Fifty-Hertz low frequency magnetic field modifies sarcoplasmic reticulum function

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Abstract. Among the putative mechanisms, by which extremely low frequency magnetic field (ELF-MF) modify calcium metabolism is that of affecting Ca^{2+} fluxes across cell membrane or internal Ca^{2+} stores. To test this hypothesis, whether ELF MF can modulate Ca^{2+} fluxes of isolated skeletal muscle sarcoplasmic reticulum vesicles (SR) was investigated in the present study. The Ca^{2+} transport and Ca^{2+} - Mg^{2+} -ATPase activity were observed by means of dynamic Ca^{2+} dye spectrum, the function of ryanodine receptor (RyR1) was monitored by [^3H]-ryanodine binding assay. The membrane fluidity of SR was measured by fluorescence polarization. 50 Hz 0.4 mT MF exposure for 30 min led to a significant decrease in the Ca^{2+} uptake initial rate and Ca^{2+} - Mg^{2+} -ATPase activity compared to sham exposed SR. These results strongly suggest that prevention of SR Ca^{2+} uptake by ELF MF exposure was due to the inhibition of Ca^{2+} - Mg^{2+} -ATPase activity, and the increase of SR Ca^{2+} release was due to the activation of RyR1.

1 INTRODUCTION

With increasing use of electric appliances, high voltage power transmission and magnetic resonance imaging, it has resulted in the increased exposure to a complex mix of artificially elevated extremely low frequency (ELF) magnetic field (MF). Epidemiological data suggest that there is an association between ELF MF exposure and the incidence of a few types of cancers, particularly in childhood leukemia, brain and breast cancers [1], but these results have not been confirmed in other studies [2-3]. However, any possible harmful effects could not be excluded because of many positive results which were independently confirmed.

In vitro studies, ELF MF have been reported to affect several basic cellular processes, such as cell proliferation [4], apoptosis [5], DNA and RNA synthesis [4,6], and gene transcription [7]. Since all these effects can be related more or less directly to the signal transduction pathways, Ca^{2+} as an intracellular critical second message has been of focus to scientists for many years [8-12]. In many types of cell the signal transduction from plasmalemma to intracellular targets is linked to the oscillatory behaviour of intracellular Ca^{2+} . Experimental investigations of ELF MF on living systems seem to indicate that calcium signaling pathways, and a cytosolic Ca^{2+} oscillator in particular, may be the processes which are affected by external ELF MF. A few scattered studies have not detected an effect [8] or even decreased in cytosolic $[\text{Ca}^{2+}]_c$ [9], whereas the majority literatures have reported an increase in cytosolic $[\text{Ca}^{2+}]_c$ on exposure to ELF MF [6,10-12]. Although many studies

of the effects of ELF MF on cytosolic $[\text{Ca}^{2+}]_c$ have been completed, the results apparent lack of consistency.

Changes of cytosolic $[\text{Ca}^{2+}]_c$ can be regulated by a network of reactions, such as receptors for Ca^{2+} influx from extra-cellular medium or Ca^{2+} release from internal Ca^{2+} stores; as well as calcium pumps, eliminating the $[\text{Ca}^{2+}]_c$ in the cytosol. It has been reported that $[\text{Ca}^{2+}]_c$ is mainly influenced by ELF MF either through cell membrane or internal Ca^{2+} stores [5,10,12]. However, there is very little information about how and at which point ELF MF could affect internal Ca^{2+} stores. The majority studies of the effects of ELF MF on cytosolic $[\text{Ca}^{2+}]_c$ have used various types of cells, such as leukemia cells (U937) [5], erythrocytes [8], T-lymphocytes (Jurkat E 6.1) [9,11,12], pituitary cells et al [10]. Experiments with different cells have the disadvantage that they represent a system with different Ca^{2+} stores, Ca^{2+} transporters and Ca^{2+} regulating receptor sides. In addition, it has been reported in the literature that ELF MF penetrate through cells or tissues without significant attenuation [13], if ELF MF could influence internal Ca^{2+} stores on cellular level, it is very likely to induce some effects on isolated Ca^{2+} stores models. Furthermore, contraction of skeletal muscle provides one of the best studied examples of calcium signalling. Muscle can be homogenized to give a preparation of sealed vesicles derived from the Sarcoplasmic reticulum (SR) that can accumulate Ca^{2+} from the external medium in the presence of ATP, following uptake of Ca^{2+} , SR vesicles will spontaneously release a fraction of the accumulated Ca^{2+} [15]. For all these reasons, using isolated internal Ca^{2+} stores (SR vesicles) while not cells to investigate

the effects of ELF MF on the basic mechanisms of Ca^{2+} regulation system in the present study. SR is the storage site for the bulk of Ca^{2+} and its transport across the SR membrane is managed by two molecules: the ryanodine receptor (RyR1) and Ca^{2+} -Mg $^{2+}$ -ATPase.

