FULL PAPER *Physiology*

Effects of Exposure to a 50 Hz Electric Field on Plasma Levels of Lactate, Glucose, Free Fatty Acids, Triglycerides and Creatine Phosphokinase Activity in Hind-Limb Ischemic Rats

Shinji HARAKAWA[1,3], Noboru INOUE[1], Takuya HORI[1,3], Koji TOCHIO[3], Tatsuya KARIYA[1], Kunihito TAKAHASHI[3], Fuyuki DOGE[3], David E. MARTIN[4], Atsushi SAITO[2], Hiroshi SUZUKI[1] and Hideyuki NAGASAWA[1]

[1]National Research Center for Protozoan Diseases and [2]Department of Veterinary Physiology, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080–8555, [3]Hakuju Institute for Health Science, 1–37–5 Tomigaya Shibuya-ku, Tokyo 151–0063, Japan and [4]M-CAM, 207 Fifth Street N.E. Charlottesville, Virginia 22902, U.S.A.

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ABSTRACT. We previously reported that extremely low frequency electric fields (ELF-EFs) affect energy metabolism in stressed conditions. To further confirm this, the effect of exposure to ELF-EFs on the experimental ischemic rat was examined. The test was based on a comparison of rats treated with EF alone, ischemic surgery alone, the combination of EF with ischemic surgery, or no treatment (double sham). The EF condition used in this study was an alternating current of 50 Hz EF at 17 500 V/m intensity for 15 min per day. The exposure to EF in ischemic rats significantly decreased plasma levels of free fatty acids and triglycerides, compared to those of the no treatment or EF alone group. The plasma lactate levels of two ischemic groups peaked on experimental day-4 and gradually decreased until the end of the study. The changes in the lactate levels induced by ischemia did not show any difference between rats treated with ischemia alone or a combination of ischemia with an EF. Any changes in plasma levels of glucose and creatine phosphokinase activity were not influenced by EF treatment. These results indicate that the EF effect on glycolysis parameters, plasma lactate or glucose levels, does not appear in a highly stressed condition and that EF effects varied dependent on the condition of organism but ELF-EF used in this study have impact on lipid metabolism parameter in a hind-limb ischemic rat. However, further studies are needed to elucidate the association of ELF-EF with the lipid metabolism system.

KEY WORDS: bioelectromagnetism, electric field, 50 Hz, ischemia, lipid metabolism.

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The biological effects of an extremely low frequency electric field (ELF-EF) have become a matter of interest and debate as worldwide distribution of electricity, which markedly increased during the last half century, resulted in increased human exposure to electromagnetic fields (EMFs) [36]. Clinical techniques using electric fields (EFs) have been developed in disciplines such as complementary and integrative medical therapies [13–16, 27]. In Japan, EF therapy has been utilized since 1972, when Hara *et al.* developed EF exposure equipment (Healthtron™, Hakuju Institute for Health Science, Tokyo, Japan) [15], which was approved by the Ministry of Health, Labour and Welfare (Approval number 14700BZZ00904). Recently, the palliative effects of EF therapy on several types of pain (headache, stiff shoulders and stomachache) in over a thousand cases have been reported [16]. Publications explaining the mechanism(s) of the response of an exposure to an EF have suggested that either the EF-induced electric current or the perception of the EF through the skin surface acts as a trigger on cellular, humoral or behavioral responses [17, 18, 22–24, 32, 40, 44, 45].

In a recent study, we reported evidence that an EF participates in the response to stress and also affects energy metabolism by suppressing the anabolism of lactate, not the acceleration of the catabolism of lactate [17]. However, when mice were exercised on a treadmill, we found no influence of exposure to an EF on plasma lactate levels (unpublished data). The aim of the present study was to reproduce the results of EF influences on energy metabolism parameters using an animal model in which marked changes in plasma lactate levels can be observed [12, 21, 35]. In order to identify the kind of condition of organisms that an exposure to EF influences on plasma lactate level, experimental ischemic rat model was applied in presence study. Hind limb ischemic rat is known to be able to occur the transient increase of plasma lactate level.

