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Mechanism of Action of Extremely Low Frequency or Static Magnetic Fields on Cells: Role of Oxidative Activation of TRPM2

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Running title: Magnetic field and activation of TRPM2.

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Abstract

There have been a great number of investigations about the influence of weak magnetic fields on biological systems, such as isolated cells and whole organisms. This is also a subject of considerable medical concern since old epidemiologic observations have indicated a possible tumorigenic effect of these fields. Their mechanism of action, however, is not firmly established. A large number of biological effects of electromagnetic fields have been attributed either to the production of reactive oxygen species (ROS) or to the entrance Ca^{2+} in the cell. A new biochemical pathway is proposed that covers these two possibilities: the primary effect of the magnetic field would be by the mechanism of radical pairs resulting in the production of ROS; these could activate the ion channels TRPM2 producing cellular inflow of Ca^{2+}, which would induce the calcium dependent effects. Thus, a large number of biological effects observed up to the present could be explained.

Key words: magnetic field effect; TRPM2; melastatin; calcium channel; reactive oxygen species; radical pairs.

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INTRODUCTION

Life exists on Earth since 3.8 billion years, according indications about primitive forms of life [Brack, 1998]. During most part of this time, cells and more complex organisms were subjected to the geomagnetic field (0.5 Gauss). Only recently, around 150 years, with the use of electric energy, living organisms have been subjected to more intense magnetic fields. These arise from lines of electricity transmission with very low frequencies (50-60 Hz), and the use of electrical domestic appliances, such as electric razors, hair dryers, electric blankets, video displays and radio communications systems (cell phones) and industrial equipments. That is a novel influence on life; cells and whole organisms have had no time for adaptation if they could have any effect. It is thus reasonable to investigate the possible biological effects of magnetic fields [Hardell and Sage, 2008].

An increased interest in these studies arose since some epidemiological reports from different countries indicate an increase in the incidence of leukemia and other malignant diseases in children living in houses near high voltage lines of electric transmission of 50-60 Hz [Lacy-Hulbert et al., 1998; Ahlbom and Feycht, 1999; Kheifets et al., 2010]. Conflicting results have appeared, however, some confirming and other rejecting this possibility [Ahlbom et al., 2001; Shupak et al., 2004]. It is difficult to interpret these studies, since they involve simple correlations about a subject that may be influenced by many variables and the multifactorial nature of these diseases. Thus, only well controlled laboratory experiments could clarify the issue, which has evident medical importance and are necessary to establish the acceptable level of exposure of the population.

Apart from the possible health hazards produced by EMF, there is also an interest in their possible medical beneficial effects [Basset, 1993; DiCarlo et al., 1999; Selvan et al., 2007].

One should also note that non-ionizing EMF cover a great frequency spectrum, from electric power distribution operating at 50 or 60 Hz to the radio frequency region up to 300 MHz used in cell phones, the microwave region with millimeter wavelength and the far infrared radiation. All these frequency ranges evidently deserve a separate study and may cause different biological effects by different mechanisms. Moreover, it is necessary to identify in

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different conditions the target or targets of the fields on cells, tissues or whole organisms.

At present there is no established biophysical mechanism to explain the biological effects of weak EMFs of 50-60 Hz or others usually found in the environment because the energy they bring is very small compared to the thermal or electrical noise produced by the movement of ions and molecules as well as endogenous processes operating in the cells. [Valberg et al., 1997]. Since many biologic affects have been reported with isolated cells as well as in vivo experiments with animals [Simkó, 2007; Akan et al., 2010; Pall 2013; Ross and Harrison 2013; Kleij et al., 2016], we should consider that possible non-thermal mechanisms might be involved.

Possible mechanisms for biologic effects of EMF

Generally, an electric field is also present when organisms are subjected to an EMF. However, tissues are surrounded by an electrolytic conducting medium that makes an efficient shielding for its penetration. The magnetic field is thus the most important agent since it is very difficult to be blocked.

