Original Article

EFFECTS OF ACUTE EXPOSURE TO MAGNETIC FIELD ON IONIC COMPOSITION OF FROG SCIATIC NERVE

Amara S[1], Abdelmelek H[2] & Sakly M[3]

ABSTRACT:
Objective: To evaluate the possible interaction between magnetic field and cadmium on ionic composition of frog sciatic nerve.
Design: The combined effect of magnetic field and cadmium (1.5mg/kg; in lymphatic sac) were studied in frog sciatic nerves (*Rana Esculenta*). Sciatic nerve samples were extracted, weighed and mixed in bidistilled water in order to analyze by inductively coupled plasma (ICP) ionic composition.
Setting: Frogs (*Rana Esculenta*) were reared in swimming-pool (Faculté des Sciences de Bizerte, Tunisia). Frogs were cared for under the Tunisian Code of Practice for the Care and Use of Animals for Scientific purpose and the Experimental Protocols were approved by the Ethics Committee.
Subjects: Treated and control groups (n=6) weighing 50-100g at the time of experiments were housed in the same condition three weeks before the beginning of the experiments.
Main outcome measures: Ionic composition will be used as a diagnostic marker of bio-effects of magnetic field.
Results: Acute exposure to magnetic field increased significantly the calcium (+298%, $p<0.05$) and iron (+50%, $p<0.05$) contents of frog sciatic nerve, whereas magnesium and copper contents remained unchanged. The association between magnetic field and cadmium, induced marked increase of calcium (+360%, $p<0.05$), whereas magnesium content remained stable.
Conclusions: Magnetic field exposure alters the ionic composition in the frog sciatic nerve, especially calcium and iron. Magnetic field magnifies the effect of cadmium on calcium homeostasis.

KEYWORDS: Magnetic field, cadmium, ionic composition, sciatic nerve.

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INTRODUCTION

There is growing concern about the increase in environmental pollution due to the emission of electromagnetic waves[1]. The mechanism of interaction between magnetic field and biological structures is still obscure. Epidemiological studies have failed to find a correlation in live subjects between the continuous presence of magnetic field (MF) at different intensities and the appearance of any particular pathology[2-5]. However, several investigations have demonstrated an increase in childhood leukemia and other related diseases in children from populations exposed to extremely low (50-60 Hz) frequency electromagnetic fields[6].

There is a vast literature on the effects of magnetic field on isolated nerve cells, cultured nervous tissue, living brain slices and behavioral measures of brain function[7-11]. Seaman and Wachtel[12] also described a decrease in spontaneous activity of neurons isolated from the

1-3. Laboratoire de Physiologie Animale,
Faculté des Sciences de Bizerte,
7021 Jarzouna,
Tunisia.

Correspondence:

Dr. Amara Salem
E-mail : sloumans@yahoo.fr

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marine gastropod Aplysia at relatively high intensities of electromagnetic waves. On the other hand, Chou and Guy[13] found no obvious electrophysiological changes in the frog sciatic nerve exposed to 2.45 GHz continuous or pulsed at modest intensities. Moreover, Nair[14] and Cleary[8] have reported, that the flux of positively charged sodium, calcium and potassium ions across cell membrane can also be affected by radio-frequency exposure, over a wide range of frequencies (27 MHz to 10 GHz). *In vitro* and in *vivo* studies[15] have showed that magnetic fields including those in the extremely low frequency regions affect cell membrane functions, particularly the movement of calcium. Collis et al[16] suggested that pulsed electromagnetic fields can have a direct effect on the movement of sodium across tissue and trans-epithelial potentials. The mechanism may depend on several factors, such as induced changes in certain ion pumps, the membrane potential and the surface charge of cell wall proteins[16]. While membrane permeability for mineral elements can be affected by cadmium[17]. Cadmium is a non-essential trace element, and its toxicity may be due to induced alterations in cellular homeostasis of essential metal ions, such as copper, zinc, and calcium[17]. Severe pathologies of the central nervous system are elicited by the high blood-brain barrier permeability to cadmium[18-21]. It is suggested that cadmium plays an important role in regulating the transmembrane flux of calcium in cells[22]. Interestingly, literature analysis showed a lack of data regarding the combined effects of MF and heavy metals in the biological system.

