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Whole body exposure to low frequency magnetic field: No provable effects on the cellular energetics of rat skeletal muscle

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Abstract

On the basis of previous experience with biological effects of electromagnetic fields a potential effect of homogeneous sinusoidal magnetic field (50 Hz, 10 mT) on energy state of rat skeletal muscle was investigated. Two different total body exposures to magnetic field were selected: (1) repeated 1 hour exposure, 2 times a week for 3 months, and (2) acute 1.5 hour exposure (and the appropriate control groups). Important energy metabolites (adenosine triphosphate – ATP, creatine phosphate, creatine, lactate, pyruvate and inorganic phosphate) were analysed by enzymatic and spectroscopic methods in *musculus gracilis cranialis.* On the basis of the concentration of important energy metabolites the apparent Gibbs free energy of ATP hydrolysis and creatine charge was calculated. Our results demonstrate no influence of this low frequency magnetic field on the level of important energy metabolites in rat skeletal muscle. The conclusion of this study is that neither repeated exposure nor the acute exposure of rats to the sinusoidal magnetic field of given parameters has any important influence on the energy state of the skeletal muscle. (Mol Cell Biochem **284:** 111–115, 2006)

Key words: ATP, adenosine-5′-triphosphate, energetics, extremely low frequency magnetic field (ELF MF), PCr, phosphocreatine, skeletal muscle

Introduction

Electromagnetic fields (EMF) are one of the most common environmental factors that can influence living systems. With the progressive development of emerging technologies, the systems that generate EMF are rapidly increasing and potentially influencing humans and animals. The research of biomedical effects of EMF is therefore also in progress as there are many examples documenting either positive or negative influences of EMF on health. At present, the research involving the effects of EMF on biological systems is often orientated towards the influence of high frequency EMF produced by mobile phones on the central nervous system or the auditory system but the experimental findings are not unambiguous. Some reported effects are small as long as the radiation intensity remains in the nonthermal range [1–3], other works refer nerve cell damage in mammalian brain after exposure to microwaves from mobile phone [4].

In some cases exposures to extremely low frequency magnetic fields often show provable physiological effects, e.g. changes in the production of some hormones (for example melatonin), modification of cellular signalling processes, help in delayed fracture healing, etc. [5, 6].

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Therapeutic applications of electromagnetic fields (involving bone repair, reduction of pain, improvement of hip movements, etc.) usually involve exposing patients to relatively high flow density levels with low frequency or static fields. Pulsed fields, based on signals with low frequency and low energy (non-thermal) are also effectively therapeutically used [7].

Our interest in this issue was orientated to total body irradiation of laboratory rats by magnetic fields (MF) of low sinusoidal frequency (50 Hz) under well-defined conditions of exposure. One of the aspects connected with the motivation of this work were positive effects of EMF on the transport of the manganese in the brain of rats [8, 9].

Possible health risks of exposure to EMF can be evaluated in many ways but we preferred to concentrate on important aspects of energy metabolism. Some *in vitro* studies revealed important influences of EMF on the function of enzymes of the mitochondrial respiratory chain, e.g. cytochrome oxidase [10]. But cellular or whole body energetic aspects after exposure to MF have not been frequently studied so far, despite the fact that the energy status of the cell is very important for its survival and proper function.

In our study, we therefore decided to test the possible influence of the total body exposure to the low frequency sinusoidal magnetic field on the energy metabolism of skeletal muscle.

Materials and methods

Male Wistar rats (body weight 350–450 g) were maintained at a temperature of 20–22 °C with a 12 h light – 12 h dark cycle. Food and water were provided *ad libitum*. All handling of rats was carried out in full compliance with the guidelines on the care and management of animals established by the European Community and the experiments were approved by the Committee of the Ministry of Education of the Czech Republic.

The exposure level of MF to rats was derived from the literature [11] so that the actual exposure (magnetic field induction) was higher than hygienic limits. A homogeneous magnetic field (MF) was produced by the solenoid (f = 50 Hz, the magnetic induction B = 10 mT) (Fig. 1). The two groups of rats were exposed inside the solenoid for the defined period of time in diverse periods. Two different exposures were selected: *group R* (14 animals) – repeated 1 hour exposure, twice a week for 3 months (with the control group – 11 animals kept under the same conditions but without any exposure to artificial MF) and *group A* (7 animals) – acute 1.5 hour exposure (and the appropriate control group of 5 animals).

