

Effect of alternating the magnetic field on phosphate metabolism in the nervous system of *Helix pomatia*

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ABSTRACT

The effect of extremely low frequency magnetic fields (50 Hz, 0.5 mT) - ELF-MF, on phosphate metabolism has been studied in the isolated ganglions of the garden snail *Helix pomatia*, after 7 and 16 days of snail exposure to ELF-MF. The influence of ELF-MF on the level of phosphate compounds and intracellular pH was monitored by ³¹P NMR spectroscopy. Furthermore, the activity of enzymes involved in phosphate turnover, total ATPases, Na⁺/K⁺-ATPase and acid phosphatase has been measured. The exposure of snails to the ELF-MF for the period of 7 days shifted intracellular pH toward more alkaline conditions, and increased the activity of investigated enzymes. Prolonged exposure to the ELF-MF for the period of 16 days caused a decrease of PCr and ATP levels and decreased enzyme activity, compared to the 7-day treatment group. Our results can be explained in terms of: 1. increase in phosphate turnover by exposure to the ELF-MF for the period of 7 days, and 2. adaptation of phosphate metabolism in the nervous system of snails to prolonged ELF-MF exposure.

Key terms: ELF-MF, garden snail, ³¹P NMR spectroscopy, phosphate turnover enzymes.

INTRODUCTION

In recent decades scientific interest in the effect of static and alternating magnetic fields on the biological systems has increased. Various strengths of magnetic fields are investigated for their biological effects, but mostly at the strength of the Earth's magnetic field (35 - 70 μ T) and, much higher, man-made magnetic fields that are normally present in our environment. This interest arises from the fact that all living organisms are constantly exposed to the Earth's and man-made magnetic fields (Table 1).

The ability of biological systems to detect the Earth's magnetic field is found in diverse invertebrates and vertebrates and is important for compass orientation of animals (Katz and Yilks, 1979; Mather and Baker, 1981; Zoeger et al., 1981; Blakemore, 1982; Mathis and Moore, 1984; Lohman and Willows, 1991; Wang et al., 2002). Furthermore, numerous studies have explored the interaction of man-made static and alternating magnetic fields with biological systems. Previous research showed that these magnetic fields can induce changes in behavior (Rudolph et al., 1985; Prato et al., 1996; Janac et al., 2005), enzyme activity (Nossol et al., 1993; Blank and Soo, 1996; Liboff et al., 2003; Chen et

al., 2009), the synthesis and release of neurohormons (Peric-Mataruga et al., 2008), biophysical properties of neurons (McLean et al., 1995; Calvo and Azanza, 1999; Ye et al., 2004; Todorovic et al., 2007), synaptic transmission (Rosen, 1992) and ion channel currents (Shen et al., 2007). Furthermore, magnetic field influence on nucleic acids and protein synthesis has been found (Cridland et al., 1999; Ciombor et al., 2002; Hirai et al., 2002; Schmitz et al., 2004). However, little is known about magnetic field effect on the phosphate metabolism of the nervous system.

In order to test the effect of magnetic fields on nervous system metabolism, we chose the nervous system of the snail *Helix pomatia*, which is a well-described model system for neurophysiological studies (Rozsa 1984, Altrup 2004). As we have already shown, neuronal membrane properties of *Helix pomatia* are influenced by static magnetic fields (Nikolic et al., 2008). Other studies have documented that magnetic fields induce changes in the bioelectric properties of snail neurons (Balaban et al., 1990; Moghadam et al., 2008; Ayrapetyan et al., 2004). As is known, the firing properties of neurons and electrical signaling between cells imply specific energy demands (Magistretti, 2003). Furthermore, maintenance of the electrochemical gradient,

Table 1
Magnetic flux densities of Earth and some man-made magnetic fields

Natural magnetic field	(μ T) Man-made Magnetic fields
Earth magnetic field 35-70	Hair dryers 6 - 2000
	Electric shavers 15 - 1500
	Vacuum cleaners 200 - 800
	Mixers 60 - 700
	Industrial processes 0.7 - 6000*

Examples of some magnetic flux densities (units in μ T) near various home appliances measured at the distance of 3 cm; * distance measured at 0.1-2 m.