MATERIALS AND METHODS

Electric field exposure system: The exposure system (Fig. 1) is composed of three major parts, namely a high voltage transformer (Fig. 1, Hakuju Institute for Health Science, Tokyo, Japan), a constant voltage unit (Fig. 1, Tokyo Seiden, Tokyo, Japan) and EF exposure cages (Fig. 1), which have been previously described [17]. Briefly, the exposure cage, which is designed for a rat or a smaller animal, is composed of a cylindrical plastic cage (diameter: 400 mm, height: 400 mm) with two electrodes made of stainless steel (1,200 × 1,200 mm) placed over and under the cylindrical cage. In order to form a 50 Hz sine waveform EF of 17 500 V/m intensity in the cage, a stable alternating current (7 000 V) was applied to the upper electrode. Experi-

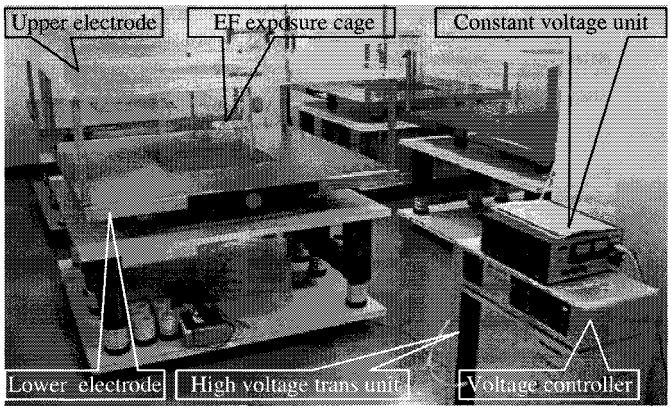

Fig. 1. The Electric Field (EF) Exposure System. A cylindrical plastic cage is placed between one upper and three lower stainless steel electrodes. The cylindrical cage has slits (length: 100 mm; width: 5 mm) at 5 mm intervals. The slits prevent contamination due to feces and saliva from disturbing the formation of a stable EF.

ments were carried out at room temperature (25 ± 0.4°C). In this study, we used four device sets: two sets for exposure to an EF and another two for sham-exposure to an EF. Each exposure cage housed only one rat during each experimental session in order to avoid an imbalance of EF distribution induced by housing two or more rats at the same time.

Animals: Male, eight weeks old Sprague-Dawley (SD) rats, weighing 270–330 g, were purchased from Japan SLC Inc. (Tokyo, Japan) and were maintained in group housing (five rats per cage) in a conventional, air-conditioned animal room. The room temperature and humidity were 21 ± 2°C and 50 ± 10% with 12 hr of artificial illumination daily (07:00–19:00). Rats had free access to standard laboratory chaw (F-2, Funabashi Farms, Chiba, Japan) and water *ad libitum* except for the period of EF exposure sessions.

Preparation of animal model: Under pentobarbital anesthesia (50 mg/kg, i.p.), the abdominal aorta was isolated free from surrounding tissue and the vena cava just above the bifurcation to the iliac arteries and occluded by a thread [12]. Sham-operated rats underwent the same surgery, but the aorta was not occluded.

Experimental design: Forty SD rats were randomly divided into four groups of ten: ischemia alone group (ischemia + sham EF); double treatment group (ischemia + EF); double sham group (sham ischemia and + sham EF); and EF alone group (sham ischemia + EF). All rats were exposed to an EF or sham EF in a fully conscious condition. Within 60 min of the ischemic or sham-ischemic surgery, rats were exposed or sham exposed to 50 Hz 17 500V /m for 15 min. All of exposure sessions were conducted within 4 hr, 10:00–14:00. Subsequently, the rats were exposed to the EF or sham EF once a day for 14 days. Exposure sessions for the 14-day exposure always were done in the same sequence.