The muscles (*musculus gracilis cranialis*) from the animals with repeated exposure (and corresponding controls)

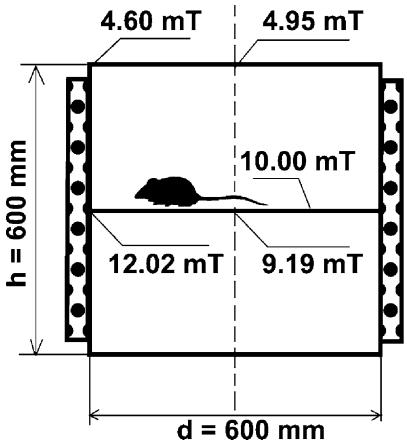

Fig. 1. A scheme of solenoid used for rat exposure to sinusoidal magnetic field (50 Hz, 10 mT).

were fixed several hours after the last exposure to MF with the Wolenberger tongs cooled in liquid nitrogen under deep anaesthesia (Thiopental i.p.). The muscles from the animals with acute exposure were removed immediately after the end of the exposure (also under deep anaesthesia induced after the end of the exposure) by the same technique. The muscles after fixation were stored at −80 °C until analysis.

Energy metabolites (adenosine triphosphate – ATP, creatine phosphate – PCr, creatine – Cr, lactate – Lac, pyruvate – Pyr, inorganic phosphate – Pi) were analysed by enzymatic and spectrophotometric methods to evaluate the potential effect of two different exposures to extremely low frequency magnetic fields on the cellular level of these metabolites in muscle (the details of analysis of muscular metabolites are described in [12]).

In addition, we evaluated the energy status of skeletal muscle fibres and its possible changes after MF exposure. This parameter, which can be most easily expressed by a "Creatine charge" (Cr-charge; Cr-charge = [PCr]/([PCr] + [Cr])) provides the possibility of describing, in a simple manner, the actual energy reserves of the cell that are necessary for the proper functioning of living tissue [13].

Another possibility is the calculation of the apparent Gibbs free energy of ATP hydrolysis ($\Delta G'_{ATP}$): this calculation is dependent on the knowledge of the free adenosine diphosphate (ADP) concentration in the cell; it is not possible to measure free ADP level, but it can be calculated from other analysed substrates.

The tissue levels of free adenosine 5′-diphosphate (ADP) were calculated from the equilibrium of the creatine kinase (CPK) reaction catalyzed by ATP: creatine *N*-phosphotransferase (EC 2.7.3.2) on the supposition of equilibrium of this reaction. The apparent equilibrium constant is given by Lawson and Veech [14]: apparent $K'_{38\,^\circ C} = 166$ for

CPK reaction. Free ADP was calculated from the formula:

$$[\mathrm{ADP}] = [\mathrm{ATP}][\mathrm{Cr}]/(K'[\mathrm{PCr}]).$$

The expressions in square brackets are concentrations given in μmol g^{-1} wet weight of tissue.

The apparent change of Gibbs free energy of ATP hydrolysis was then calculated according to the equation:

$$\Delta G'_{\mathrm{ATP}} = \Delta G^0_{\mathrm{ATP}} + R\mathrm{T}\ln([\mathrm{ADP}][\mathrm{Pi}]/[\mathrm{ATP}])$$

where T is the absolute temperature in Kelvin, R the gas constant (8.314 J K^{-1} mol^{-1}) and $\Delta G^0_{\mathrm{ATP}}$ is the standard apparent Gibbs free energy change of the reaction ATP + $H_2O \rightarrow$ ADP + Pi. The value $\Delta G^0_{\mathrm{ATP}} = -31.9$ kJ mol^{-1} (Lawson and Veech [14]) was used assuming pH 7, [Mg^{2+}] $= 10^{-3}$ M, and ionic strength 0.25 M.

The results are expressed as means ± standard errors of the mean (S.E.M.). Differences among groups were compared by the analysis of variance (ANOVA) after confirmation of the normal distribution of a variable (χ^2-test). In a case in which the normal distribution was not confirmed, a non-parametric Kruskal-Wallis test was used instead.