Many hypothesis have been proposed for the mechanism by which magnetic fields might produce biologic effects [Valberg et al., 1997; Simkó, 2007; Pall, 2013; Grundler et al., 1992; Eichwald and Walleczek. 1997; Panagopoulos et al., 2002; Goodman and Blank, 2002; Funk and Monsees 2006; Hore, 2012]. Presently two are the most likely mechanisms based on experimental findings and plausible physical processes: the mechanism mediated by calcium channels, and the radical pair mechanism.

A great variety of biologic effects has been associated with EMF. There is considerable evidence that calcium channels may be involved in many of these effects, since calcium channels inhibitors can block them [Pall, 2013]. On the other hand, there is also good evidence that magnetic fields may induce or increase the production of oxygen reactive species (ROS) in a sort of cell types, and that many of these effects can be blocked or decreased by ROS scavengers [Simkó, 2007; Simkó and Mattson, 2004].

There is no a priori ground to suppose that magnetic fields have a unique biophysical mechanism of action upon cells or whole organisms. Despite the experimental evidence for the participation of calcium channels on their effects there is no established explanation for the primary physical process of their action. As to the

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effects of ROS, there is a physical process that can explain their production by the presence of a magnetic field – the radical pair mechanism. It has a solid theoretical and experimental evidence of being able to influence the rate and yields of same chemical reactions. The aim of this article is to present a biochemical pathway that connects the biological effects via calcium channels with the radical pair mechanism.

Radical- pair mechanism

Free radicals are atoms or molecules with one or more unpaired electrons. Many radicals are highly reactive with a tendency to pairing their electrons, so that they can react with another molecule and pick up or donate electrons, forming other radicals including reactive oxygen species [Simkó, 2007]. This may set up a chain of reactions that can produce biological effects.

The best understood physical mechanism by which magnetic fields can produce chemical effects is their influence on radical pair recombination. This effect may be observed even with a very low field intensity such as the geomagnetic field (around 0.5G). A detailed description of this physical mechanism can be found in [Grissom, 1995]. Shortly, when a pair of radicals is produced in a sequence of reactions, they remain for a short time in a cage of solvent, and may undergo a recombination or escape from the cage. The presence of a magnetic field can increase the lifetime of the radicals before recombination and thus favor their escaping from the solvent cage. Then, a sequence of radical reactions can take place including the formation of the superoxide ion and other oxygen reactive species that may cause biological effects.

THE PROPOSAL

A BIOCHEMICAL PATHWAY THAT CONNECTS THE RADICAL PAIR MECHANOSM WITH THE ACTIVATION OF TRPM2 CHANNELS

A great variety of biologic effects observed could be dependent of a unique action of the magnetic field, which has a firmly established physical basis. This proposal is based on the properties of the transient receptor potential, melastatin (TRPM2). It is an oxidant-activated channel belonging to the family of TRP cation channels

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[Kraft et al., 2004; Hecquet et al., 2008; Wehage et al., 2002]. The oxidative activation of this receptor induces calcium entry into cells eliciting calcium dependent cellular processes. The diagram of figure 1 illustrates this possibility.