According to environmental health criteria of the World Health Organization, Geneva, <http://www.inchem.org/documents/ehc/ehc/ehc69.htm#SubSectionNumber:3.2.1>) a

particularly for Na^+ and K^+ ions by Na^+/K^+ -ATPase, is the main energy consuming process in neuronal cells. Moreover, the energy status of the cell influences bioelectrical properties of the membrane (Lara et al., 1999).

We wanted to test whether magnetic fields of extremely low frequency (50 Hz) and of a magnetic flux density of 0.5 mT (ELF-MF) would have effects on the nervous system metabolism of *H. pomatia*. As can be seen from Table 1, alternating magnetic fields of similar strength can be encountered in the vicinity of various home appliances.

In order to explore whether exposure to ELF-MF can cause changes in the level of phosphate compounds in the nervous system of the snail, we used the ^{31}P NMR spectroscopy, which provides direct information about tissue energy metabolism and indirect data from intermediary metabolism. To investigate ELF-MF influence in more detail, some of the enzymes involved in phosphate (P) turnover were also explored. So far, several reports about magnetic field influence on the activity of enzymes involved in P turnover have been reported (Blank and Soo, 1996; Chen et al., 2009). We have chosen to explore the total ATPases, Na^+/K^+ -ATPase and acid phosphatase enzymes in the snail nervous system.

METHODS

Experimental Design

The ^{31}P NMR and enzyme activity analysis were performed on the isolated ganglion complex of the garden snail *Helix pomatia* (Pulmonata: Helicidae). Snails used for all experiments were collected at spring, placed in polycarbonate boxes and kept in the cold chamber at 7 °C. The experiments were conducted in the winter period, because snail activity and variations in snail physiology, as

seen by variations in the texture and rigidity of connective sheets, are minimal in the winter months. In this way the effects of seasonal changes in snail physiology are greatly minimized. Four weeks prior to the experiments, snails were acclimated at 22 °C, kept in an active state, and fed regularly.

An experimental group composed of randomly selected snails of similar age (with a shell diameter of approximately 4 cm) was placed in a polycarbonate box (26 cm wide x 43 cm long x 15 cm high) in a temperature controlled room (22 \pm 1 °C) and exposed to the ELF-MF as shown in Figure 1.

For the purpose of ^{31}P NMR analysis, the single group of snails (n = 20) was exposed to the ELF-MF. After 7 days of exposure, 10 randomly selected snails were used for recording NMR spectrum, another 10 snails were further exposed to the ELF-MF up to 16 days, and another NMR spectrum was recorded. Immediately afterwards, a control group was formed by placing 8 snails at the same position as the exposed group, but the source of ELF-MF was turned off and unplugged from its power supply (*sham* exposure) for 7 days, after which the control NMR spectrum was recorded.

A similar procedure was used for biochemical analysis. The only difference was the number of snails: 3 animals out of group of 7 snails were used for enzyme activity analysis after 7 days of ELF-MF exposure, and the remaining 4 snails were used for enzyme activity analysis after 16 days. The control group (n = 5), placed at the same position as the ELF-MF treated group, was *sham* exposed for a period of 7 days, immediately after the 16-day treatment.

Ganglion complexes were isolated as previously described in Nikolic et al (2008). Briefly, after the snail foot was separated, it was pinned onto a cork plate in the extended position. Incision at the dorsal anterior surface of the snail's foot enabled

