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Electromagnetic field of extremely low frequency decreased adenylate kinase activity in retinal rod outer segment membranes

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Abstract

Adenylate kinase activity in rod outer segment membranes of bovine retina decreased of about 55% when exposed to an extremely low frequency electromagnetic field of 75 Hz and 250 μT. The effect was independent of the time of permanence in the field. Negligible effects of the field were found on the enzymatic activity of a soluble isoform of adenylate kinase or of rod outer segment membranes solubilized with Triton, suggesting the importance of the membrane in determining the conditions of the enzyme inactivation.

Keywords: ELF electromagnetic fields effects; Adenylate kinase; ATP production; Phototransduction; Enzyme inactivation

1. Introduction

The effects of extremely low frequency electromagnetic fields (ELF-EMFs) on simple systems like colloids and particles in solutions as well as on living matter have been under investigation for years [1–8]. However, despite the large number of reports on these effects, an unifying physico-chemical understanding is still lacking not only for living matter [9–15] but also for the more simple precipitation processes of carbonates and oxalates [16–19]. However, it has been recently shown that the weak electromagnetic energy carried by ELF-EMFs can account for the change in the surface energy of a body simulated fluid solution enhancing the vaporization of the CO_2 [20]. Thus, if the ELF-EMFs are acting on the interfacial regions, such a mechanism may be active also in more complex systems such as the biological membranes. As ELF-EMFs were found to affect the activity of membrane enzymes such as Na,K-ATPase or cytochrome oxidase [21], this hypothesis could be checked on enzyme complexes bound to biological membranes.

The rod outer segment (ROS) of bovine retina contains two forms of adenylate kinase activity, one bound to membranes and the other one soluble in the cytosol [22]. This system seems very suitable for investigating the effects of ELF-EMFs on the activity of an enzyme in different conditions, membrane-bound or free in solution.

The applied ELF-EMF was effective on the adenylate kinase activity bound to membranes but gave no effect on soluble isoforms of the enzyme or a small effect on Triton solubilized disk membranes, suggesting that the membrane structure is crucially involved.

2. Experimental

2.1. Rod outer segments and disk membranes preparations

Rod outer segments (ROS) were isolated from bovine retina in dim red light by following the method of Schnetkamp and Daemen [23] by sucrose gradient centrifugation. Intact ROS were first washed in an isotonic medium containing 40 mM Tris–Maleate pH 7, 120 mM KCl and then centrifuged for 10 min at 1500 × *g*.

The pellet was resuspended in distilled water and homogenized in a Potter apparatus for 10 min at ice temperature. Homogenated ROS were then centrifuged for 60 min at 20,000 × *g*. The supernatant was withdrawn and the pellet was washed twice and centrifuged again. The pellet (ROS membrane preparation) was then resuspended in the initial

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volume of distilled water and the second supernatant was added to the first (ROS soluble fraction).

Mitochondrial contamination seems absent in the ROS preparations as no activity was found of the mitochondrial marker succinate dehydrogenase.

2.2. ATP formation

Adenylate kinase activity was measured in the presence or in the absence of the field for 1 min after ADP addition to the reaction mixture containing the disk membranes kept in the presence or in the absence of the field for different time intervals of 0, 2, 5 and 10 min. The reaction mixture contained 40 mM Tris–HCl pH 8, 120 mM KCl, 5 mM $MgCl_2$, 2.5 mM ADP and 100 μg/ml of disk protein. The Ca^{2+} concentration of the mixture was kept 1 μM with a Ca^{2+}-EGTA buffer [24] (EGTA was 2 mM). Before and after 1 min of incubation 100 μl of the reaction mixture were withdrawn and added to 50 μl of 25% PCA.

Each sample was centrifuged shortly at 14,000 rpm and then 100 μl of supernatant was withdrawn and neutralized with 50 μl K_2CO_3. The centrifugation was repeated to remove potassium perchlorate. Aliquots of 100 μl of each neutralized extract were used to assay ATP.

In the experiments where a standard adenylate kinase activity was measured, a soluble adenylate kinase purified from rabbit muscle (Sigma) was used.

2.3. ATP assay

ATP was assayed enzymatically, following the methods of Bârzu and Michelson [25] with minor modifications. For ATP assay, the medium contained 0.1 ml of neutralized perchloric extract, 50 mM Tris–HCl pH 8.0; 1 mM NADP; 10 mM $MgCl_2$; 5 mM glucose in 1 ml final vol. Samples were analyzed spectrophotometrically before and after the addition of 4 μg of purified Hexokinase/glucose-6-phosphate dehydrogenase (Boehringer). The rise in absorbance at 340 nm, due to NADPH formation, was proportional to the ATP concentration.

Protein concentrations were determined using the Bradford method [26].

2.4. Magnetic field production

ELF EMFs were produced by the equipment "Biostim" (IGEA, Modena, Italy) mainly used for clinical application such as that to accelerate the healing of bone fractures. The equipment supplies a square wave with a maximal applied tension of 180 V, a period of 13.3 ms (75 Hz of frequency), a duty cycle of 10%, to a couple of Helmoltz coils (each with 1000 turns of copper wire of 0.2 mm of diameter) with internal and external diameter of 72.5 and 82.5 mm, respectively. Measurements of the intensity of the magnetic field B with a gaussmeter, showed that it was fairly constant for the entire distance between the coils, giving values of about 250 μT when the distance between the coils were 12 cm. The sample for the enzymatic activity measurements was always placed at the center of the distance between the two coils. The temperature variation of the sample in the presence of the applied field was found constant within the experimental error of ± 0.1 K.

