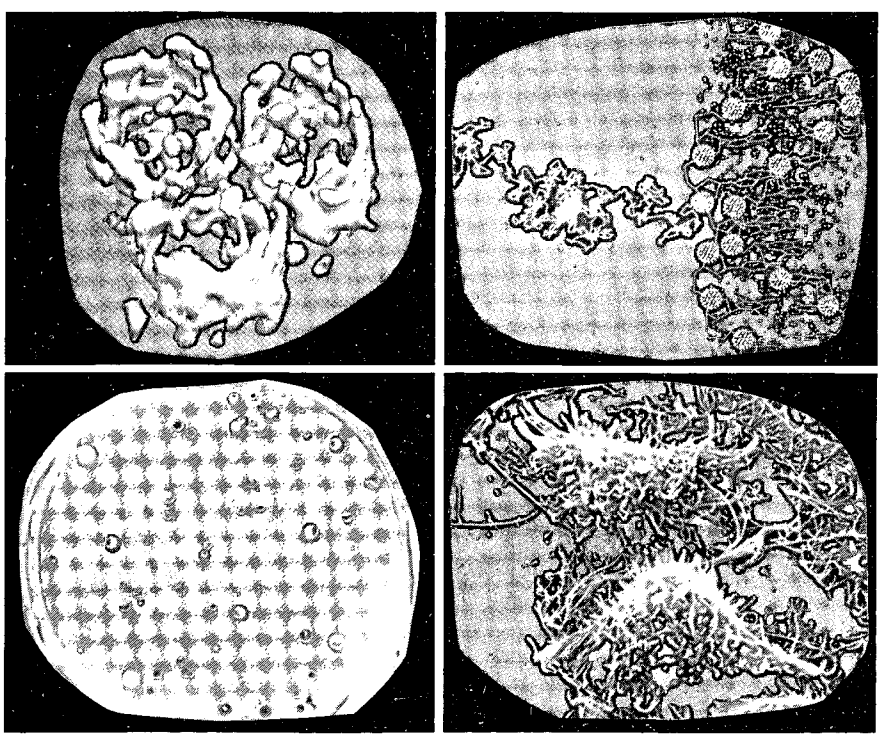
Ultrahigh Static Magnetic Fields and Signal Transduction in the Lymphocyte

R.P. Liburdy, M.S. Falk, and D.J. de Manincor

February 1993



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ABSTRACT Effects of ultrahigh static magnetic fields of 2.3, 7.5, and 9.0 Tesla on calcium transport in resting and in mitogen-activated lymphocytes were evaluated. Cells were maintained in static fields at 37°C ± 0.5°C for 30 minutes and calcium transport was followed using $^{45}Ca^{+2}$ in the absence or presence of mitogen (1 μg/ml Con-A). 2.3 Tesla fields did not influence calcium transport. 7.5 Tesla field exposure was associated with a 45% increase in calcium transport in resting cells (p=0.001) compared to control cells treated simultaneously in an isothermal, μ-metal-shielded waterbath located remote from the magnet (<30mG static magnetic field flux density). 9.0 Tesla resulted in a 26% decrease in calcium transport for resting cells and 15% for mitogen-activated cells. An apparent threshold for static magnetic field effects exists above 2.3 Tesla.

INTRODUCTION Motivation for performing biosafety and bioeffects studies employing ultrahigh static magnetic fields derives in part from the use of NMR imaging technology in the medical setting [1]. Current FDA guidelines permit the use of static fields up to 2.0 Tesla. There is interest in improving the spatial and temporal resolution of NMR imaging and this can be achieved through the use of static field in excess of 2.0 Tesla.

Previously we investigated the effects of a complete NMR imaging environment on calcium transport in resting and mitogen-activated rat and in human lymphocytes [2]. Lymphocytes from rats and humans were found to be unresponsive to static fields in the resting state; they transported calcium in a manner identical to that for isothermal control cells. When mitogen-activated rat and human lymphocytes were placed in NMR fields, however, they experienced a 22%, and 30% increase in mitogen-activated calcium influx, respectively, compared to isothermal control cells [2].

These findings led to studies in which two NMR imaging field components were evaluated separately. The RF (pulsed 64μT, 100 MHz carrier) and static (2.3 Tesla) field components separately did not influence calcium transport [3]. In light of these findings we decided to investigate whether static magnetic fields greater than 2.3 Tesla would influence calcium transport. The results of these studies are presented here.