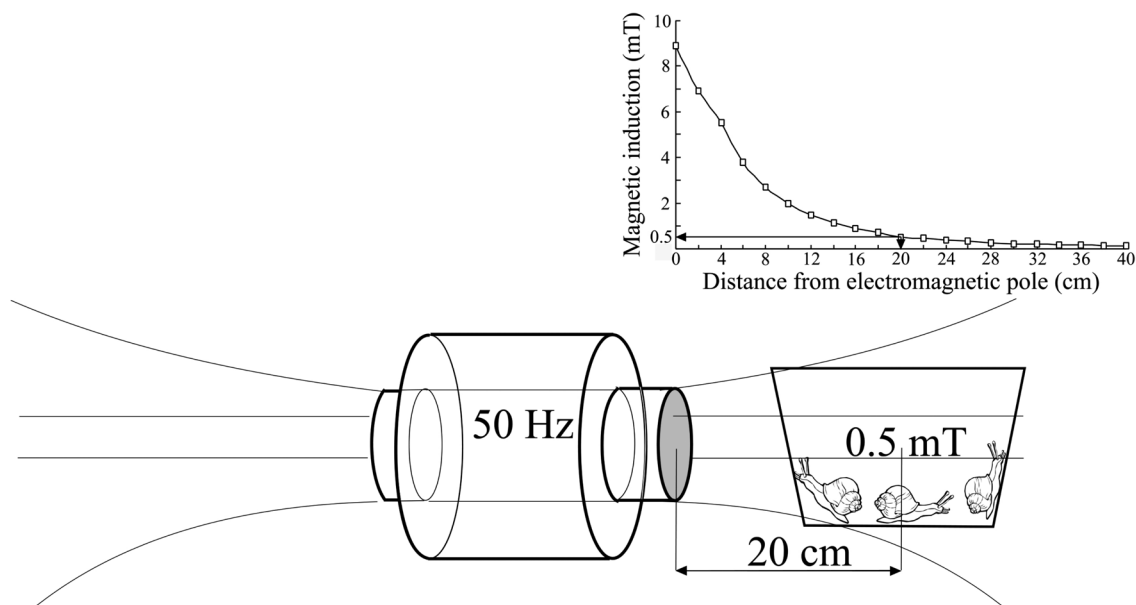


Figure 1. ELF-MF exposure system (electromagnet and box with snails) with marked magnetic force lines. Inset: change of the magnetic induction within the box with snails.

access to the ganglion complex. In order to prevent tissue degradation, the isolated ganglions were kept in snail physiological solution on ice prior to measurements.

The source of ELF-MF was placed approximately 20 cm from the center of the box with snails (Fig. 1). Alternating magnetic fields were generated by a solenoid-type electromagnet with a regular laminated transformer core and pole dimensions of 9.5 cm × 9.5 cm. A 50 Hz sinusoidal current (40 V, 4.5 A) was passed through the magnet. The alternating magnetic field was not uniform in the exposure space (Fig. 1), but an average magnetic induction was 0.5 mT at the middle of the polycarbonate box, a value in the magnitude found in the vicinity of home appliances (Table 1) (measured by a Hirst GM05 Gaussmeter, using a PT2837 probe). The temperature difference between ELF-MF treated and *sham* exposed groups, in the exposure space, was less than 0.3 °C measured in the air, and no difference was found measured in the liquid.

Magnetic force lines were parallel to the horizontal component of the local geomagnetic field. Earth magnetic field strength, measured by a GSM 10 proton magnetometer (Geomagnetic Institute - Grocka, Belgrade), was within the normal range throughout experiments in the area of study (44°38' N, 20°46' E). The background magnetic field did not exceed the value of 10⁻⁵ mT.

Exposure time was chosen based on the previous experiments, showing that a 0.5 mT alternating magnetic field can induce behavioral effects, as well

as biochemical changes in the brains of rats exposed for as little as 7 days (Janać et al., 2005; Jelenković et al., 2006).