Blood analysis: Blood samples were collected from the tail vein just before surgery on experimental day-1 and just after exposure to EF on experimental day-4 and day-7 in order to measure hematological properties and plasma lactate levels. Blood samples were also taken from the abdominal aorta under pentobarbital anesthesia on experimental day-14 in order to measure hematological properties and plasma levels of lactate, glucose, triglyceride (TG), free fatty acids (FFA) and creatine phosphokinase activity. To analyze hematological properties, aliquots of the blood samples collected were treated with K2-EDTA (1 mg/m*l*).

Red blood cell (RBC), white blood cell (WBC) and platelet (PLT) counts, as well as hematocrit values (HCT) and hemoglobin levels (HGB) were measured using the automatic multi-hemocytometer F-800 (SYSMEX Co. Ltd., Hyogo, Japan). The mean corpuscular volume (MCV), mean corpuscular HGB (MCH) and mean corpuscular HGB concentration (MCHC) were calculated from the RBC, HCT and HGB values. Plasma levels of lactate, glucose, TG, FFA and creatine phosphokinase activity were examined after treating blood samples with sodium heparin (0.1 mg/m*l*) and isolating plasma by centrifugation at 1,670 × g at 4°C for 10 min. Levels of each substance, except lactate, were measured with an automatic analyzer (7170, Hitachi Co. Ltd., Tokyo, Japan). Plasma lactate levels were measured by using the Determiner-LA (Kyowa Medex Co. Ltd., Tokyo, Japan).

Statistical analyses: The statistical significance of differences among groups and/or throughout the experimental period was calculated by one-way analysis of variance (ANOVA) or by two-way ANOVA for plasma lactate levels and hematological properties. The statistical significance of differences between groups was calculated by the Student's *t* test or Aspin-Welch *t* test or one-way ANOVA for plasma glucose, TG, FFA and CPK activity levels. The level of significance was defined as $P<0.05$. All computations for the statistical analyses were carried out in Prism Version 4.0b (GraphPad Software Inc., San Diego, CA).

RESULTS

Hematological properties: WBC counts on experimental day-1, -4, -7, and -14 are shown in Fig. 2. The differences between all groups were observed by both factors of treatment and period (P<0.01, two-way ANOVA). WBC counts in the two ischemic groups showed transient increases until experimental day-7 and recovers on day-14 (P<0.01, one-way ANOVA); WBC counts of ischemic groups on day-7 were higher than those of the double sham and EF alone groups (P<0.05, Student's t test). Among all groups, the other parameters measured do not show any marked significant changes (data not shown).

Glucose and lactate levels: Plasma glucose levels on experimental day-14 were measured. The values for all groups varied from 172.9 ± 3.2 (mean ± standard error of the mean (SEM)) to 181.6 ± 2.8 mg/d*l* (P=0.71, one-way ANOVA); there were no significant differences among all groups.

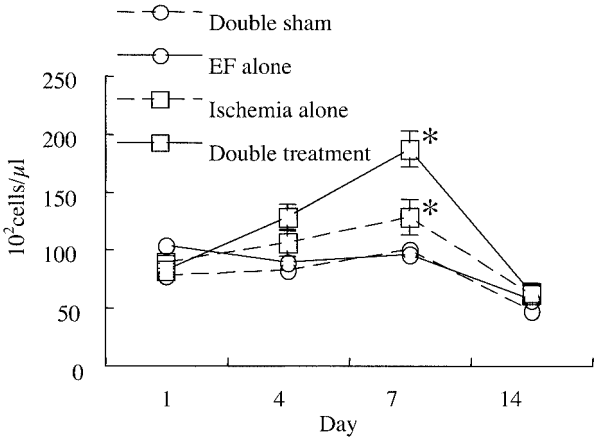

Fig. 2. White blood cell (WBC) counts. WBC counts were measured just before and on day-4, -7, and -14. The data are expressed as the mean ± standard error of the mean (SEM). The differences between all groups were observed by both factors of treatment and period (P<0.01, two-way ANOVA). WBC counts in the two ischemic groups showed transient increases until day-7 and recovers on day-14 (P<0.01, one-way ANOVA); WBC counts on day-7 were higher than those of the double sham and EF alone groups (*: P<0.05, Student's t test).