METHODS. Preparation of rat thymocytes and procedures for performing the $^{45}Ca^{+2}$ transport assay are described in [2,4]. The superconducting magnet employed for 7.5 Tesla and 9.0 Tesla field exposures is described in [5]. Six Sprague-Dawley rats (250-375gm) were employed in these studies over a period of approximately 3 weeks. Three exposures to 7.5 Tesla and three exposures to 9.0 Tesla were performed with exposures randomly alternated on different days. Results presented here are pooled across these randomized experiments. Cells at 1 x 10^7/ml were placed into eppendorf tubes secured on plastic rods and placed into a polyethylene bottle that served as a waterbath reservoir for distilled, deionized water circulated from a remote waterbath circulator. This bottle was positioned in the middle third volume of the warm horizontal-bore magnet. Cells were maintained at 37°C for 30 minutes in the absence or presence of Con-A (1 μg/ml) during treatment in the magnet or sequentially in a remote isothermal waterbath This waterbath was shielded in μ-metal and cells inside experience a static magnetic field of < 30 mG.

RESULTS. The importance of maintaining rat thymocytes at 37 ± 0.5°C is illustrated in **Figure 1**. The temperature dependence of calcium transport in thymocytes at temperature between 25-40°C in the absence or presence of Con-A (1 μg/ml) is different. Resting cells (no mitogen) are relatively temperature insensitive. Mitogen-activated cells are strikingly temperature dependent with a maximal increase in calcium uptake observed at the physiologically significant temperatures of 37 and 38°C. For this reason we controlled temperature to 37 ± 0.05°C during magnetic field and control treatments.

The effect of exposure of thymocytes to a 7.5 Tesla static magnetic field is shown in **Figure 2**. Resting cells in the magnetic field displayed an approximate 45% increase in basal calcium transport compared to isothermal control cells ($p = 0.001$)(left-hand side). This observation suggests that the resting state is potentially influenced by 7.5 Tesla fields. A comparison of the two open bar graphs indicates that Con-A itself resulted in a modest 16% increase in calcium influx in response to mitogen. During mitogen-activation in the absence or presence of the magnetic field cells exhibited statistically indistinguishable levels of calcium

influx (right-hand side). This finding is consistent with the null effect on mitogen-activated calcium influx we previously obtained at 2.3 Tesla [3].

When thymocytes were exposed to 9.0 Tesla both resting and activated cells experienced a statistically significant reduction in calcium transport (**Figure 3**). Resting cells displayed a 26% inhibition of basal calcium transport ($p = 0.000006$)(left-hand side). Activated cells showed an inhibition in calcium influx of 15% ($p = 0.0128$)(left-hand side). Comparison of the open bar graphs indicates that Con-A itself resulted in an approximate 17% increase in calcium influx. This is comparable to that seen for control cells in the 7.5 Tesla experiments (**Figure 2**). Comparison of closed bar graphs shows that cells in the 9.0 Tesla magnetic field experienced an approximate 34% increase in calcium influx due to Con-A. The levels of activation in the absence and presence of the field are modest and not believed to be biologically significant. The above findings suggest that 9.0 Tesla magnetic fields inhibit basal calcium transport and inhibit calcium influx during mitogen activation.

DISCUSSION. Static magnetic fields can interact with bilayer membrane structures by inducing a conformational change in membrane elements that possess a significant magnetic moment [5,6]. Biological membranes are complex, however, and the localized magnetic susceptibility tensor associated with the lymphocyte calcium pump or calcium ion channels in the bilayer are not yet defined. In our studies we have the added experimental consideration that the lymphocytes were not immobilized with regard to the direction of magnetic flux density during exposures; cells were free to orient in suspension. The transit time for a calcium ion moving through the bilayer however is significantly less that the rotational diffusion constant associated with cell rotation.

Given these considerations, the most likely site of interaction remains the cell membrane and, specifically the ordered structures associated with the calcium pump and calcium ion channels. Ordered, repetitive domains are candidates for large summed magnetic susceptibility tensors. Future studies are required to completely characterize the dose-response of lymphocytes to static magnetic fields at intermediate magnetic flux densities, such as 4.0 Tesla. We plan to perform such studies.

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FIGURE LEGENDS

Figure 1. Effect of Temperature on Calcium Transport in Rat Thymocytes. Mitogen-activated (1 μg/ml Con-A) calcium influx exhibits temperature sensitivity. Mean ± S.E. ($n > 7$).