³¹P NMR spectroscopy

³¹P NMR measurements were performed using a Bruker MSL 400 (Germany), Apollo upgraded (Tecmag, USA) spectrometer operating at 161.978 MHz for ³¹P. The other experimental conditions were: 15 μs pulse width (45°), relaxation delay of 300 ms, 8 KHz spectral width. Under such conditions, peaks are partially saturated, however, data were analyzed only in terms of relative changes and there are no reasons to assume that the relaxation times of different components change. Each spectrum represents 4000 acquisitions averaged over 20 minutes. The line broadening of 25 Hz was applied with Fourier transformation. The isolated snail ganglions (about 5.8 g) were packed in the 10 mm NMR tube filled with physiological snail solution (80 mM NaCl, 5 mM MgCl₂, 10 mM CaCl₂, 4 mM KCl, and 5 mM Tris (hydroxymethyl) aminomethane. Capillary with methylenediphosphonate, MDP (25 mM) was used as an external chemical shift (17.05 ppm relatively to 85% H₃PO₄) and peak intensity standard. It should be mentioned that during experiments there were no changes in the chemical shift and line width of the MDP reference signal, which is in agreement with reported independence of these parameters on the magnetic susceptibility of the solution in the

outer tube under similar experimental conditions (Fabry and San George, 1983). All chemicals used for ^{31}P NMR spectroscopy were supplied from Merck (Germany). The ^{31}P NMR spectra for the control and 7-day and 16-day ELF-MF exposed groups of snails were obtained from 8 to 10 ganglions, and represent average status of the ganglions used.

Intracellular pH value was estimated according to Mimura and Kirino (1984).

Enzyme assays

Analysis of enzyme activity was performed on isolated, individual snail ganglions. Each ganglion with a weight of approximately 61 mg was analyzed in triplicate. Ganglion tissue was homogenized in 0.7 mL Tris-HCl buffer at pH of 7.4 with 0.01% Triton X-100. The homogenate was centrifuged in refrigerated in an Eppendorf microcentrifuge, model 5415R, at 10000 rpm. The supernatant was used for enzyme activity measurements. Protein content was determined by the method of Bradford using BSA as standard proteins (Bradford, 1976). The activity of investigated enzymes was measured using a Shimadzu UV-2501 PC spectrophotometer (Shimadzu Scientific Instruments, Japan).

ATPase activity was determined by measuring ATP decomposition to inorganic phosphate and ADP. Three reaction mixtures were set, one experimental and two controls. The reaction was started by adding 70 μg of total proteins from isolated ganglions into the final volume of the reaction mixture. The experimental reaction mixture contained: 40 mM KCl, 10 mM ATPMg, 10 mM MgCl_2 , 240 mM NaCl, and 25 mM Tris-Cl buffered at pH 7.4. First the control experiment was performed with the addition of 2 mM ouabain and without KCl, and the control experiment was performed without ATPMg. The reaction mixture was incubated at 22 °C for one hour and the reaction was stopped by adding SDS to a final concentration of 1%. Inorganic phosphate was quantified by the method of Ohnishi et al. (1975) modified for microplate reader. The activity of ATPases was obtained as follows: the total ATPases were calculated by subtracting the probe absorbance from the total ATPases absorbance; by subtraction of the ouabain insensitive absorbance values from the total ATPases we obtained ouabain sensitive ATPases (Na^+/K^+ -ATPase).

Acid phosphatase activity was measured by the rate of p-nitrophenyl phosphate hydrolysis for 5 minutes by following absorbance at 405 nm. The reaction was started by adding 200 mg of total protein from isolated ganglions in the final volume of reaction mixture. The reaction mixture contained

10 mM acetate buffer (pH 5.5), 1 mM MgCl_2 , and 5 mM p-nitrophenyl phosphate. The p-nitrophenol concentrations were estimated by using extinction coefficient 18.5 $\text{cm}^2/\mu\text{mol}$.

All chemicals used for enzyme assays were supplied from Merck (Germany).

^{31}P NMR and enzyme assays data analysis

In data analysis, the results of the control group of snails (7-day sham exposed) were compared to the results of 7-day treatment group. In order to monitor the effect of the ELF-MF for a longer period, the 16-day treatment group was compared to the group of snails treated for 7 days.

Intensity of each ^{31}P NMR signal was calculated by NTNMR software (Tecmag, USA), and normalized to the intensity of the MDP signal. The errors of measurements were calculated as absolute errors. The error D_i of normalized signal intensities R_i was calculated as $D_i = R_i * ((a / \text{MDP intensity}) + (a / \text{signal intensity}))$, where a is the amplitude of noise, measured as Ω peak to peak, and R_i is the signal intensity/ MDP intensity. We considered that ^{31}P NMR spectra normalized intensity values between compared groups were different if the sum of absolute errors was smaller than the difference between the intensities measured.