Results

Direct effects of high energy magnetic fields on biological systems are often mixed with the effect of increasing temperature linked with the device in which the objects are exposed. We performed a control test in the solenoid which was used. The changes in air temperature and water temperature (in water volume 250 mL) measured in the centre of the solenoid during active 50 Hz sinusoidal magnetic field with density flow 10 mT are shown in Fig. 2. There is a considerable increase in air as well as water temperature in the active solenoid in comparison with the surrounding laboratory temperature. This indicates that long exposure of animals to MF could have a dominant effect on physiological functions due to extremely high thermal stress. Therefore we used only short time exposures of rats – 1 hour in repeated exposures and 1.5 hour in acute exposure. For possible suppression of the action of increasing temperature, the control animals of *group A* were placed in the metabolic chamber with the temperature comparable to the air temperature in the active solenoid for the same time as animals exposed to MF. In experiments with repeated exposures for 3 months the metabolite analysis was made several hours after the last exposure to MF, so at that time the exposed animals and the controls were also localized in the same (laboratory) temperature. We can therefore suggest that the eventual different findings between exposed rats and controls could, in actuality, be explained by the influence

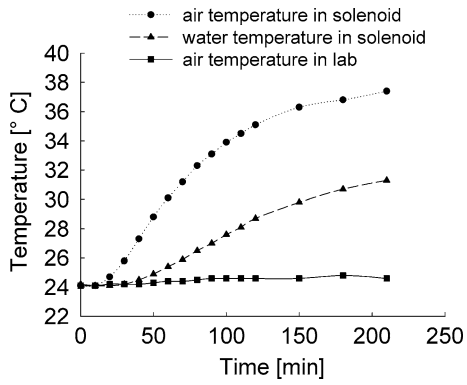

Fig. 2. Temperature changes in air and water (250 ml) localized in solenoid producing sinusoidal magnetic field (50 Hz, 10 mT) in comparison with surrounding laboratory air temperature.

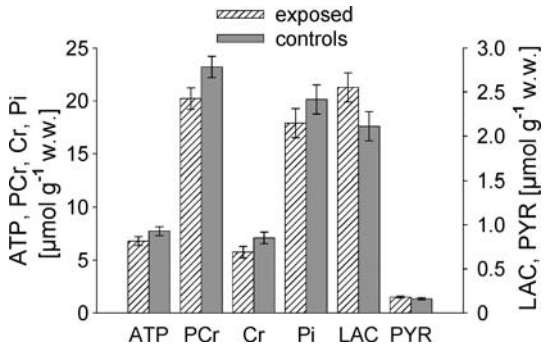

Fig. 3. Cellular levels of metabolites of rat skeletal muscle after acute 1.5 h exposure of rats (*group A*) to magnetic field (50 Hz, 10 mT) and in control animals localized for the same time in similar surrounding temperature. ATP, adenosine-5′-triphosphate; PCr, phosphocreatine; Cr, creatine; Pi, inorganic phosphate; LAC, lactate; PYR, pyruvate. Means ± SEM in [μmol g^{-1} wet weight tissue] (exposure: $n = 7$, controls: $n = 5$); ANOVA or Kruskal-Wallis non-parametric test did not prove any significant difference between exposed animals and controls for all analysed metabolites.

of the magnetic field and not by the effect of temperature changes.

Nevertheless, the levels of important energy metabolites in the skeletal muscle of both experimental groups are demonstrated in Figs. 3 and 4 and we cannot prove any substantial change in the concentrations of analysed cellular substrates. Evaluation of results (comparing exposed animals with their appropriate controls) did not show any significant differences in the tissue concentrations of all analysed metabolites between exposed animals and controls. Neither did the comparison of both experimental groups show any significant difference in the concentrations of cellular metabolites with the exception of lactate. The lactate levels were significantly higher in *group A* than in *group R* ($P < 0.05$). One possible explanation of this lactate difference between group 1 and group 2 is the placement of rats in different temperatures