To understand how and at which point ELF MF exposure alter intracellular calcium homeostasis, we observed the effects of ELF MF on the Ca^{2+} transport with isolated SR vesicles.

2 Materials and methods

2.1 Isolation of rabbit skeletal muscle SR vesicles

SR vesicles were isolated from hind leg and back skeletal muscle from New Zealand White rabbits by the method of MacLennan with small modifications [15]. Fifty micromolar dithiothreitol and 0.2 $\mu\text{g}/\text{ml}$ leupeptin were added to all buffers except for the final SR resuspension buffer. The final samples were flash-frozen in liquid nitrogen and stored at -80°C until use. The protein concentration was determined by absorption spectroscopy [16].

2.2 Magnetic field exposure

The MF exposure system is composed of three major parts: a pair of circular horizontal Helmholtz coils with 150 turns of a copper wiring (20 cm in height and 20 cm in radius), a signal generator and a power amplifier. The whole equipment is placed in a cell culture incubator, and is shielded from external field contamination.

Measurements of the intensity of the magnetic field with a Gauss-meter (HT 22, shanghai) showed that it was fairly constant for the center area (10 cm in height and 5 cm in radius) between the coils. Our preliminary experiments indicated that the field intensity of 0.1 mT had no obvious effects on SR Ca^{2+} transport, whereas a higher intensity (0.4 mT) would lead to the sharp decrease in Ca^{2+} transport. In the present study, a 0.4 mT 50 Hz magnetic field was used. The uniformity of the field over the experimental region is (0.400 ± 0.012) mT, calculated from the values measured in the middle of the field and at the eight corners of cube box ($5\text{cm} \times 5\text{cm} \times 5\text{cm}$).

Purified SR (10 mg/ml) in 0.5ml-Eppendorf tubes were randomly divided into two groups: the sham-exposed group (Control), the MF-exposed group (+MF). Each group contained three samples. The +MF groups were exposed to a 0.4 mT 50 Hz magnetic field for 30 min. The Control groups were sham exposed for the same time in a MF exposure system with power off which was shielded from external field contamination.

2.3 A-23187- stimulated Ca^{2+} -Mg $^{2+}$ -ATPase activity

A-23187-stimulated Ca^{2+} -Mg $^{2+}$ -ATPase activity was determined according to the method of Ref [17]. The

standard assay buffer contained 100 mM KCl, 20 mM Hepes, 3 mM NADH, 1 mM EGTA, 1 mM MgCl_2 , 0.5 mM Mg^{2+} -ATP, 1 mM phosphoenol pyruvate, 5 units of lactate dehydrogenase, 5 units of pyruvate kinase, at pH 7.0 in a 1.5-ml volume. Background ATPase activity was initiated by the addition of SR (0.15 mg) to the cuvette in the absence of Ca^{2+} , and the absorbance changes of the oxidation of NADH at 340 nm were recorded for 2 min (25°C). Maximal Ca^{2+} -stimulated activity was determined in the presence of 8 μM free Ca^{2+} (the free Ca^{2+} concentrations in the presence of 1 mM EGTA were calculated by WinMaxc) and 3 μM A-23187 (A-23187 is a Ca^{2+} ionophore), and absorbance changes were monitored for 5 min until the reaction was run to completion. A-23187 was added to prevent back inhibition that results from overloading Ca^{2+} of vesicles with. Ca^{2+} -Mg $^{2+}$ -ATPase activity was calculated as the differences between maximal ATPase and background ATPase activities. Enzyme activity was expressed as μmol NADH. $\text{mg}^{-1} \cdot \text{min}^{-1}$. The data are the average of four independent experiments.

2.4 Measurement of Ca^{2+} uptake

Ca^{2+} fluxes across SR vesicles were monitored by using anti-pyrilazo III Ca^{2+} chelometric dye [17]. The standard procedure was as follows, Ca^{2+} uptake into SR vesicles (0.2 mg/ml) was carried out in a buffer containing 100 mM KCl, 20 mM Hepes, 1 mM MgCl_2 , 200 μM APIII, 20 μM free Ca^{2+} , pH 7.0. Ca^{2+} uptake was initiated by the addition of 0.5-1 mM Mg^{2+} -ATP and followed continuously on a Cary 100 spectrophotometer operating at a measuring wavelength of 710 nm and a reference wavelength of 790 nm. In all experiments, the free extravesicular Ca^{2+} concentration was recorded as a function of time and stored in the computer. The Ca^{2+} uptake capacity and the initial Ca^{2+} uptake rate was determined from the uptake curve of extravesicular Ca^{2+} concentration versus time.