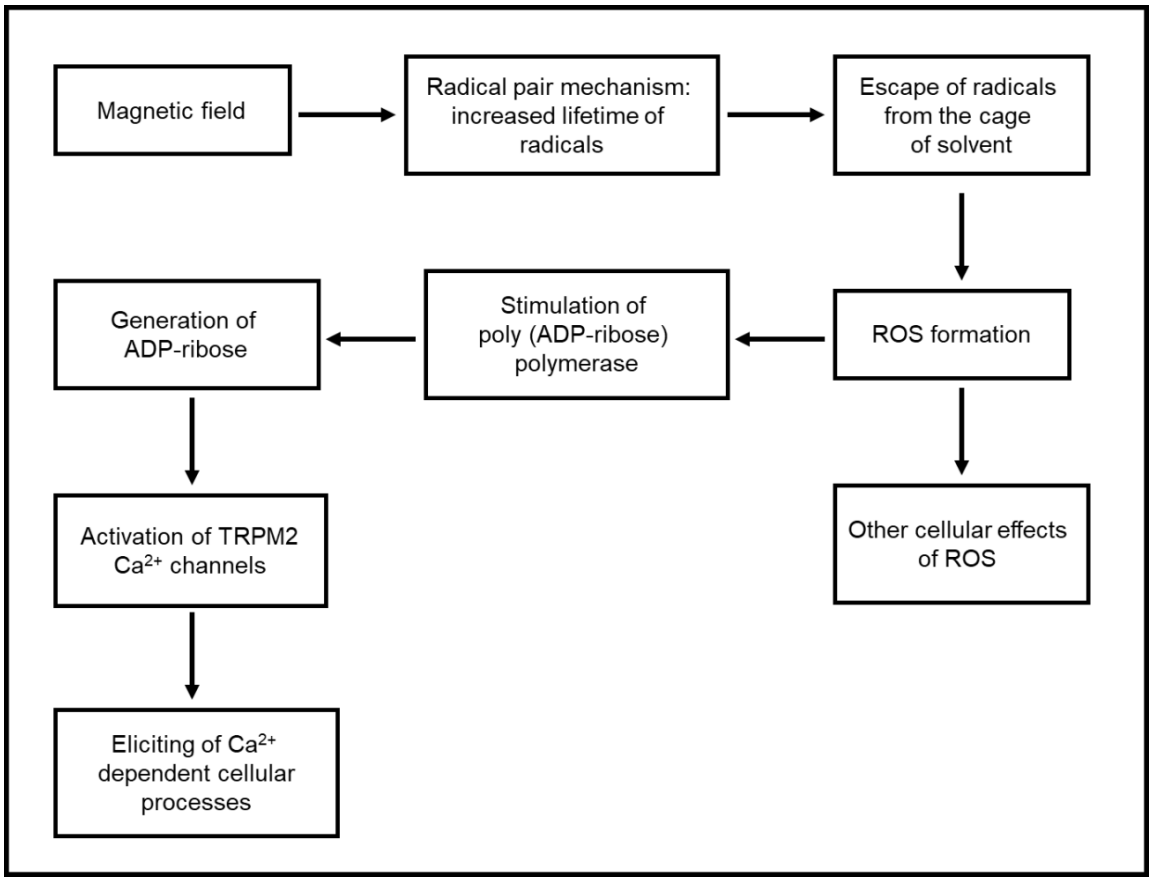

Figure 1 - Mechanism of action of magnetic fields on cells. The primary effect would be on the recombination of pairs of radicals formed in cellular reactions. This induces an increase in the lifetime of radicals, which could then escape from the cage of solvent resulting in the formation of reactive oxygen species (ROS); these stimulate the enzyme poly(ADP-ribose) polymerase to generate ADP-ribose which activates TRPM2 calcium channels eliciting calcium dependent cellular processes.

TRPM2 channels is widely expressed in mammalian tissues, including the brain, peripheral blood cells, bone marrow, spleen heart, liver and microglia [Kraft at al., 2004; Sano et al., 2001; Vehrhahn et al., 2010]. Evidently, there might be other independent effects of ROS as signaling molecules.

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There are indications that TRPM2 activation is mediated by the stimulation of poly (ADP-ribose) polymerase to generate ADP-ribose which binds to TRPM2 channel [Fonfria et al., 2004; Perraud et al., 2005]. However, it was also reported that the activation of this calcium channel could in some cases be induced independently of ADP-ribose [Wehage et al., 2002].

There have been described biological effects with oscillating as well static magnetic fields. One should note that with very low frequency (60 Hz or lower alternate fields), the time- scale of the variation of the magnetic field is much slower than the lifetime of the radical pairs (around 1-100 ns) [Eichwald and Waleczek, 1997].Thus, the action on radical pairs will be effective with extremely low frequency as well as with static fields.