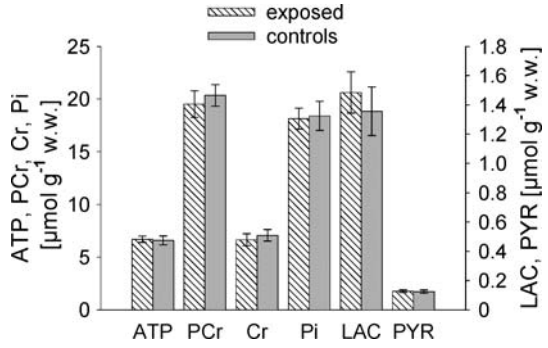

Fig. 4. Cellular levels of metabolites of rat skeletal muscle after repeated 1 hr exposure of rats twice per week for three months (*group R*) to magnetic field (50 Hz, 10 mT) and adequate control animals. ATP, adenosine-5′-triphosphate; PCr, phosphocreatine; Cr, creatine; Pi, inorganic phosphate; LAC, lactate; PYR, pyruvate. Means ± SEM in [μmol g $^{-1}$ wet weight tissue] (exposure: $n = 14$, controls: $n = 11$); ANOVA or Kruskal-Wallis non-parametric test did not prove any significant difference between exposed animals and controls for all analysed metabolites.

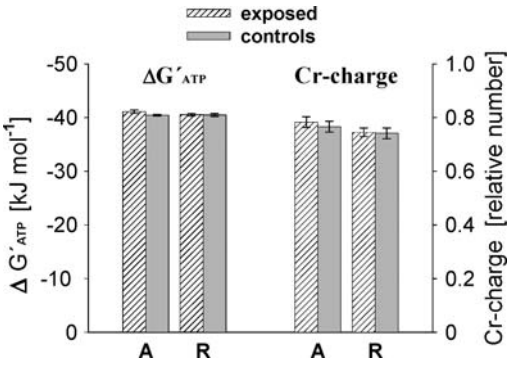

Fig. 5. Evaluation of possible energy state of skeletal muscle of rats *group A* and *group R* after exposure to sinusoidal magnetic field (50 Hz, 10 mT) and their relevant controls (means ± SEM). $\Delta G'_{\mathrm{ATP}}$ is calculated free Gibbs energy of ATP hydrolysis in units [kJ mol^{-1}] – see Methods for details; Cr-charge is a relative number (a ratio of creatine phosphate to the total creatine concentration). Statistical analysis did not prove any significant difference between exposed rats and controls in both quantities.

immediately before removing the muscle; but the MF exposure (repeated as well as acute) had no influence on the lactate concentration.

We used creatine charge (Cr-charge) and apparent Gibbs free energy of ATP hydrolysis ($\Delta G'_{\mathrm{ATP}}$) for the evaluation of the energy state of the skeletal muscle; the results in Fig. 5 document that exposure of rats to MF has no effect on the energy state of the muscle. Statistical evaluation of Cr-charge and $\Delta G'_{\mathrm{ATP}}$ proves that there is also no significant difference in the energy state of *musculus gracilis cranialis* between rats subjected to MF and controls in *group A* as well as in *group R*.

Discussion

Works studying regulation of cellular respiration distinguish the 'first mechanism of respiratory control' that is connected with the proton gradient of the inner mitochondrial membrane from another 'second mechanism of respiratory control' which is independent of the proton motive force [15]. A 'second mechanism of respiratory control' has been discovered based on allosteric ATP-inhibition of cytochrome c oxidase at high intramitochondrial ATP/ADP ratios [15–17]. ATP or ADP binds to the same site at subunit IV. The allosteric ATP-inhibition of cytochrome c oxidase is reversibly turned on by cAMP-dependent phosphorylation and turned off by Ca^{2+}-activated dephosphorylation [18, 19]. These described facts indicate that any possible change in these important cellular molecules that might be induced by extremly low frequency magnetic fields could alter the metabolic regulation and ultimately the energy state of skeletal muscle. The evaluation of the potential influence of low frequency magnetic fields on skeletal muscle energetics is therefore of interest from this point of view.

