Low frequency MFs increased inositol 1,4,5-trisphosphate levels in the Jurkat cell line

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Abstract **We have earlier reported that when a weak 50 Hz MF (magnetic field) was applied, the leukemic T-cell line Jurkat responded with intracellular calcium oscillations [Lindström, et al., *J. Cell Physiol.*, 156 (1993) 395–398]. The result suggested that the MF interfered with the signal transduction, although neither target molecules nor molecular mechanisms are at present known. In this study we found that application of a MF to Jurkat cells resulted in significant increase of inositol 1,4,5-trisphosphate (IP_3) levels. Chelation of intracellular calcium ions by BAPTA/AM, did not block the increase of IP_3 induced by MF. This result implied that MF-induced Ca^{2+} oscillations were not due to direct stimulation of the Ca^{2+}-dependent phospholipase C-γ1 (PLC-γ1).**

Key words: Inositol 1,4,5-trisphosphate; Jurkat T-cell line; Ionomycin; BAPTA/AM; Low frequency magnetic field (50 Hz)

1. Introduction

Low frequency magnetic fields (MF) have been reported to affect several basic cellular functions such as growth, differentiation, macromolecular synthesis and to cause chromosomal aberrations [2–9]. Epidemiological studies have implied an increased risk for malignant tumours in individuals exposed to MF [10–13]. Previous studies of effects on intracellular calcium have demonstrated effects on Ca^{2+} uptake after prolonged exposure, using ^{45}Ca, which gives an estimation of effects on intracellular calcium pools [14,15].

No molecular mechanism has been established to explain how these biological effects are achieved. In a previous report from our laboratory it was shown that an applied sinusoidal 50 Hz MF caused oscillatory increases of $[Ca^{2+}]_i$ in the leukemic T-cell line Jurkat. The effect was in the same order of magnitude as that caused by stimulation of the CD3 receptor complex with an anti-CD3 monoclonal antibody, and occurred within minutes after application [1]. These type of changes in $[Ca^{2+}]_i$ are typical for an established signalling pathway from cell surface receptors to the nucleus [16].

The regulation of $[Ca^{2+}]_i$ in T-cells is receptor-regulated and the influx of Ca^{2+} through the plasma membrane does not occur by classic voltage-dependent calcium channels [17]. Upon T-cell receptor stimulation, tyrosine phosphorylation of the PLC-γ1 activates this enzyme, leading to hydrolysis of phosphatidylinositol 4,5-biphosphate (IP_2) to diaglycerol and IP_3 [18–20]. The role of calcium signalling and $[Ca^{2+}]_i$ regulation by IP_3 has been extensively reviewed [16]. While it is generally accepted that IP_3 releases Ca^{2+} from intracellular stores through IP_3-activated channels, the mechanisms that regulate the sustained Ca^{2+} entry across the plasma membrane during the precommitment period are controversial. The activation of T-cells requires prolonged increases in $[Ca^{2+}]_i$, derived from extracellular sources [22]. Patch-clamp studies have showed that IP_3 activated, in a dose dependent fashion, a Ca^{2+}-permeable channel in the plasma membrane of Jurkat cells [23]. Confocal microscopic studies have further shown that, upon receptor stimulation and during calcium increase, the IP_3 receptors are located close to the TCR [24]. As a result $[Ca^{2+}]_i$ is increased, both from intracellular and extracellular sources, via IP_3-sensitive channels in the plasma membrane and the endoplasmic reticulum; the so-called IP_3 hypothesis [25]. In contrast, the depletion hypothesis asserts that IP_3 only liberates Ca^{2+} from the endoplasmatic reticulum and that the ensuing depletion of this Ca^{2+} store generates a second signal that activates Ca^{2+} influx [26]. The signal in Jurkat cells that couples depletion of stores to activation of influx can not be Ca^{2+} itself, as patch-clamp-measured Ca^{2+} currents developed in the presence of the calcium chelating agent BAPTA before any increase in $[Ca^{2+}]_i$ [27]. The PLCγ-1 activity is thought to be regulated by tyrosine phosphorylation upon TCR stimulation [28]. There are also other signalling pathways, in addition to the phosphoinositide pathway, which may increase $[Ca^{2+}]_i$ in these cells [29].