The present study performed an experimental approach to determine the effects of acute exposure to magnetic field on mineral elements content of frog sciatic nerve, and the possible interaction between magnetic field and cadmium treatment.

METHODS

Animals and exposure scenario

Frogs (Rana Esculenta) weighing 50 -100g at the time of experiments were used in this study. Frogs were exposed in the Plexiglas cage between the two bobbins of the Lake Shore System, one hour/day (between 9h–12h) during three days at a static magnetic field (67mT). The cage in the Lake Shore contains two frogs for each exposure. The control frogs were positioned in the Lake Shore system one hour/ day (between 9h–12h) during three days without applying magnetic field.

The intensity of magnetic field was measured and standardized in the total floor area of the Plexiglas cage at 67mT. The cage have the seize of 20cm length, 10cm width and 20cm height. The two bobbins of the Lake shore System were separated by the distance of 12 cm.

Frogs were cared for under the Tunisian Code of Practice for the Care and Use of Animals for Scientific purposes and the Experimental Protocols were approved by The Ethics Committee (Faculté des Sciences de Bizerte, Tunisia).

Exposure system

Lake Shore Electromagnets (Lake Shore Cryotronic, Inc, Westerville Ohio, USA) are compact electromagnets suited for many applications such as magnetic resonance demonstrations. Water-cooled coils provide excellent field stability and uniformity when high power is required to achieve the maximum field capability for the electromagnet. We have an accurate pole alignment by precise construction of the air gap adjustment mechanism[23]. In the present study we used a static magnetic field generated by the Lake Shore Model.

Chemical assays

Sciatic nerve samples of treated and control frogs were extracted, weighed and mixed in bidistelled water. Minerals were assayed simultaneously by inductively coupled plasma (ICP). The homogeneous solutions were transformed by spraying in aerosol ionized with gasified argon plasma in high temperature about 6000°C at 10000°C. An ionic detector record ionic transmission, ions measurement permits to calculate directly the concentration of analyzed element after logical treatment[23].

Data presentation and statistical analysis

Data are reported as the mean ± SEM. Differences between means were evaluated by one-way analysis of variance (ANOVA). Statistical significance of the differences between means was assessed by Student's t-test. The level of significance was set at $p<0.05$.

RESULTS

As shown in figure 1 & 2, acute exposure to MF (67mT) during one hour / day for three consecutive days increased significantly the calcium (1.95 ± 0.33 ng / mg vs. 0.49 ± 0.07 ng / mg, $p<0.05$) and iron (0.09 ± 0.006 ng / mg vs. 0.06 ± 0.008 ng / mg, $p<0.05$) content in frog sciatic nerve. Magnesium content (0.35 ± 0.11 ng / mg vs. 0.21 ± 0.091 ng / mg, $p>0.05$) and copper content (0.08 ± 0.01 ng / mg vs. 0.071 ± 0.01 ng / mg, $p>0.05$) remained constant. Cadmium treatment (1.5mg / kg, in lymphatic sac) increased the calcium content of sciatic nerve (1.78 ± 0.22 ng / mg vs. 0.49 ± 0.07 ng / mg, $p<0.05$) (figure 3), whereas magnesium remained stable (0.09 ± 0.02 ng / mg vs. 0.21 ± 0.09 ng / mg, $p>0.05$) (figure 4). When cadmium treatment was associated with magnetic field (67mT) during one hour / day for 3 consecutive days, we note a marked increase of calcium (2.26 ± 0.24 ng / mg vs. 0.49 ± 0.07 ng / mg, $p<0.05$) in the sciatic nerve (figure 3). As shown in figure 4, the same treatment induced a non significant increase in magnesium contents (0.10 ± 0.02 ng / mg vs. 0.21 ± 0.09 ng / mg, $p>0.05$).