The effects of low frequency magnetic fields on cell bioenergetics have been shown in some studies describing increase of cellular respiration [20, 21] or activation of cytochrome-c oxidase [22]. On the other hand, the work studying cytochrome oxidase activity in extremely low magnetic fields has shown that ELF stimulatory effect depends on the basal enzymatic action; with normal enzymatic activity the effect of the magnetic field is lower [10]. Study of biochemical parameters of human erythrocytes (glycolytic enzymes, glycolytic metabolic pathways, energy and oxido-reduced potentials) did not find significant changes between exposed and non-exposed erythrocytes to 50 Hz electromagnetic field [23]. These differences show that it is difficult to predict about the potential effects of low frequency magnetic fields upon some physiological functions (as, for example, the energy status of skeletal muscle) without appropriate experimental analysis.

Possible effects of magnetic fields to biological functions are studied under broad conditions, involving the frequency of changes, the extent of induction of MF and the total exposition time. Primarily the frequency and induction values are considered as the dominant, because many papers document important therapeutic effect under exposition to low frequency or static magnetic field (see in [7, 24]). The comparison of influence of total time exposition to MF is difficult. Some *in vitro* studies [10, 22] used only a single short exposure time (in minutes) to MF and documented its influence to the enzyme activity. Many other studies (see [24]) demonstrated different biological effects of the low frequency magnetic fields during different exposure times. It shows that comparison and forecast any possible effect of MF requires specification of the exposure data (induction, frequency, and

exposure time). In our work we aim our effort to compare the possible effects of MF after acute one-shot exposure and sparsely repeating several months continuing exposure with non exposed animals. This repeated exposure scheme was induced by important effect of the same magnetic field (50 Hz, 10 mT) on the increasing the manganese content in the brains of rats after intratracheal giving of the manganese sulphate in saline [8].

Our research evaluated the potential effect on the skeletal muscle bioenergetics in rats which were exposed *in toto* to sinusoidal low frequency (50 Hz, 10 mT) magnetic field. But as shown in Results, the levels of important metabolites reflecting muscle energy state were not changed after actual exposure as well as after repeating exposure of rats to low frequency magnetic fields. It indicates that the repeated exposure for three months (1 hour/day, 2 days/week) as well as the actual exposure (1.5 hour) of rats to the low frequency sinusoidal homogeneous magnetic field ($f = 50$ Hz, the magnetic induction $B = 10$ mT) has no influence on the energy metabolism of skeletal muscle.