We undertook this study to investigate whether a 50 Hz MF could affect the levels of IP_3 in the T-cell line Jurkat. Our data suggest that MF effects signal transduction events upstream of release of IP_3.

2. Materials and methods

2.1. Exposure system for the IP_3 measurement

The magnetic field was generated by a pair of Helmholtz' coils with 20 turns (diameter 20 cm), with the magnetic field in a vertical direction. Since only a sinusoidal 50 Hz MF was used the coils were fed from a variable transformer connected to the mains. The magnitude of the applied field, 0.10 mT, was set by monitoring the current in the coils on an amperemeter, connected in series with the coils and then calculating the field, and by measuring with a small, calibrated, pick-up coil. The sham and the exposure coils were placed inside Forma incubators, modified to give low stray MF below 0.2 μT. No attempt was made to control the geomagnetic field, which was measured with a Bell Gauss meter. The values for the exposure incubator were 17 μT for the total geomagnetic field and 14 μT for the vertical component. The corresponding values for the sham exposure incubator were 35 and 22 μT, respectively.

Temperature (37°C) was the same in the incubators both for exposed and sham exposed cells.

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Abbreviations: BAPTA, 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetate; TCR, T cell receptor; MF, magnetic field; $[Ca^{2+}]_i$, intracellular calcium concentration; IP_3, inositol 1,4,5-trisphosphate; PLC-γ1, phospholipase C-γ1; MAb, monoclonal antibody.

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2.2. Exposure system for the intracellular calcium measurements

A homogenous vertical magnetic field was set up by using a pair of Helmholtz coils supplied from the mains via a low voltage transformer [1]. The total geomagnetic field was 62 μT with a vertical component of 55 μT. The applied vertical sinusoidal 50 Hz field was 0.15 mT. Environmental stray fields were controlled as well as the temperature (37°C) in the chamber.

2.3. Cells and chemicals

The leukemic T cell line Jurkat was grown in RPMI 1640 medium containing 10% FCS, 100 U/ml penicillin and 100 μg/ml streptomycin. The monoclonal antibody (MAb) UCHT-1, specific for the CD3 receptor is available from DAKO A/S; BAPTA/AM was purchased from Calbiochem and ionomycin from Sigma.

2.4. Measurement of intracellular free cytosolic Ca^{2+}

Cells were put on cover glasses coated with poly-L-lysine solution (0.01%) and incubated in an CO_2 incubator for 60 min at 37°C, with fura-2/AM at 2 μM [30,31].

2.5. Measurement of IP_3 in Jurkat cells

The cell concentration was adjusted to approximately 10^6/ml. Cells were collected at different time points, as indicated in the figures (Figs. 2–4), in 0.4 or 0.5 ml aliquots after the different treatments. IP_3 was assayed using a commercially available kit (Amersham International). The assay is based on competition between unlabelled and tritium-labelled IP_3 for binding sites on a bovine adrenal binding protein preparation. The data represent the mean of several experiments as indicated in figure legends.

2.6. Chelation of intracellular free Ca^{2+}

Jurkat cells were pretreated by adding BAPTA/AM to incubation media for 30 min before exposure to MF. Different BAPTA/AM concentrations were tested by fura-2 measurements to find the optimum that completely blocked Ca^{2+} oscillations induced by MF (Fig. 1A,B). As a positive control for increase of $[Ca^{2+}]_i$ we used addition of the anti-CD3 antibody UCHT-1, or the calcium ionophor, ionomycin, as previously described [1].