Figure 2. Effect of a 7.5 Tesla Static Magnetic Field on Calcium Transport in Rat Thymocytes. Mean ± S.E.

Figure 3. Effect of a 9.0 Tesla Static Magnetic Field on Calcium Transport in Rat Thymocytes. Mean ± S.E.

Figure 1

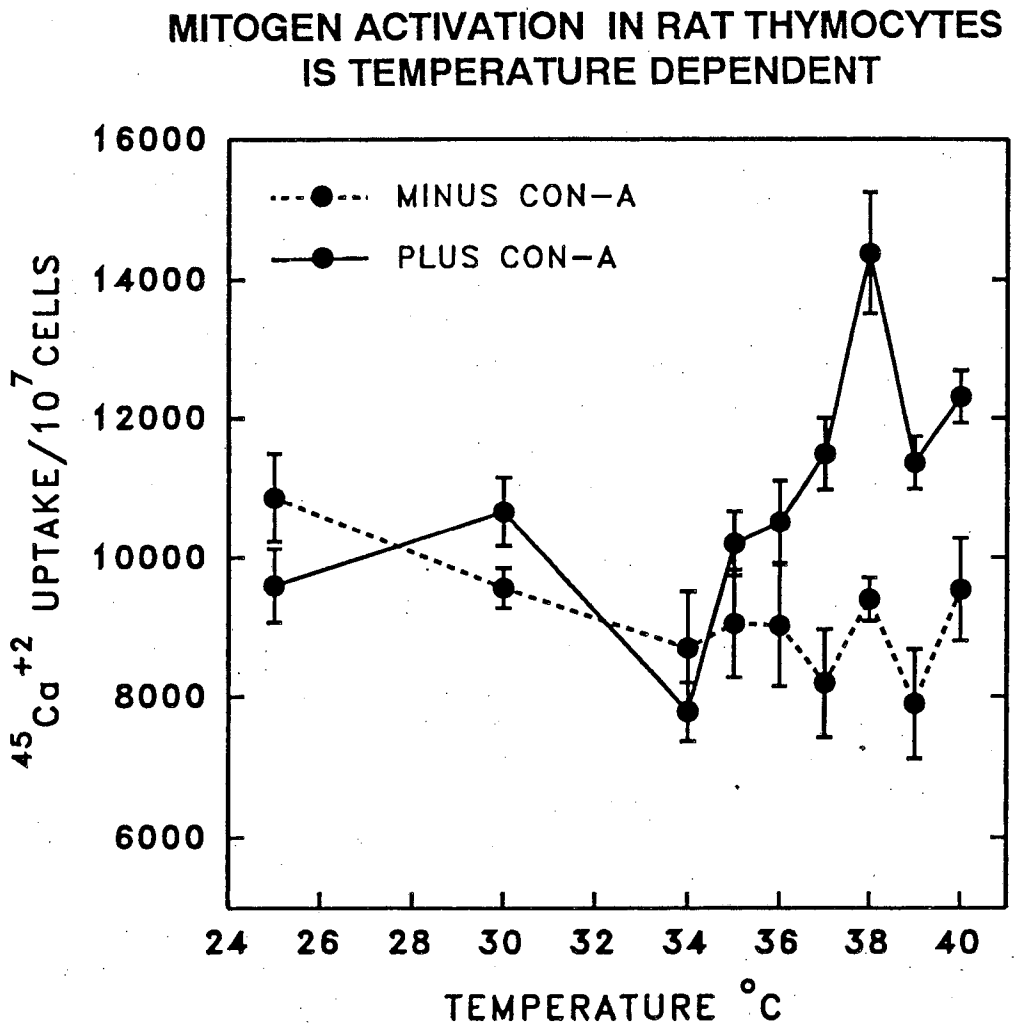

MITOGEN ACTIVATION IN RAT THYMOCYTES
IS TEMPERATURE DEPENDENT
MINUS CON-A
PLUS CON-A
16000
14000
12000
10000
8000
6000
$^{45}Ca^{+2}$ UPTAKE/10^7 CELLS
24
26
28
30
32
34
36
38
40
TEMPERATURE °C