Considering that net Ca^{2+} uptake is an equilibrium between Ca^{2+} entry into the vesicles and Ca^{2+} efflux through the release channel, 10 μM ruthenium red (RyR specific inhibitor) was added to the assay buffer in order to observe the effects of MF on single aspect of Ca^{2+} -ATPase uptake. That is sufficient to inhibit Ca^{2+} efflux through the release channel. The independent experiment was repeated more than eight times.

2.5 Measurements of Ca^{2+} release

Assays of Ca^{2+} release were performed with two different methods from actively loaded and passively loaded SR vesicles. During the actively loaded SR assay in the absence of ruthenium red, upon completion of Ca^{2+} uptake, at which time the Ca^{2+} concentration had reached a steady state, Ca^{2+} release was initiated by the addition of RyR modulator (1 mM NADH). At the end of Ca^{2+} release, 3 μM A-23187 was used to release the rest Ca^{2+} in SR lumen.

In order to obtain the effects of MF on the RyR initial Ca^{2+} release rate under conditions of the same SR Ca^{2+}

uptake capacity, we also carried out the passively loaded SR Ca^{2+} release assays. The procedure was as follows: SR vesicles (10 mg/ml) were passively loaded for 90 min in a medium containing 1.0 mM CaCl_2 , 100 mM KCl, 20 mM Hepes, pH 7.0 at 25 °C. Then divided the sample into MF group and sham group, MF group was exposed to 50 Hz、0.4 mT MF for 30 min at 25 °C, and sham group was placed the same time at the same temperature. Passively loaded SR vesicles were diluted 50-fold into the assay buffer (200 μM APIII, 100 mM KCl, 20 mM Hepes, pH 7.0). The free extravesicular Ca^{2+} concentration was recorded as a function of time and the Ca^{2+} initial release rate was determined from the initial slope of extravesicular Ca^{2+} concentration versus time.

2.6 Statistical analysis

Each independent experiment was repeated certain times as indicated in corresponding sections. The results were expressed as means $\pm$ standard deviation (SD). of n repeating times. Statistical comparisons between groups were performed with Student's t test. Differences were considered statistically significant when $p < 0.05$.

3. Results and conclusions

3.1. Effects of MF exposure on SR Ca^{2+} -ATPase activity.

The ATPase studies were conducted in the presence of A-23187, Ca^{2+} -ionophore to ensure that maximal catalytical activity was not prone to “back inhibition” of the pump or stimulation of ATPase activity via opening of the RyR1. SR vesicles were exposed to 50 Hz 0.4 mT MF for 30 min caused the hydrophobic activity of Ca^{2+} -Mg $^{2+}$ -ATPase to decrease significantly, which reduced to (0.91 ± 0.13) from that of sham exposure SR vesicles (1.25 ± 0.16) , as shown in Fig.1. Thapsigargin (TG) was the Ca^{2+} -Mg $^{2+}$ -ATPase specific inhibitor, the addition of 1.5 μM TG resulted in both MF exposure (0.22 ± 0.05) and sham exposure (0.37 ± 0.08) decreasing to nearly their baseline activity. Ca^{2+} -Mg $^{2+}$ -ATPase activity was determined by subtracting the rate of oxidation NADH in the presence of TG from the rate of oxidation NADH in the absence of TG, namely the Ca^{2+} -Mg $^{2+}$ -ATPase activity of MF group (0.69 ± 0.12) reduced to 78% from sham exposure group (0.88 ± 0.10) .

Fig 1. Effects of ELF MF (50 Hz、0.4 mT) on SR Ca^{2+} -Mg $^{2+}$ -ATPase activity. MF intensity : 0.4 mT, exposure time: 30 min, TG concentration: 1.5 μM , SR concentration: 0.1 mg. ml-1. (n=4, $P < 0.05$)

3.2 Effects of MF exposure on SR Ca^{2+} uptake.

When cytosolic $[\text{Ca}^{2+}]_i$ is elevated, the Ca^{2+} -ATPase will actively transports Ca^{2+} back into the SR lumen. Ca^{2+} -Mg $^{2+}$ -ATPase is the major SR protein that transports 2 mol of Ca^{2+} across the SR bilayer membrane with hydrolysis of 1 mol ATP.