3. Results and discussion

The effects of an ELF-EMF of 75 Hz and 250 μT on the adenylate kinase activity of ROS membranes were measured as follows. The reaction mixture containing the disk membranes but not the substrate ADP was previously kept in the presence or in the absence of the field for different time intervals of 0, 2, 5 and 10 min. At the end of the incubation time, ADP was added to the reaction mixture and the enzymatic activity was measured for 1 min in the presence or in the absence of the field. The results of these measurements are shown in Fig. 1. A dramatic decrease of about 55% in the adenylate kinase activity of ROS membranes appears due to the field exposure. In fact, the mean value of the nanomoles of ATP produced/min/mg of protein, obtained by summing the activity values of intact ROS membranes at different exposure times, dropped from 75 ± 3 (S.D.) to 34 ± 3 (S.D.) in the presence of the field. The enzymatic activity appears constant within the experi-

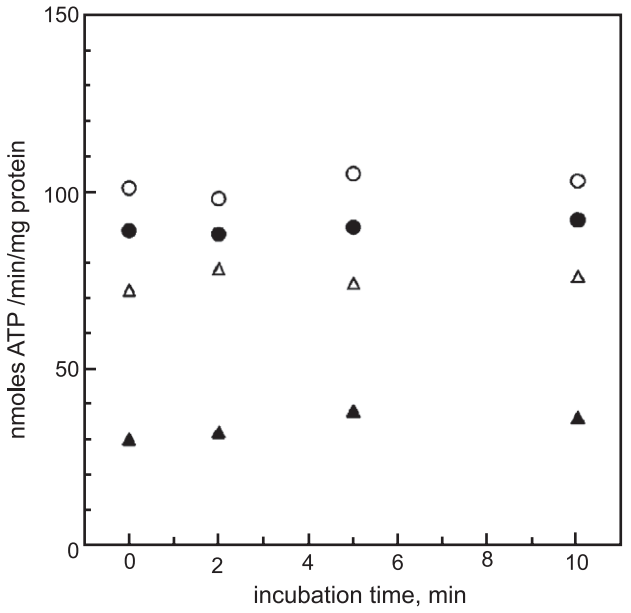

Fig. 1. Time course of the adenylate kinase activity of ROS membranes in the absence (Δ) or in the presence (▲) of an alternate magnetic field of 75 Hz and 250 μT; of ROS membranes dissolved in 0.1% Triton in the absence (○) or in the presence (●) of the same field. The enzymatic activity, expressed as nmols of ATP produced/min/mg protein, was measured for one min in the absence (Δ,○) or in the presence (▲,●) of the field. Each point represents the mean of five measurements. A standard deviation of about 10% was calculated for each mean value but was not drawn for a better reading of the data.

mental errors and independent of the time of permanence in the field. Instead, when the same experiments were performed on ROS membranes solubilized by 0.1% Triton, the adenylate kinase activity was slightly affected by the field (see Fig. 1). The higher values (about 26%) of enzymatic activity found in ROS membranes solubilized by Triton are usually observed when membrane bound enzymes are solubilized in low concentration of a mild detergent [27]. Moreover, when the experiments were run on a soluble isoform of adenylate kinase present in ROS cytosol or on the soluble adenylate kinase purified from rabbit muscle, no effect of the field on the enzymatic activity was found (data not shown).

In order to investigate further about the field effect, the enzymatic activity of the adenylate kinase of the ROS membranes kept in the field for a maximum of 30 min was measured immediately after the withdrawal of the field. The results were identical to those of the controls, indicating that there is no remanent effect of the previous field exposure on the enzymatic activity.

The data reported in this paper show that an ELF-EMF of 75 Hz and 250 μT produces a decrease of about 55% of the adenylate kinase activity of retinal ROS. As shown in Fig. 1, the effect of the field on enzyme activity starts within the first minute and remains constant during the experiment. When the enzyme is removed from the field, full activity is observed. However, the field had no effect on the soluble isoforms of the same enzyme, suggesting that the field action is mediated by the membrane organization and structure.

The effects of ELF-EMFs on enzyme functions [28–31] are surprising in comparison with the very small amount of energy (about 10^{-12} Kcal/mol) carried by the applied field. Although there is evidence that animals can detect small changes in the Earth's magnetic field with a mechanism which coud be based on magnetic sensitive chemical reactions [32], however, for the ELF-EMFs to be effective for biochemical reactivity, amplification is required such as that of the classical signal transduction cascades on biological membranes [33].

Current theories and models on the effects of ELF EMFs on enzyme activities refer to changes of motion of ions such as calcium at the active site [34] or alterations in the binding parameters of ligand-receptor [35,36]. However, the activity decrease of disk adenylate kinase in the presence of ELF field, does not seem to depend on changes of motion of ions such as calcium or magnesium. In fact, these ions are necessary for the enzymatic activity of adenylate kinase of both soluble or bound-to-membrane isoforms. On the contrary, the effect of the field could be on the membrane organization and structure which would limit in some way the binding of the substrate to the active site of the enzyme. Frequency dependent physical interactions with subsystems such as cell membranes have been often emphasized [6,13]. Important results involving the biological membranes were reported for cell cultures exposed to 50 Hz magnetic fields [37–39]. In particular changes in lipid molecular dynamics of the cell membrane were measured by florescent probes [38], while electron microscope images of freeze-fractured membranes indicate that the effect of the field was that of a significant clustering of the distribution of the intramembrane proteins [39]. Therefore, it seems very likely that the effects of the field on the adenylate kinase could involve changes of interfacial parameters on the disk membranes which would alter the enzyme functionality. Experiments are in progress on different membrane-bound enzymes exposed to ELF-EMFs to verify the generalization of this membrane hypothesis.

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