Figure 2

CALCIUM TRANSPORT IN RESTING AND MITOGENICALLY ACTIVATED RAT THYMOCYTES: 7.5 TESLA EXPOSURE

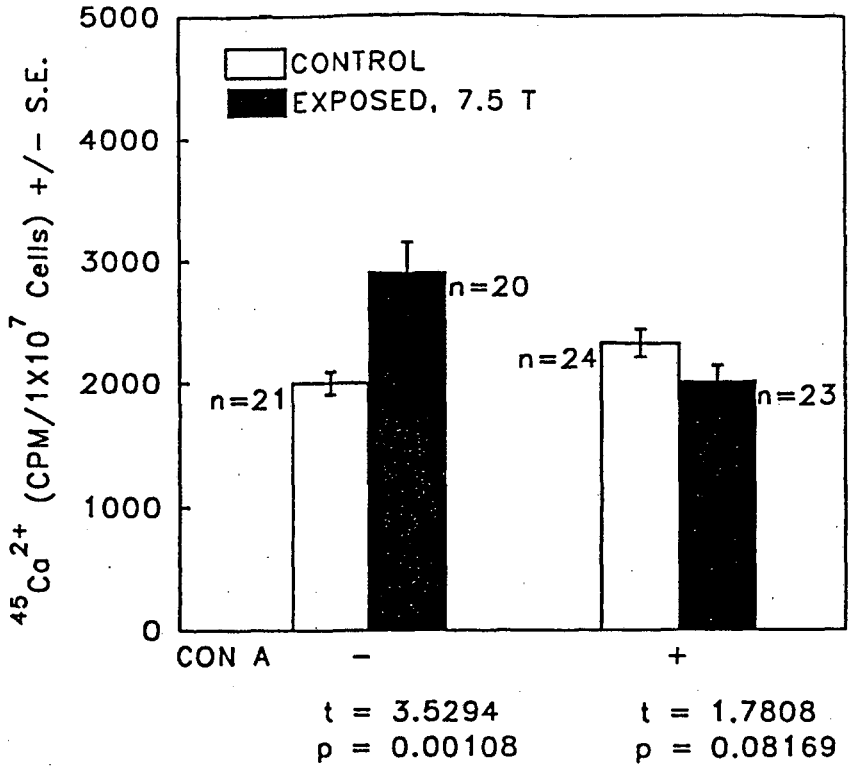
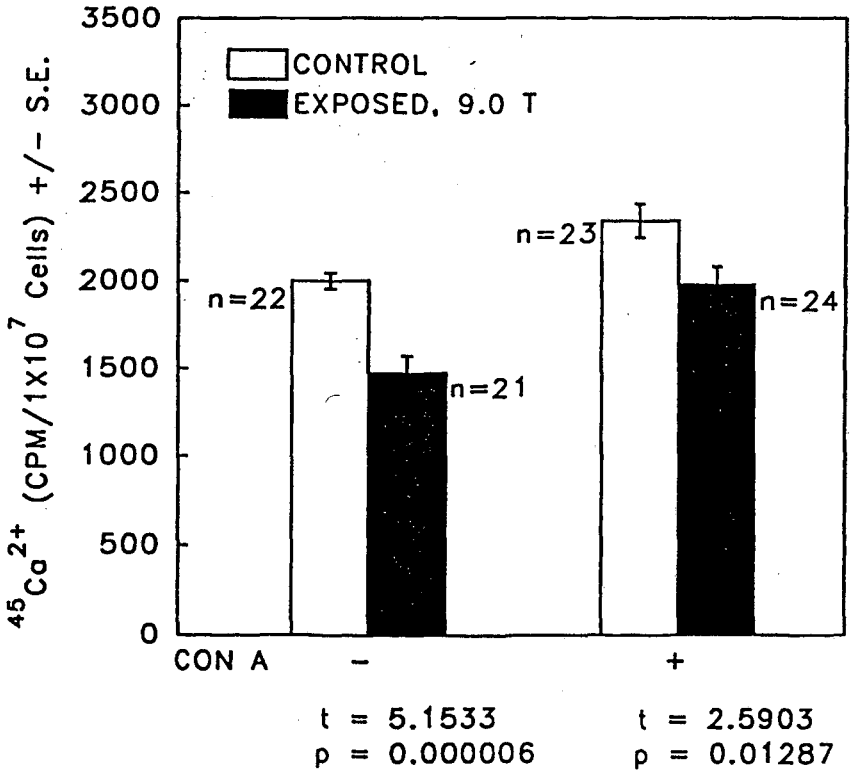


Figure 3

CALCIUM TRANSPORT IN RESTING AND MITOGENICALLY ACTIVATED RAT THYMOCYTES: 9.0 TESLA EXPOSURE



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