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Effect of a 50 Hz Electric Field on Plasma ACTH, Glucose, Lactate, and Pyruvate Levels in Stressed Rats

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The effect of extremely low frequency electric field (EF) on stress induced changes of plasma ACTH, glucose, lactate, and pyruvate levels was examined in ovariectomized rats. The rats were exposed to 50 Hz EF (17500 V/m) for 60 min and were restrained for the latter half (30 min) of the EF exposure period. The restraint stress significantly increased the plasma ACTH and glucose levels ($P < .05$: Student's t test). Restraint induced increase of plasma ACTH and glucose levels tended to be suppressed by exposure to the EF. Meanwhile, the EF exposure also affected plasma lactate level. Thus, the EF exposure significantly decreases plasma lactate levels in the stressed rats ($P < .05$: Student's t test). Although the precise mechanisms in the restraint dependent alteration in plasma ACTH, glucose, lactate, and pyruvate levels are not fully understood, our results demonstrate that the 50 Hz EF alter both stress responses and energy metabolism in stressed rats. Bioelectromagnetics 25:346–351, 2004.

Key words: hormone; blood chemistry; ovariectomized rats; restraint stress

INTRODUCTION

All terrestrial living organisms are constantly exposed to electromagnetic fields (EMF) in varying degrees. Although the argument whether an electric field (EF) or magnetic field (MF) is harmless to a human being or not has often been put forward, various medical instruments, which harness an EF and MF, are widely utilized. Percutaneous electric nerve stimulation (PENS) and transcutaneous electric nerve stimulation (TENS) have an effect on patients with chronic low back pain [Lee et al., 1993; Ghoname and William, 1999] and can help repair soft tissue, respectively.

In TENS and PENS, electrodes contact and an electric current directly stimulates the affected part. However, it is not necessary to keep electrical contact in order to expose an object to EF and MF. When a living body is exposed to EMF, an induced electric current is generated. Recently, it has been reported that both an EMF-induced electric current and perception of an EMF exposure on skin surface trigger cellular and humoral responses to the EMF [Weigel et al., 1987; Kato et al., 1989]. Romo et al. [1998] also reported that a mechanical vibration (5–50 Hz) of skin surface, which is similar to the perception of an EMF exposure on skin surface, activated neurons of the primary somatosensory cortex corresponding to the site of stimulation.

In Japan, EF therapy has been utilized since Hara [1961] developed the EF exposure equipment named Healthtron™ (Hakuju Co., Ltd., Tokyo, Japan), which was approved by the Ministry of Health, Labour, and Welfare (Approval number 14700BZZ00904) in 1972. Recently, we reported that the curative effect of the EF therapy on several pains (headache, stiff shoulders, and stomach ache) in over a thousand cases [Harakawa et al., 2002]. Our observation suggests that the 50 Hz EF can modulate some biological functions, such as endocrine system, immune system, and cell signaling.

In fact, evidence has been reported that exogenous EF change in intracellular calcium ion concentration and protein synthesis in vitro [McLeod et al., 1987; Cho et al., 1999]. However, quantitative or qualitative

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analyses of effects of exogenous EF on a living body have not been examined yet. In this regard, it is necessary to establish methods for quantitative or qualitative assay of effects of exogenous EF. In addition, how the EF exposure modulates biological functions also has not been understood. In terms of curative effects of 50 Hz EF [Harakawa et al., 2002], the EF may alter stress responses and energy metabolisms in patients.

In this study, in order to clarify effects of the 50 Hz EF on stress responses and energy metabolisms, plasma levels of ACTH, glucose, lactate, and pyruvate were examined in stressed rats under the EF exposure.

MATERIALS AND METHODS

Electric Field Exposure System

The EF exposure system is composed of three major parts, namely, a high voltage trans unit (Healthtron^{TM}, maximum output voltage: 9000 V; Hakuju Co. Ltd., Tokyo, Japan), a constant voltage unit (TOKYO SEIDEN, Tokyo, Japan), and EF exposure cages (Fig. 1). The exposure cage is composed of a cylindrical plastic cage (400 mm diameter, 400 mm height) and two electrodes made of stainless steel (1200×1200 mm) placed over and under the cylindrical cage (Fig. 2A). In order to establish the EF (50 Hz 17500 V/m) in the cage, stable alternating current (50 Hz, 7000 V) was applied to the upper electrode.

Experimental Animals

Female 7-week-old Wistar rats, 300–350 g of body weight, were purchased from Charles River Japan, Inc. (Tokyo, Japan) and were maintained in a conventional animal room which conforms to the Japanese Good Laboratory Practice guideline. The room temperature and humidity were 24 ± 2 °C and $55 \pm 10\%$ with 12 h of artificial illumination daily (07:00–19:00).