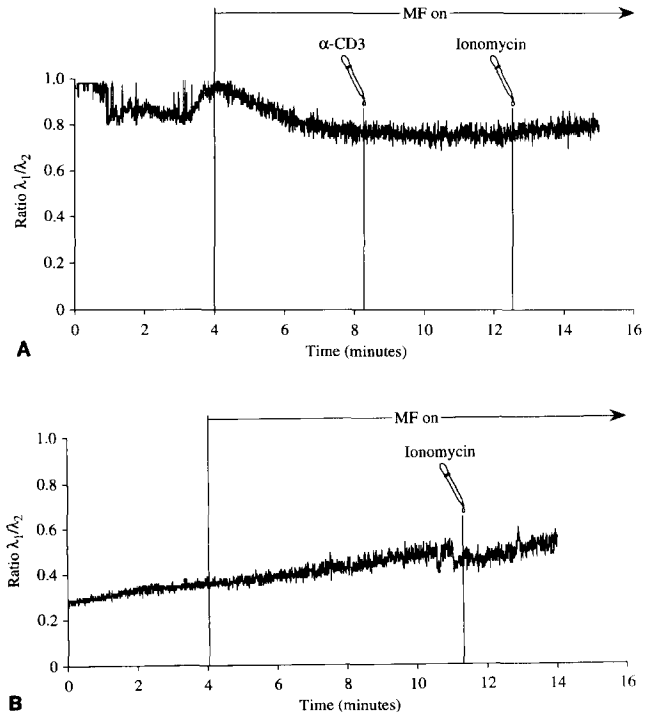

Fig. 1. Measurements of $[Ca^{2+}]_i$ in single cells after 30 min pretreatment with 37 μM (A) or 75 μM (B) BAPTA/AM. At the times indicated, anti-CD3 (0.5 μg/ml) and ionomycin (1 μg/ml) was added. The traces are representative of seven experiments.

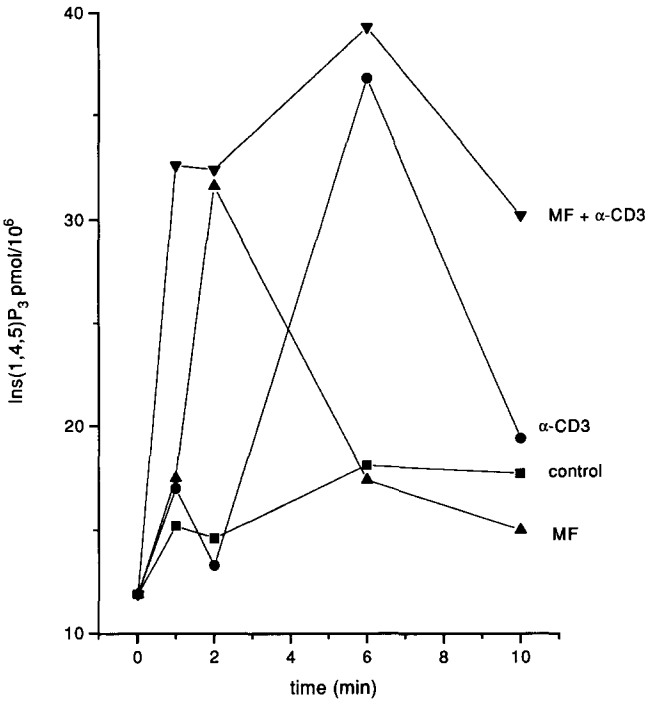

Fig. 2. Levels of IP_3 in lysates of Jurkat cells exposed to MF (50 Hz, 0.10 mT), the anti-CD3 MAb UCTH-1 (0.5 mg/ml), or to both stimuli. The figure represents the mean of six experiments. ■, sham exposed control; ●, anti-CD-3 MAb; ▲, 50 Hz, 0.10 mT; ▼, 50 Hz, 0.10 mT + anti-CD3 MAb. S.E.M. was < 30%.

3. Results

Jurkat cells were exposed to MF (50 Hz, 0.1 mT) and samples for determination of IP_3 levels were taken during the first 10 min as indicated in Fig. 2. An optimised concentration of the anti-CD3 monoclonal antibody was used as a positive control, as upon binding to the CD3 complex it initiates a signalling cascade through the phosphatidylinositol pathway, resulting in $[Ca^{2+}]_i$ oscillations [1,32].

In repeated experiments the level of intracellular IP_3 increased almost immediately after application of MF, with a maximum at 2 min. The magnitude of the increase was the same as that for the anti-CD3 MAb, which, however, peaked at 6 min. The combination of the monoclonal antibody and MF gave an additive effect after 2 and 6 min of treatment. However, the combined treatment seemed to give a synergistic effect after 1 min.