The differences in the enzyme activity (mmol/mg \cdot min) were evaluated by a one-way ANOVA (followed by Fisher LSD test). The significance was set at * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. Error bars represent the standard error of the mean (SEM).

RESULTS

^{31}P NMR spectroscopy

The ^{31}P NMR spectrum of the isolated ganglion complex of the *Helix pomatia* snail is presented in Figure 2 and, to the best of our knowledge; this is the first recorded spectrum of the snail nervous system. Therefore, assignment of spectra signals was made by comparison to previously reported ^{31}P NMR spectra of the nervous systems of other species (Kauppinen and Williams, 1994; McNamara et al., 1994; Tsuji et al., 1995; Buck et al., 1998; Tsao et al., 1999). Obtained spectrum consists of signals that were assigned, from downfield, to: phosphomonoesters (PME), inorganic phosphate (Pi), energy storage compound phosphocreatinin (PCr), and nucleotide phosphates (α , β , γ -ATP) involved in energy metabolism. The phosphomonoester peak consists mainly of phosphorylethanolamine (PE), with a smaller contribution from phosphorylcholine (PC).

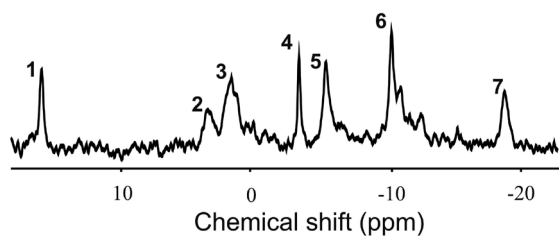


Figure 2. ^{31}P NMR spectrum of *Helix pomatia* isolated ganglion complex ($n = 8$). The signals are assigned to the following compounds: (1) Methylene diphosphonate (MDP, 17.05 ppm); (2) Phosphomonoesters (PME, 4.4 ppm); (3) Inorganic phosphate (P_i , 2.6 ppm); (4) Phosphocreatine (PCr, -2.7 ppm); (5) γ -ATP (-4.7 ppm); (6) α -ATP (-9.8 ppm); (7) β -ATP (-18.4 ppm).

The spectra of ganglion complexes for the control and 7-day and 16-day ELF-MF exposed snails are presented in Figure 3A, while quantitative representation is presented in Figure 3B.

As can be seen from the Figure 3B, the changes in the normalized intensities of phosphate compounds were not found between the control and the 7-day ELF-MF exposed group of snails (white and black bars). The important difference, between signals from the ganglions of control and 7-day ELF-MF exposed snails was the change in the chemical shift of the P_i signal which is commonly used as an indicator of intracellular pH changes (Kauppinen and Williams, 1994) indicating intracellular pH change toward alkaline conditions, from about 6.9 to 7.1 in the 7-day ELF-MF exposed snails (Fig. 3A, vertical line).

However, when we compared the normalized signal intensities of the 7- and 16-day treatment groups (Fig. 3B, black and gray bars), the decrease in the intensity of the PCr and the ATP signals in the 16-day exposed group of snails was found. The intensity of the β -ATP signal was used for monitoring the energy status (ATP content), since γ and α ATP signals overlap considerably with the β and α resonance of ADP (Stubbs et al., 1996).

Enzyme activity

To further explore the influence of ELF-MF on the phosphate metabolism of the snail nervous system we investigated the activity of enzymes: total ATPases, Na^+/K^+ -ATPase, and acid phosphatase.