Acknowledgement

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References

1. Hossmann K-A, Hermann DM: Effects of electromagnetic radiation of mobile phones on the central nervous system. Bioelectromagnetics 24: 49–62, 2003
2. Aran JM, Carrere N, Chalan Y, Dulou PE, Larrieu S, Letenneur L, Veyret B, Dulon D: Effects of exposure of the ear to GSM microwaves: *in vivo* and *in vitro* experimental studies. Int J Audiol 43: 545–554, 2004
3. Foster KR: Do ELF bioeffects studies have relevance to RF bioeffects? In: Forschungsgemanschaft Funk, Berufsgenossenschaft der Feinmechanik und Elektrotechnik and COST 244 Bis (eds), Workshop: Biological and Biophysical Research at Extremely Low- and Radio Frequencies. Berufsgenossenschaft der Feinmechanik und Elektrotechnik, Köln, pp. 27–33, 2003
4. Salford LG, Brun AE, Eberhardt JL, Malmgren L, Persson BR: Nerve cell damage in mammalian brain after exposure to microwaves from GSM mobile phone. Environ Health Perspectives 111: 881–883, 2003
5. Brendel H, Niehaus M, Lerchl A: Direct suppressive effects of weak magnetic fields (50 Hz and 16 2/3 Hz) on melatonin synthesis in the pineal gland of Djungarian hamsters (Phodopus sungorus). J Pineal Res 29: 228–233, 2000
6. Johnston S: Studies on effects of ELF and non-thermal modulated radiofrequency on biological molecules and subcellular fractions. In: Forschungsgemanschaft Funk, Berufsgenossenschaft der Feinmechanik und Elektrotechnik and COST 244 Bis (eds), Workshop: Biological and Biophysical Research at Extremely Low- and Radio Frequencies. Berufsgenossenschaft der Feinmechanik und Elektrotechnik, Köln, pp. 35–42, 2003
7. Stavroulakis P: Therapeutic effects of electromagnetic fields. In: Stavroulakis P (ed). Biological effects of electromagnetic fields. Springer Verlag, Berlin, Heidelberg, New York, pp. 624–732, 2003
8. Vojtisek M, Jerabek J, Knotkova J, Hornychova M, Formanek J, Hulinska D, Bittnerova D: The influence of a magnetic field on manganese transport into rat brain. Rev Environ Health 11: 229–233, 1996
9. Vojtisek M, Knotkova J, Kasparova L, Hornychova M, Frantik E, Hulinska D, Svandova E: Influence of a chemical and/or physical factor/s on the brain. In: Pokorny J (ed). Abstract Book: Coherence and Electromagnetic Fields in Biological Systems. Neoset, Prague, pp. 72–73, 2005
10. Blank M, Soo L: Enhancement of cytochrome oxidase activity in 60 Hz magnetic field. Bioelectrochem Bioenerg 45: 253–259, 1998
11. Musil J: The Czech limits and the European prestandards. In: Klauenberg BJ, Miklavcic D (eds). Proceedings of the NATO Advanced Research Workshop on Radio Dosimetry and its Relationship to the Biological Effects of Electromagnetic Fields, Oct. 12–16, 1998, Goyd Martuljek, Slovenia NATO Science Partnership Sub-Series 3: High Technology, Vol. 82, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 541–544, 2000
12. Stefl B, Mejsnar JA, Janovska A: Energy metabolism of rat skeletal muscle modulated by the rate of perfusion flow. Exp Physiol 84: 651–663, 1999
13. Connett RJ: Analysis of metabolic control: new insight using scaled creatine kinase model. Am J Physiol Regulatory Integrative Comp Physiol 254: R949-R959, 1988
14. Lawson JWR, Veech RL: Effects of pH and free Mg^{2+} on the K_{eq} of the creatine kinase reaction and other phosphate hydrolyses and phosphate transfer reactions. J Biol Chem 254: 6528–6537, 1979
15. Arnold S, Kadenbach B: Intramitochondrial ATP/ADP-ratios control cytochrome c oxidase activity allosterically. FEBS Lett 443: 105–108, 1999
16. Arnold S, Kadenbach B: Cell respiration is controlled by ATP, an allosteric inhibitor of cytochrome c oxidase. Eur J Biochem 249: 350–354, 1997
17. Kadenbach B, Arnold S: Minireview. A second mechanism of respiratory control. FEBS Lett 447: 131–134, 1999
18. Bender E, Kadenbach B: The allosteric ATP-inhibition of cytochrome c oxidase is reversibly switched on by cAMP-dependent phosphorylation. FEBS Lett 466: 130–134, 2000
19. Lee I, Bender E, Kadenbach B: Control of mitochondrial membrane potential and ROS formation by reversible phosphorylation of cytochrome c oxidase. Mol Cell Biochem 234/235: 63–70, 2002
20. Detlavs IE *et al.*: Electromagnetotherapy of traumas and diseases of locomotor apparatus. Riga – RMI 1987; pp. 110–123 (Cited by Jerabek J: The First World Congress of Magnetotherapy, London, 1996)
21. Pafkova H, Jerabek J, Tejnorova I, Bednar V: Developmental effects of magnetic field (50 Hz) in combination with ionizing radiation and chemical teratogens. Toxicol-Lett 88: 313–316, 1996
22. Nossol B, Buse G, Silny J: Influence of weak static and 50 Hz magnetic fields on the redox activity of cytochrome-C oxidase. Bioelectromagnetics 14: 361–372, 1993
23. Dacha M, Accorsi A, Pierotti C, Vetrano F, Mantovani ROG, Conti R, Nicolini P: Studies on the possible biological effects of 50 Hz electric and/or magnetic fields: evaluation of some glycolytic enzymes, glycolytic flux, energy and oxido-reductive potential in human erythrocytes exposed in vitro to power frequency fields. Bioelectromagnetics 14: 383–391, 1993
24. Shupak NM: Therapeutic uses of pulsed magnetic-field exposure: a reviw. Radio Science Bulletin 307: 9–32, 2003