Pretreatment with the cytosolic calcium chelator BAPTA/AM resulted in an initial lower $[Ca^{2+}]_i$ of 40–50 nM, compared to untreated Jurkat cells that have a base level of 50–100 nM. Almost all intracellular calcium was bound using a BAPTA/AM concentration of 37 μM (Fig. 1A). Anti-CD3 MAb binds to the TCR complex, and is a well-known stimulator of the phosphatidylinositol pathway and the second messenger Ca^{2+} [18]. Addition of anti-CD3 in BAPTA-loaded cells could not increase the $[Ca^{2+}]_i$. Ionomycin, that normally rapidly increases intracellular calcium levels, had no effect. As much as 75 μM BAPTA/AM resulted in extremely low basal $[Ca^{2+}]_i$, approximately zero values (Fig. 1B). Neither MF nor ionomycin could overcome this strong BAPTA-chelation during the measurements.

As the MF could still induce an increase of IP_3 in the presence of high concentrations of BAPTA/AM (Fig. 3), we concluded that the applied MF induced an increase in IP_3 independent of increases of the $[Ca^{2+}]_i$ levels. Ionomycin treatment resulted in a prompt increase in the levels of IP_3 already after 30 s, and high levels of IP_3 were maintained for 6 min (Fig. 4). These results suggests that the applied MF interferes with the receptor-triggered signal transduction pathway, which results in release of intracellular $[Ca^{2+}]_i$, and that the target for MF is PLC-γ1, or molecules upstream of that enzyme, and not calcium channel interactions.

4. Discussion

Cells of the immune system have been shown to be responsive to MF as discussed by Walleczek [2]. Cellular responses to MF of the type applied in our study have been hypothesised to be due to interactions with molecules in the cell membrane, although they have not been identified.

In a previous publication it was shown that MF increased $[Ca^{2+}]_i$ in a similar pattern, both in respect to amplitude and oscillation [1]. Here we provide data that the observed transient increases in free intracellular Ca^{2+} might be a consequence of activation of PLC-γ1. The role of this enzyme in cellular signalling in T lymphocytes is well established [18]. The amplitude of the IP_3 increase was of the same order as that caused by the addition of anti-CD3 antibodies, and the time course fits with the observed start of $[Ca^{2+}]_i$ oscillations [1].

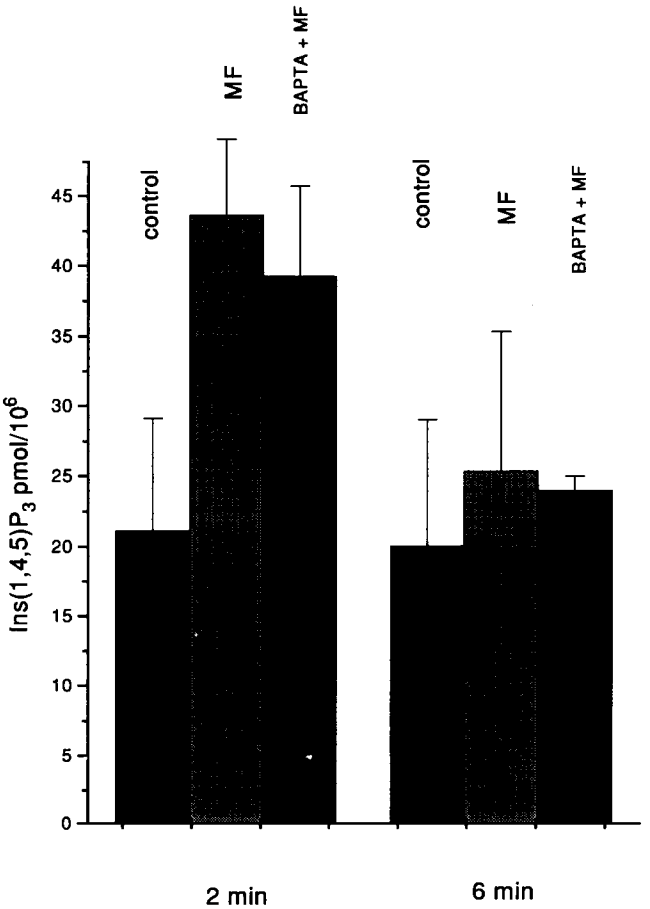

Fig. 3. Levels of IP_3 in lysates of Jurkat cells exposed during indicated times to the Ca^{2+} chelator BAPTA/AM (50 μM) and MF (50 Hz, 0.10 mT). The figure represents the mean of three experiments.