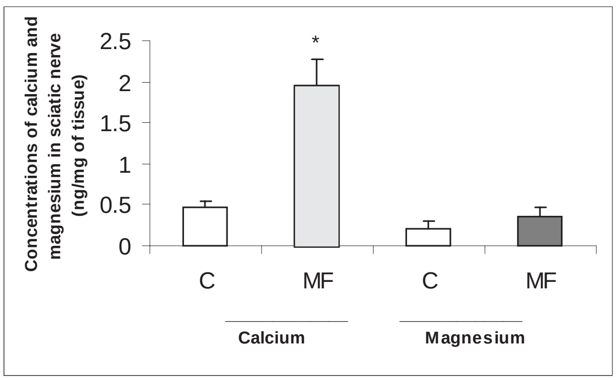

Fig. 1: Effects of acute exposure to magnetic field (MF, 67mT) on calcium and magnesium contents in frog sciatic nerve. C (control), MF (magnetic field).
* $p<0.05$

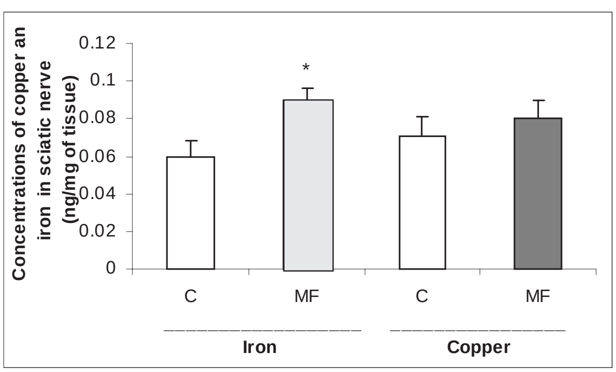

Fig. 2: Effects of acute exposure to magnetic field (MF, 67mT) on iron and copper contents in frog sciatic nerve. C (control) ; MF (magnetic field).
* $p<0.05$

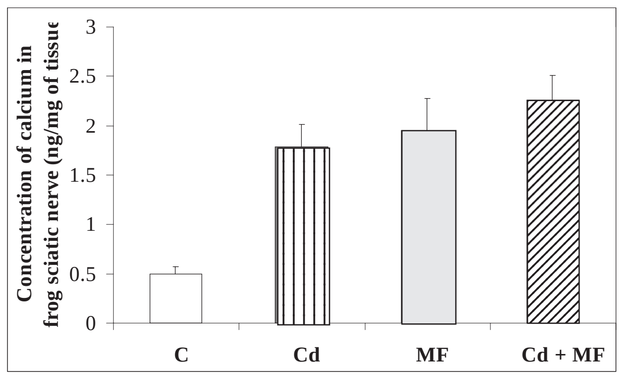

Fig. 3: Effects of acute exposure to magnetic field (MF, 67mT) and / or cadmium treatment on calcium content in frog sciatic nerve. C (control), MF (magnetic field), Cd (cadmium)
* $p<0.05$

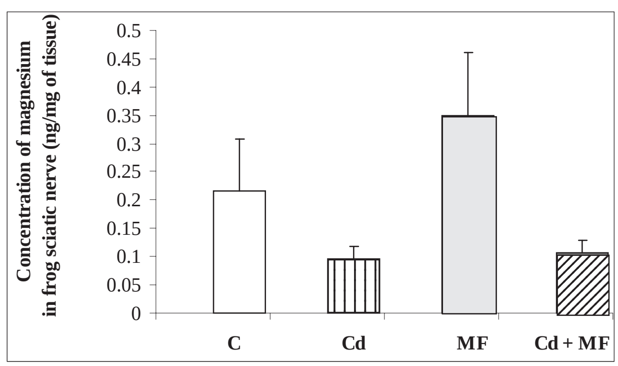

Fig. 4: Effects of acute exposure to magnetic field (MF, 67mT) and / or cadmium treatment on magnesium content in frog sciatic nerve. C (control), MF (magnetic field), Cd (cadmium)
* $p<0.05$

DISCUSSION

The major finding of this study was that the MF exposure disrupts the mineral content in the sciatic nerve, especially calcium and iron. The combined effect of MF and cadmium treatment elicited a marked increase of calcium. According to our findings, MF exerted probably a partial effect on biological systems via minerals.