Inhibition of SR Ca^{2+} -Mg $^{2+}$ -ATPase activity resulted in a parallel reduction in the Ca^{2+} uptake capacity (CUC) and the initial Ca^{2+} uptake rate (IUR). The CUC and the IUR of MF group decreased to 80% and 69% of sham exposure SR, respectively. Because Ca^{2+} uptake was a dynamic process between Ca^{2+} entry into the vesicles and Ca^{2+} effluxes through RyR1, the inhibition of Ca^{2+} uptake on MF exposure is caused by either the Ca^{2+} -Mg $^{2+}$ -ATPase influxes decrease or the RyR1 effluxes increase. To investigate the effects of MF on SR Ca^{2+} -Mg $^{2+}$ -ATPase net Ca^{2+} uptake, the addition of 10 μM RR in assay buffer to inhibit RyR1 Ca^{2+} release. Results shown that the net CUC and IUR of MF group only recovered to 86% and 84% that of control. These results demonstrated that MF exposure decreased CUC and IUR of Ca^{2+} -Mg $^{2+}$ -ATPase by 14% and 16%.

The decrease percentage caused by MF exposure are only partially recovered from 80% to 86% for CUC and from 69% to 84% for IUR, in the presence of RyR1 inhibitor, which indicated that MF exposure may also activated SR RyR1 Ca^{2+} release activity.

3.3. Effects of MF on SR Ca^{2+} release.

The analysis from above results indicate, that the original decreases of MF group CUC (20%) and IUR (31%) was due to the decreases in Ca^{2+} -Mg $^{2+}$ -ATPase influx (CUC 14%, IUR 16%) and the increase in RyR1 Ca^{2+} release (CUC 6%, IUR 14%). In order to investigate the effects of 50 Hz 4 G MF on RyR1 Ca^{2+} release, we carried out Ca^{2+} effluxes assays from actively loaded SR and passively loaded SR.

MF activates the SR Ca^{2+} release channel/RyR1 and induces rapid release of Ca^{2+} from actively loaded vesicles. What's more, the effects of MF on the RyR1 Ca^{2+} release obviously magnified by 1 mM NADH, which caused the initial Ca^{2+} release rate (IRR) to

increase 17% and 23% in the absence or presence of 1 mM NADH. Considering the inhibitor effects of MF on SR Ca^{2+} - Mg^{2+} -ATPase may be affected the RyR1 Ca^{2+} release, we also carried out the passively loaded SR Ca^{2+} release assay.

Data from passively loaded SR further demonstrated that MF markedly increase the IRR, from control group (8.58 ± 0.70) increase to (10.81 ± 1.61) ($n=5$). If the whole process of passively loaded SR 2h were exposed to MF, the IRR increased to (10.13 ± 0.97)

($n=5$). Moreover, passively loaded SR with exposing to MF 0.5 h, if not measured immediately and placed at room temperature for another 2 h, MF activating effect on RyR is significantly weakened (data not shown). These results implicated that MF increased RyR Ca^{2+} release may be time-dependent and can be reversible.

4. Discussions and conclusions

Cellular studies have demonstrated that ELF MF can influence processes, such as DNA, RNA or protein synthesis in various cell types [6-7]. The cellular and molecular mechanisms by which these fields trigger biological effects are still unknown. Data reported in the literature suggested that cell membrane mediated signal transduction pathway, especially those involving calcium transport might be candidates for ELF field interactions.

In this study, we evaluated the effects of 50 Hz MF exposure at field intensity of 0.4 mT on isolated internal Ca^{2+} stores (SR) activity. Results of Ca^{2+} uptake experiments showed that 50 Hz sinusoidal MF reduced CUC and IUR to 80% and 69% of control respectively. the addition of ruthenium red could only partially restore the decreased Ca^{2+} uptake by MF exposure. These finding provide insight into the role Since Ca^{2+} is an integral part of various cellular signaling pathways, the effects of ELF MF on various cells intracellular [Ca^{2+}]_i or calcium signal transduction have been widely carried out. Studies suggest that ELF MF exposure influences intracellular Ca^{2+} movement and signal transduction pathways. The exposure to ELF MF causes oscillation in fibroblasts and lymphocytes. Changes in Ca^{2+} influx across the cell membrane of lymphocytes and decreases in cytoplasmic Ca^{2+} oscillation are also reported. But for all this, neither the effect itself, nor the corresponding mechanism is clarified.

Our results showed that ELF MF significantly decreased SR Ca^{2+} loading, leading to a 16% decrease of the initial Ca^{2+} loading rate. Decreased the initial Ca^{2+} loading rate was paralleled by a significant decrease of the percentage of Ca^{2+} -ATPase activity.

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