Plasma lactate levels were measured on experimental day-1, -4, -7, and -14 and are shown in Table 1. Plasma lactate levels of both ischemic groups showed a day-dependent changes compared to those of non-ischemic groups (P<0.01, two-way ANOVA). EF-dependent changes were not shown in every measurement point.

Creatine phosphokinase: Plasma creatine phosphokinase activity levels were measured on experimental day-14. A one-way ANOVA analysis on the four groups did not show any treatment-dependent changes (data now shown).

TG and FFA: Plasma levels of TG and FFA were measured on experimental day-14 and are shown in Figs. 3 and 4. Plasma TG levels showed treatment-dependent changes (P<0.05, one-way ANOVA); 159.5 ± 14.4, 149.3 ± 12.9, 139.1 ± 18.5 and 101 ± 20.1 mg/d*l* for double sham, EF alone, ischemia alone and double treatment groups, respectively (Fig. 3). Results of the Student's t test indicate that the TG level of the double treatment group was significantly lower than that of the double sham and EF alone groups (P<0.05). There were not any statistically significant differences between the double sham and EF alone groups and between the ischemia alone and double treatment groups. Plasma FFA levels (Fig. 4) in the double sham, EF alone, ischemia alone and double treatment groups were 0.21 ± 0.02, 0.20 ± 0.02, 0.17 ± 0.01 and 0.12 ± 0.02 mEq/*l*, respectively (P<0.01, one-way ANOVA). The FFA level of the double treatment group was significantly lower compared to those of the EF alone (P<0.01, Student's t test) and double sham group (P<0.01, Student's t test). In the comparison double treatment group to ischemia alone group, the P value by Student's t test was 0.06. The differences between FFA levels for the double sham and EF alone groups were not statistically significant.

DISCUSSION

We previously reported that exposure to ELF-EF has an impact on cellular Ca^{2+} regulation [18, 40] and also on the response to stress. These include effects on energy metabolism, specifically glycolysis, in restriction stressed rats [17]. In addition, we have also found that lipid peroxide level increase with the oxidizing agent was reduced by EF [19]. Therefore, we hypothesized that an EF might have an effect on the energy metabolism of organisms undergoing stress.

Table 1. Plasma lactate levels just before and on day-4, -7 and -14

Treatment	Experiment Day			
	1	4	7	14
Double sham	24.3 ± 3.2	18.4 ± 1.5	18.3 ± 1.0	19.2 ± 1.3
EF alone	20.7 ± 2.3	23.0 ± 2.2	19.0 ± 1.4	21.8 ± 1.5
Ischemia alone	23.7 ± 1.3	44.6 ± 7.4**,#	34.9 ± 3.7*,##	26.3 ± 2.2**,##
Double treatment	22.7 ± 1.6	44.6 ± 6.2**,#	33.5 ± 5.3*,#	24.2 ± 1.9*,#

Unit: [mg/d*l*]. The data are expressed as the mean ± standard error of the mean (SEM) (n=10). An ischemia-dependent increase and transient recovery was observed compared to the levels of the non-ischemic group (P<0.01, two-way ANOVA). No EF-dependent changes were found. * and **: P<0.05 and 0.01 vs. double sham groups. # and ##: P<0.05 and 0.01 vs. EF alone group.