Recently, Panagopoulos et al.[24] reported that the basic mechanism of electromagnetic fields action on cells is the forced-vibration of all the free ions on the surface of a cell's plasma membrane, caused by an external oscillating field, this coherent vibration of electric charge is able to irregularly gate electrosensitive channels on the plasma membrane and thus cause disruption of the cell's electrochemical balance and function[24]. Moreover, Kirschvink[25] proposed a model in which MF forces on magnetite particles are visualized as producing the opening and closing of pressure-sensitive ion channels in membranes. There is little information regarding the factors that control and modulate the bioeffects of metals under MF. One element of interest is cadmium, since its oral or parenteral administration has been shown to be toxic. Indeed, elevated levels of cadmium in brain[26] have been associated with several neurological diseases. Until now there are few studies dealing with the combined effects of cadmium and MF in biological systems. The present work was carried to characterize the influence of cadmium and/or MF on minerals permeabilities in the sciatic nerves.

Previous study suggests that extremely low frequency (ELF) can affect the nervous system[27]. Changes in blood-brain-barrier, morphology, electrophysiology, neurotransmitter functions, cellular metabolism, calcium efflux and genetic effects have been reported in the brain of animals after exposure to ELF[27]. These changes can lead to functional changes in the nervous system and the behavior of animals after exposure to Radiofrequency[28]. However, there is no clear understanding of how RF fields have such effects. In the present study we demonstrated that MF increased significantly calcium and iron content of frog sciatic nerve. On the contrary, MF exposure did not alter magnesium and copper content. Numerous studies showed that MF exerted a preponderate controlling influence on the flux of positively charged sodium, calcium and potassium ions across cell membranes[8,14]. The most evident effects induced by magnetic waves are the mobilization of intracellular calcium and occasionally morphological changes, although cell signals when present are extremely variable[29-31]. Likewise, Walleczek et al.[32] reported an increase in calcium influx into human Jurkat T cells after two minute exposure to 60Hz, 2mT EMFs. Interestingly, the present investigations demonstrated that membrane permeability for calcium and iron was affected by MF. The evidence that MF acts to modulate mineral permeability raises the question of how the MF regulate heavy metals (cadmium) response to different waves.

Earlier studies showed that cadmium induced alteration in cellular homeostasis of essential metal ions, such as copper, zinc and calcium[17], which in turn was implicated in many, severs neuropathologies. Our data showed that cadmium treatment increased the calcium concentration of frog sciatic nerve, whereas the magnesium content remained constant. These results were in accordance with previous study showing that cadmium treatment in dissociated mesencephalic trigeminal neurons of the adult rat elevated the intracellular calcium and induced morphological changes in neurons concentration[33]. Moreover, Jelko et al.[34] demonstrated the effect of cadmium on the homoeostasis of intracellular free calcium. Cadmium affects intracellular calcium binding proteins, and interferes with the calcium influx/efflux machinery[35]. An interpretation of the results showed that the variation of calcium content can be attributed to the interaction of cadmium with plasma membrane and calcium channels in the sciatic nerve. The hypothesis of an action of MF on heavy metals (cadmium) is reinforced by the fact that the combined effect of MF and/or cadmium treatment induced a pronounced increase of calcium (+360%). We have noted an apparent lack of Mg response to MF and / or cadmium treatment. Therefore the effect of MF and / or cadmium treatment appeared to be restricted to calcium ion. The mechanism under laying this change remained to be investigated.

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