The results presented in Figure 4 show that the activity of total ATPases significantly increased ($p < 0.01$, $n = 3$) in the 7-day treatment group of snails ($19.4 \times 10^{-6} \pm 8.4 \times 10^{-6}$ mmol/mg \times min) compared to the control group ($2.1 \times 10^{-6} \pm 0.5 \times 10^{-6}$ mmol/mg \times min). However, in the 16-day treatment group the significant decrease ($p < 0.05$, $n = 4$) in the activity of total ATPases ($6.1 \times 10^{-6} \pm 2 \times 10^{-6}$ mmol/mg \times min)

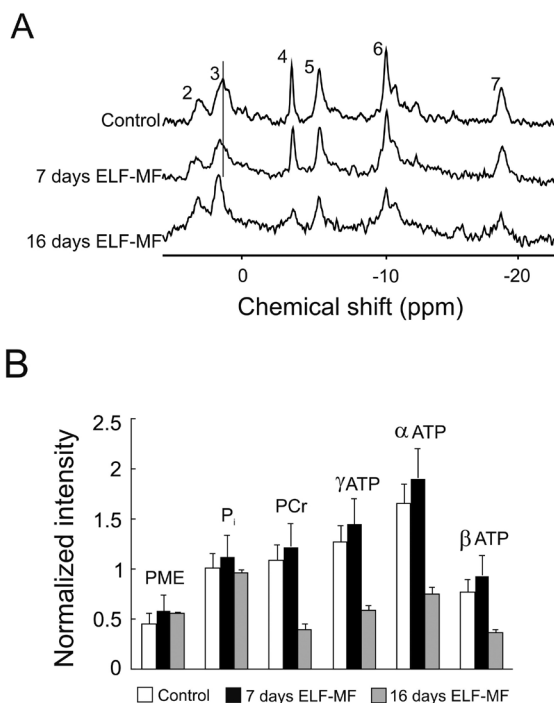


Figure 3. A. ^{31}P NMR spectra of *Helix pomatia* ganglion complex for the control ($n = 8$), 7-day ($n = 10$), and 16-day ($n = 10$) ELF-MF (50 Hz, 0.5 mT) exposed groups of snails. The vertical line represents the chemical shift of the P_i signal. B. Intensity of ^{31}P NMR signals normalized to the intensity of standard (MDP) for the control, 7-day and 16-day ELF-MF exposed groups of snails. Error bars represent the absolute errors.

compared to the 7-day treatment group was found. As well, Figure 4 shows the significant increase ($p < 0.05$, $n = 3$) in the specific activity of Na^+/K^+ -ATPase in the 7-day treatment group ($9.5 \times 10^{-6} \pm 5 \times 10^{-6}$ mmol/mg \times min) compared to the control group of snails ($1.2 \times 10^{-6} \pm 0.4 \times 10^{-6}$ mmol/mg \times min). The difference between 7- and 16-day treatment groups of snails was not significant. The Na^+/K^+ -ATPase activity in the 16-day ELF-MF exposed group of snails was $2.2 \times 10^{-6} \pm 0.6 \times 10^{-6}$ mmol/mg \times min.

We also calculated Na^+/K^+ -ATPase fraction in total ATPases for the control and exposed groups of snails. The difference between the values for the control (0.6 ± 0.1), 7-day treated group (0.5 ± 0.1) and the 16-day ELF-MF treated snails (0.4 ± 0.1) is not significant.

Figure 4, 5 Effect of the tested ELF-MF on the specific activity of the acid phosphatase is presented in Figure 5. ELF-MF applied for 7 days caused a significant increase ($p < 0.001$, $n = 3$) in the activity of acid phosphatase ($6.8 \times 10^{-7} \pm 1.3 \times 10^{-7}$ mmol/mg \times min) compared to the control group of snails ($0.5 \times 10^{-7} \pm 2.5 \times 10^{-9}$ mmol/mg \times min). A statistically significant decrease in acid phosphatase activity was