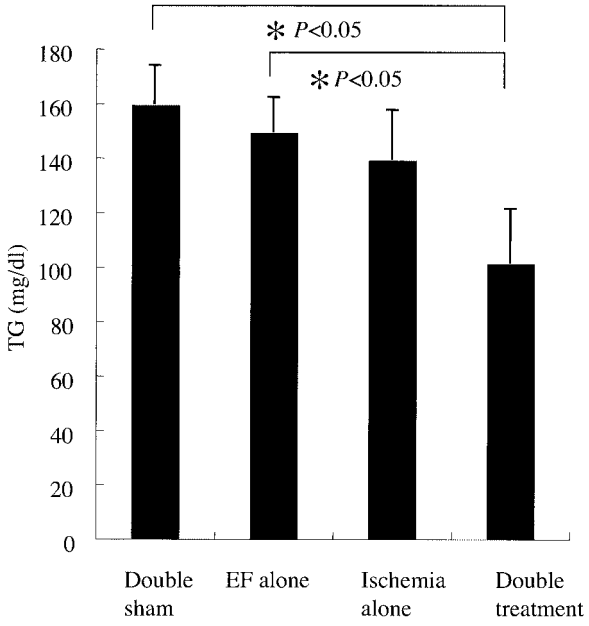

Fig. 3. Plasma triglyceride (TG) levels on day-14. The data are expressed as the mean ± standard error of the mean (SEM). Plasma TG levels showed significant changes among all groups (P<0.05, one-way ANOVA). TG level of the double treatment group was significantly lower than that of the double sham and EF alone groups (P<0.05, Student's t test). The difference in TG levels between the double sham and EF alone groups was not statistically significant.

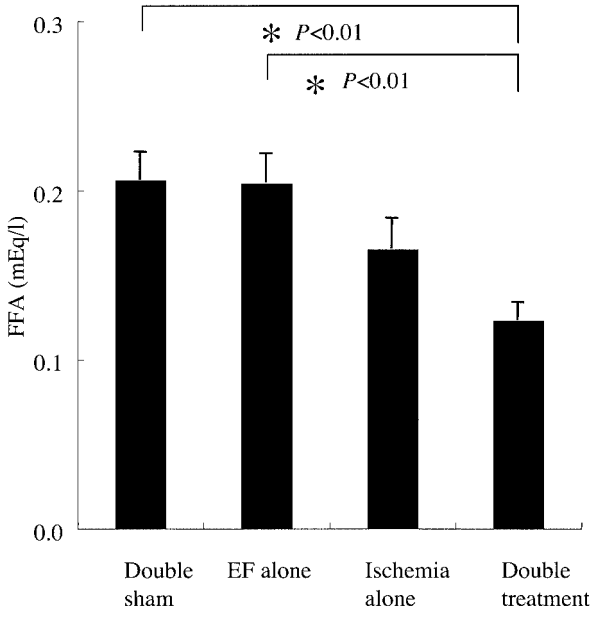

Fig. 4. Plasma free fatty acids (FFA) levels on day-14. The data are expressed as the mean ± standard error of the mean (SEM). The FFA levels suggested significant change among all groups (P<0.05, one-way ANOVA). The FAA level of the double treatment group was significantly lower than those of the double sham and EF alone groups, respectively (P<0.01, Student's t test). The difference in FFA levels between the double sham and EF alone groups was not statistically significant.

The results presented here, obtained using the rat ischemia model [12, 21, 35], enhance our understanding of the relationship between exposure to an EF and energy metabolism under the ischemic condition. Several earlier studies using this model suggested that stimulation with an EMF has an impact on the process of angiogenesis that is related to both cancer and ischemia [37, 46, 48]. The aim of this study was not to examine the relationship between an EF and blood circulation. The changes in lactate levels after ischemic surgery seen in this study may be an indirect parameter of a process in vascularization [12] and did not suggest any influences by EF exposure.