The plausibility of this proposal is based on the following elements:

a) The fact that the action of magnetic fields on radical pair's recombination is very well established physicochemical process [Grissom, 1995; Rodgers, 2009].
b) The activation of TRPM2 channels by oxidation promoted by ROS [Hecquet et al., 2008].
c) The great number of observations showing the importance of cellular calcium influx upon the exposure of cells to magnetic fields, and the inhibitory effect of calcium channel blockers [Pall, 2013].
d) Several observations on the participation of ROS in the effects of MF, which could be inhibited by treatment with ROS scavengers [Katsir and Parola 1998, Simkó, 2007; Simkó and Mattson, 2004].

EXPERIMENTAL TEST OF THIS MECHANISM

An attempt to test experimentally this mechanism might be to verify the dependence of MF effects on the formation of ADP-ribose. It has been shown that TRPM2 can be activated by ADP-ribose which binds to its cleft in the carboxyl terminus [Perraud et al., 2001; Parraud et al., 2005].

The ROS specie, H_2O_2, was shown to stimulate poly(ADP-ribose) polymerase (PARP) to generate ADP-ribose [31]; therefore, the treatment of cells with inhibitors of this enzyme, such as DPQ [Fonfria et al., 2004; Hecquet et al., 2008], could impair the effect of

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magnetic fields in those cases in which the effects of the fields has been shown to be related to the influx of calcium.

However, as indicated in figure 1, the mechanism proposed does not exclude other possible biological effects of ROS, which would be independent of activation of calcium channels.

DISCUSSION

It is important to have plausible mechanisms for the action of MF that are consistent with the principles of physics, chemistry and the present biological knowledge, in order to guide investigations in this field.

The possible direct activation of VGCC by MF is in line with a great number of observations showing Ca^{2+} influx in cells upon the action of the field, with several intensities and frequencies [Pall 2013]. Theoretical physical analysis of a possible direct effect of EMF on ion channels indicated that this was a plausible mechanism [Panagopoulos et al., 2002]. Other investigations however argued against this possibility [Valberg et al., 1997]. However, an attempt to demonstrate this possibility with 1 mT, 50 Hz MF gave negative results in experiments based on patch-clamp recordings [Grassi et al., 2004]. It has been suggested that the effect of MF could be in the modulation of channel expression or in its turnover [Piacentini et al 2008]. The question of their activation has not been addressed.

On the other hand, the radical pair mechanism has a solid theoretical and experimental evidence to interfere in the rate and yields of chemical reactions involving radical pairs as intermediates [Grissom 1995; Rodgers 2009]. Nevertheless, the detailed biochemical process where radical pairs are formed in biological systems has not been established yet. Some observations with mouse macrophages suggest that the exposure to 50 Hz electromagnetic fields at 1 mT can stimulate the NADH pathway to produce superoxide [Rollwitz et al., 2004]. There are also interesting observations with flavoproteins in model systems as well as in blue- light photoreceptor cryptochromes that might be involved in magnetosensitivity in some animals such as migratory birds [Sovov'you et al. 2010]. The possible role of flavo proteins has also been addressed in experiments with mammalian cells (rat pulmonary arterial muscle). It was shown that radio frequency magnetic field (7 MHz, at 10 μT_{rms} and 45 μT static magnetic field) can induce the production of H_2O_2; the radical pair mechanism

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proposed involves the reaction of an enzyme-bound reduced flavin with molecular oxygen [Usselman et al. 2013]. Nevertheless, there are controversies about the possible role of flavin enzymes as the site of action of MF [Messiha et al., 2015]. In view of the number of biochemical reactions in which radicals are involved, it is reasonable to continue the investigations to detect the formation of radical pairs susceptible to the action of magnetic fields.

Conclusion: The proposed biochemical pathway starting with the effect of MF on pairs of radicals formed by cellular metabolism could explain a large number of biological effects of extremely low frequency or static magnetic fields observed up to the present.

Conflict of interest

The author declares no conflicts of interest.

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