Total ATPases, Na,K-ATPase

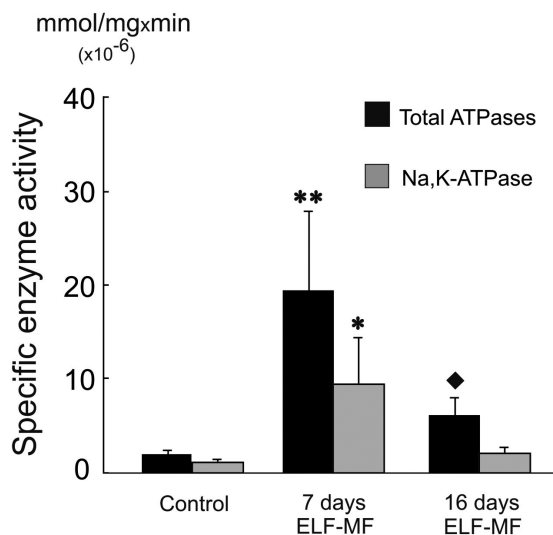


Figure 4. Specific activity of the total ATPases and Na,K-ATPase in the control (n = 5), 7-day (n = 3), and 16-day (n = 4) ELF-MF (50 Hz, 0.5 mT) exposed group of snails, presented as mean ± SEM. Note that the scaling factor on the y axis is 10⁻⁶. *p < 0.05 and **p < 0.01 indicate significant differences between control (7-day sham exposed) and 7-day ELF-MF exposed group of snails. ♦ < 0.05 indicates significant difference between 7 and 16-day ELF-MF exposed group of snails (one-way ANOVA, Fisher LSD test).

Acid phosphatase

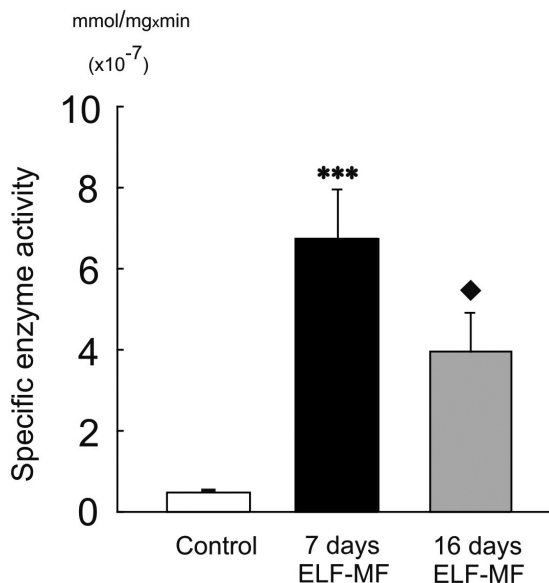


Figure 5. Specific activity of acid phosphatase in the control (n = 5), 7-day (n = 3) and 16-day (n = 4) ELF-MF (50 Hz, 0.5 mT) exposed groups of snails, presented as mean ± SEM. Note that the scaling factor on the y axis is 10⁻⁷. ***p < 0.001 indicates significant differences between control (7-day sham exposed) and 7-day ELF-MF exposed group of snails. ♦ < 0.05 indicates significant difference between 7 and 16-day ELF-MF exposed group of snails (one-way ANOVA, Fisher LSD test).

found in the 16-day exposed group ($3.9 \times 10^{-7} \pm 1 \times 10^{-7}$ mmol/mg×min) compared to the 7-day exposed group of snails ($p < 0.05$, n = 4).

DISCUSSION

In the present work, performed on *Helix pomatia* isolated ganglions, we showed that exposure of snails to the ELF-MF for a period of 7 days increased intracellular pH value and caused changes in the activity of the total ATPases, Na⁺/K⁺-ATPase and acid phosphatase, enzymes involved in phosphate turnover. Another important finding of our research is the difference between the effect of 7 and 16 days of ELF-MF exposure on snail phosphate metabolism. The level of phosphate compounds, PCr and ATP, as well as the activity of investigated enzymes, decreased in the 16-day treatment group of snails, compared to the 7-day treatment group.