In all treatment groups, plasma glucose levels were not different. The results indicate that a severe change, such as the depletion of stored glucose, would not occur even if ischemia or an EF actually had a minor impact on glycolysis. The ischemia-dependent transient plasma lactate increases on day-4 followed by decreases until day-14, suggest two things: 1) the ischemia induced the imbalance of lactate metabolism by hypoxia; and 2) the ischemia is normalized gradually via micro bypass [12]. Obvious EF-related changes were not shown in plasma lactate levels in the double treatment group compared to those of the ischemia alone group. In addition, obvious EF-related changes were not shown on lactate levels in the EF alone group compared to those of double sham group. These results indicate that the EF applied in this study did not influence plasma lactate levels. An earlier study suggests that a magnetic field (MF) stimulation of 0.1 and 2 gauss for a variety of periods results in a significant increase in neovascularization [37] but we did not test any angiographic parameters in our studies. Based on the lack of effect of EF in alterating plasma lactate level in the present study, we consider that EF does not have a significant effect on vascularization. Future studies should test applied angiographic parameters to determine an effect of EF on vascularization. In addition, differences in the electrical conditions, such as an EMF and/or EF, a sine wave or a pulse form, frequency or field intensity, etc., must be considered.

Except for the WBC count, the present results did not show any major alteration in hematologic properties. The WBC count in the two groups treated with ischemic surgery were significantly elevated by day-7 and had returned to baseline values by day-14. This may be based on the inflammatory response induced by the surgery. Although creatine phosphokinase activity is well known as an endpoint of tissue damage [20], creatine phosphokinase activity on day-14 did not change among all groups. It is clear that any tissue damage in the rats did not remain on day-14 postsurgery.

At day-14, the TG levels showed double treatment group < ischemia alone group < EF alone group=double sham

group. A similar tendency was also observed in FFA levels. Triglycerides, which are an energy source in many organisms, are synthesized in the liver and are digested into fatty acids or glycerol by lipase distributed in various tissues. Therefore, two possibilities are to be considered: 1) TG metabolism is enhanced by the EF-induced increase of energy metabolism, including a fatty metabolism; and/or 2) an EF acts to suppress the TG synthesis pathway. Our previous study about the EF-induced suppressive effect on the lactate synthetic pathway in stressed rats [17] would support the possibilities of an EF effect on energy metabolism. In addition, we have also found that even though antioxidant activity was not influenced, plasma level of lipid peroxide was reduced by EF in rats with the oxidizing agent, 2,2'-azobis(2-aminopropane) dihydrochloride (AAPH) [19]. At present, we propose a hypothesis that EF does not have catabolic effects, but does have anabolic effects on the lipid metabolism. Our findings are consistent with our hypothesis. However, to investigate EF effect on lipid metabolism, more studies are needed in the future. These studies focus on EF-induced effect on the function of the pituitary gland or humoral properties including changing of lipid metabolism-related molecules, such as TNF-α, leptin, leptin receptor or adipsin. [47]. A number of studies previously reported that cellular calcium signaling was modified by EMF, including an EF [1–11, 18, 25, 26, 28–34, 40–43]. In addition, the success of operant conditioning, with EF used as a trigger for feeding, has been reported [38, 39]. Based on this information, we predict that the EF effect appears to have two major components as follows: 1) sensing an EF stimulation and 2) processing of EF in the central nerve systems. Several questions still remain including: 1) Where is the sensor for an EF in organisms, in the skin surface or brain?; 2) Are Current intensity, Frequency, Exposure period or Unknown factor important parameters in mode-of-action of an EF treatment?; 3) Is the biological effect(s) of EF different than those of EMF or MF?

In conclusion, these results indicate that the EF effect on glycolysis parameters, plasma lactate or glucose levels, does not appear in a highly stressed condition and that EF effects varied depending on the condition of the organism. Nevertheless, ELF-EF used in this study did have some impact on lipid metabolism parameters in a hind-limb ischemic rat. Further studies are needed to elucidate the association of ELF-EF with the lipid metabolism system.

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