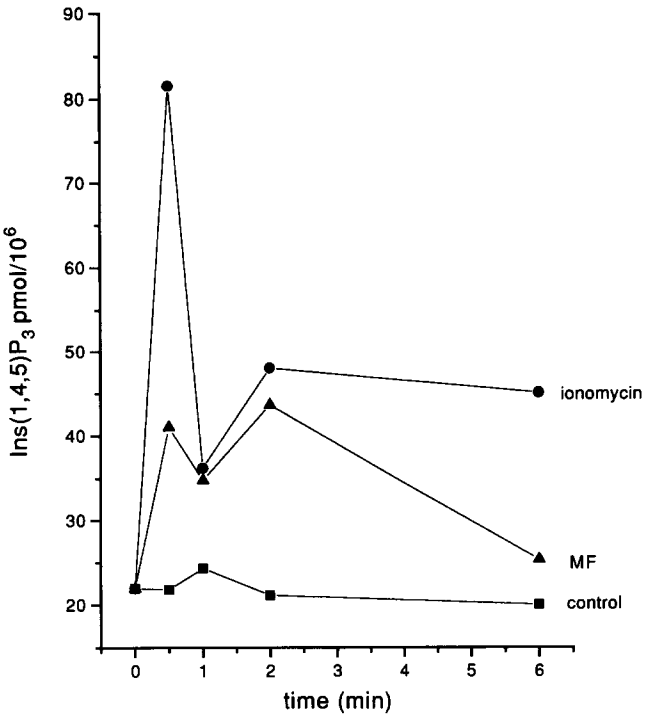

Fig. 4. Levels of IP_3 in lysates of Jurkat cells exposed to MF (50 Hz, 0.10 mT), the anti-CD3 monoclonal antibody and ionomycin. The figure represents the mean of four experiments. ■, sham exposed control; ●, ionomycin 4 μg/ml; ▲, 50 Hz, 0.10 mT. Standard errors of the mean were < 30%.

It is known that all phopholipases are activated by calcium [17], and the observed $[Ca^{2+}]_i$ increase due to MF exposure may just as well be the result of e.g. unspecified membrane effects, causing leakage of extracellular Ca^{2+} into the cells. However, our results showed increased IP_3 levels even when all the cytosolic free Ca^{2+} was chelated by BAPTA (Figs. 1 and 3). We therefore think that it is not an unspecific increase of Ca^{2+} that activates PLC-γ1 and results in the IP_3 increase. Increased IP_3 levels upon stimulation with anti-CD3 or phytohaemagglutinin in BAPTA-loaded Jurkat cells has been reported by others [33].

The fact that also the Ca^{2+}-ionophore, ionomycin, caused an increase of IP_3 might support the notion that IP_3 is generated rather by an increased $[Ca^{2+}]_i$ than the reverse, or that ionomycin has effects other than just to open calcium channels. Since the calcium chelator BAPTA/AM did not inhibit the IP_3 increase upon MF exposure, and the kinetic and the level of IP_3 increase resemble the anti-CD3 stimulation, our data suggest that the applied MF interferes with events upstream of PLC-γ1. This is further supported by ongoing experiments indicating the need for CD45 expression for MF-mediated calcium oscillations. The validity of the findings in this report and its biological significance can only be revealed by further studies of the early signal pathway events, upstream of the observed effects on IP_3 and $[Ca^{2+}]_i$ levels.