Two alternative explanations can be proposed for the obtained ³¹P NMR spectrum of ganglia from the 7-day treatment group of snails. The first is that the tested ELF-MF did not cause changes in the

metabolism of identified phosphate compounds in the snail nervous system. As can be seen from Figure 3, we did not find prominent changes in the intensities of PME, Pi, PCr and ATP signals. The second explanation of the apparent lack of change in the ³¹P NMR spectrum of snail ganglia after exposure to ELF-MF for 7 days is that an increase in both synthesis and the degradation of phosphate compounds occurred. We find the second explanation more plausible, since the increase in the activity of total ATPases and Na⁺/K⁺-ATPase, as found in the 7-day treatment group, should result in a decrease of ATP signal intensity, and we did not detect this by ³¹P NMR. We interpret this finding as an indicator that metabolic pathways involved in the synthesis of ATP increased in the ganglia from the 7-day treatment group. Furthermore, the detected increase of acid phosphatase activity, involved in the processes of catabolism (Hollander, 1971), in the 7-day ELF-MF treatment group of snails indicates that the processes of catabolism of phosphate compounds in the snail nervous system increased.

A longer period of magnetic field exposure, according to our ³¹P NMR data, decreased the

level of energy source compounds ATP and PCr. Depressed ATP levels were also reported in experiments where rat brains were subjected to low frequency microwaves (Sanders et al., 1980; Sanders and Joines, 1984) and in the experiments with 60 Hz sinusoidal magnetic field on *Physarum amoeba* (Marron et al., 1986). Overall, our data suggest that magnetic fields probably cause a transient increase in the activity of enzymes involved in the synthesis of ATP, followed by the decrease in their activity with prolonged exposure.

The increase of the Na⁺/K⁺-ATPase activity, which we found after exposure to ELF-MF *in vivo*, is in agreement with the results of the ELF-MF effect on the Na⁺/K⁺-ATPase activity obtained on the isolated enzyme preparations. The *in vitro* effect of 60 Hz magnetic field was reported for the Na⁺/K⁺-ATPase containing vesicles prepared from frozen rabbit kidneys (Blank and Soo, 1996). The increase in the activity of Na⁺/K⁺-ATPase, found in both, *in vivo* and *in vitro* research, indicates that ELF-MF interactions with biological systems as reflected in this enzyme activity might be at the protein level.

Recent research showed that the 0.5 mT 60 Hz magnetic field can increase the activity of F₀F₁-ATPase in chromatophores prepared from the cells of bacteria *Rhodospirillum rubrum* (Chen et al., 2009). The F₀F₁-ATPase is also present in the inner membrane of eukaryotic mitochondria. To what extent this mitochondrial level of the ELF-MF-biological system interaction has a determining role in the complex phenomena whose net results we measured, needs to be further explored.

The prominent decrease after 16 days exposure at the level of PCr, which serves as the source of ATP (Mellergard and Siesjo, 1998) suggests that long exposure most probably affected an energy demanding cellular processes, such as gene transcription and protein synthesis. Therefore, the possibility of magnetic field-biological system interaction on the gene level should not be excluded.

We found that with longer exposure to ELF-MF in comparison to 7 days ELF-MF treatment there was a significant decrease in the activity of total ATPases and acid phosphatase. Even though it seems that some compensatory mechanism is in place, it is hard to say what levels and mechanisms of the regulation of enzyme activity are involved. Although it is quite possible that long term effects of ELF-MF exposure involve some gene transcription and translation changes that was not the object of our present study.

Finally, on the basis of the results obtained, we propose that exposure to the ELF-MF for the period of 7 days increased the overall phosphate turnover in the snail nervous system, while after prolonged ELF-MF exposure phosphate metabolism adjusted to the tested ELF-MF by reaching a balance at the

new level. Most probably, more than one level of magnetic field-biological system interaction is involved in the detected perturbations of the phosphate metabolism.

Several aspects of nervous system energy status after exposure to ELF-MF *in vivo* were measured in this study. The results presented here, to our knowledge, are the first description of the ELF-MF influence on the phosphate metabolism in the nervous system of *Helix pomatia*. We have found that the magnetic field induced an increase in the consumption of phosphate compounds and altered the activity of some enzymes involved in phosphate turnover. Together with *in vitro* conducted research, it could contribute to further understanding of the magnetic field - biological systems interactions.

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