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References

[1] Lindström, E., Lindström, P., Berglund, A., Hansson Mild, K. and Lundgren, E. (1993) J. Cell. Physiol. 156, 395–398.
[2] Walleczek, J. (1992) FASEB J. 6, 3177–3185.
[3] Cohen, M.M., Kunska, A., Astemborski, J.A., McCulloch, D. and Paskewitz, D.A. (1986) Bioelectromagnetics 7, 415–423.
[4] Rosenthal, M. and Oboe, G. (1989) Mutat. Res. 210, 329–335.
[5] Conti, P., Gigante, G.E., Alesse, E., Cifone, M.G., Fieschi, C., Reale, M. and Angeletti, P.U. (1985) FEBS Lett. 181, 28–32.
[6] Goodman, R., Wei, L.X., Xu, J.C. and Henderson, A. (1989) Biochim. Biophys. Acta 1009, 216–220.
[7] Walleczek, J. and Liburdy, R.P. (1990) FEBS Lett. 271, 157–160.
[8] Greene, J.J., Skowronski, W.J., Mullins, J.M., Nardone, R.M., Penafiel, M. and Meister, R. (1991) Biochem. Biophys. Res. Commun. 174, 742–749.
[9] Nordenson,I., Hansson Mild, K., Andersson, G. and Sandström, M. (1994) Bioelectromagnetics 15, 293–301.
[10] Ahlbom, A. (1988) Scand. J. Work Environ. Health 14, 337–343.
[11] Feychting, M. and Ahlbom, A. (1993) Am. J. Epidemiol. 138, 467–481.
[12] Floderus, B., Persson, R., Stenlund, C., Wennberg, A., Öst, Å. and Knave, B. (1993) Cancer Causes Control 4, 465–476.
[13] Olsen, J.H., Nielsen, A. and Schulgen, G. (1993) Br. Med. J. 307, 891–895.
[14] Liburdy, R.P., Callahan, D.E., Harland, J., Dunham, E., Sloma, T.R. and Yaswen, P. (1993) FEBS Lett. 334, 301–308.
[15] Walleczek, J. and Budinger, T.F. (1992) FEBS Lett. 314, 351–355.
[16] Berridge, M.J. and Irvine, R.F. (1993) Nature 341, 197–205..
[17] Tsien, R.W., Pozzan, T. and Rink, T.J. (1982) Nature Lond. 295, 68–71.
[18] Fraser, J.D., Straus, D. and Weiss, A. (1993) Immunol. Today 14, 357–362.
[19] Abraham, R.T., Karnitz, L.M., Secrist, J.P. and Leibson, P.J. (1992) Trends Biochem. Sci. 17, 434–437.
[20] Rhee, S.G. and Choi, K.D. (1992) J. Biol. Chem. 267, 12393–12396.
[21] Berridge, M.J. (1993) Nature 361, 315–325.
[22] Desai, D.M., Newton, M.E., Kadlecek, T. and Weiss, A. (1990) Nature 348, 66–69.
[23] Kuno, M. and Gardner, P. (1987) Nature 326, 301–304.
[24] Khan, A.A., Steiner, J.P., Klein, M.G., Schneider, M.F. and Snyder, S.H. (1992) Science 257, 815–818.
[25] Kuno, M., Goronzy, J., Weyand, C.M. and Gardner, P. (1986) Nature 323, 269–273.
[26] Putney, J.W. (1990) Cell Calcium 11, 611–624.
[27] Zweifach, A. and Lewis, R.S. (1993) Proc. Natl. Acad. Sci. USA 90, 6295–6299.
[28] Weiss, A., Koretzky, G., Schatzman, R.C. and Kadlecek, T. (1991) Proc. Natl. Acad. Sci. USA 88, 5484–5488.
[29] Hakamata, Y., Nishimura, S., Nakai, J., Nakashima, Y., Kita, T. and Imoto, K. (1994) FEBS Lett. 352, 206–210.
[30] Grynkiewicz, G., Poenie, M. and Tsien, R.Y. (1985) J. Biol. Chem. 260, 3440–3450.
[31] Lindström, E., Lindström, P., Berglund, A., Lundgren, E. and Hansson Mild, K. (1994) Bioelectromagnetics, in press.
[32] Brattsand, G., Cantrell, D.A., Ward, S., Ivars, F. and Gullberg, M. (1990) J. Immunol. 144, 3651–3657.
[33] Ng, J., Fredholm, B.B., Jondal, M. and Andersson, T. (1988) Biochim. Biophys. Acta 971, 207–214.