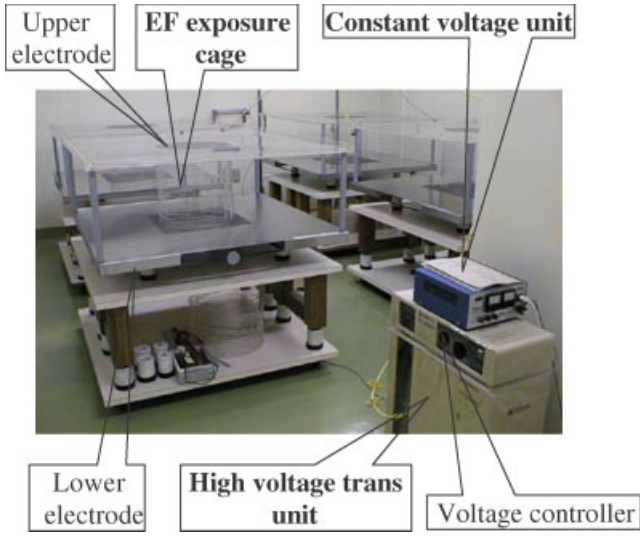

Fig. 1. The EF exposure system.

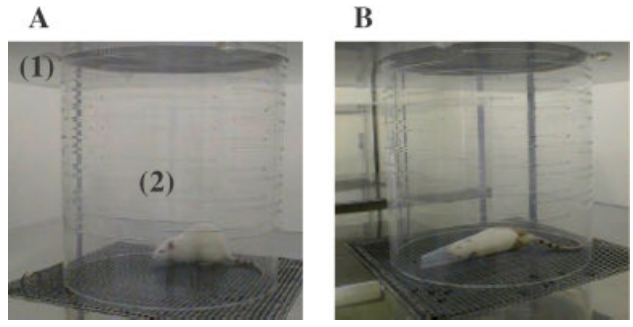

Fig. 2. The EF exposure cage. **A**: A cylindrical plastic cage (2) is placed between an upper (1) and a lower (3) stainless electrodes. The cylindrical cage has slits (length: 100 mm; width: 5 mm) around at intervals of 5 mm from each other. The slits prevent smudges, due to feces and saliva, disturbing formation of stable EF. **B**: A rat restricted by wrapping with a thin polyethylene sheet.

Restraint Stress

Each rat was restrained by wrapping with a thin polyethylene sheet and being laid on the lower electrode for 30 min (Fig. 2B).

Ovariectomy

In order to avoid effects of an estrous cycle on this experiment, effects of exposure to EF on stress responses were examined by using ovariectomized rats. An ovariectomy was performed 4 weeks before the experiments.

Experimental Designs

To assess whether the restrained rats feel stress, plasma ACTH level, which is one of many stress indicators, was measured just before and after the 30 min restraint. Six rats were divided into two groups. One group of rats was restrained for 30 min and another group of rats was intraperitoneally injected with 1 mg/kg of diazepam 30 min before the restraint period. Before and after the restraint period, plasma samples were collected from each rat and ACTH concentration was measured.

In order to examine effects of the 50 Hz EF exposure on plasma levels of ACTH, glucose, lactate, and pyruvate, 24 rats were divided into four groups (six rats/group) as follows: (1) control, (2) restrained under sham EF exposure, (3) restrained under the 50 Hz EF exposure, (4) restrained with diazepam treatment and sham EF exposure. The EF exposure and restraint treat-

ment were performed as follows. Rats were exposed to the 50 Hz, 17 500 V/m EF for 1 h. Rats were restrained with polyethylene sheets for the second half (30 min) of the EF exposure period. In control group, rats were kept in the same EF exposure cages without any treatment during the experiment. All experiments described in this article were conducted in accordance with the Guiding principles for the Care and Use of Research Animals promulgated by Panapharm Laboratories Co., Ltd. (Kumamoto, Japan).

Collecting Blood Samples

One millilitre of blood was collected from subclavian vein before the experiment, and plasma was separated by centrifugation at 1500*g* for 10 min at 4 °C. The plasma samples were stored at −80 °C until use. After the experiment, 3 ml of whole blood was collected from each rat by decapitation into a glass tube containing 9 mg EDTA. One millilitre of blood was used for hematological analyses. The remaining 2 ml of the blood was centrifuged (1500*g* for 10 min at 4 °C), and plasma was stored at −80 °C until measurement of ACTH, glucose, lactate, and pyruvate.

Blood Analysis

Plasma glucose, lactate, and pyruvate levels were measured with an automatic analyzer (7170 Hitachi, Hitachi Co. Ltd., Tokyo, Japan). Plasma ACTH level was measured by using an ACTH radio immunoassay kit (ACTH IRMA, Mitsubishi Chemical Co. Ltd., Tokyo, Japan) and a gamma counter (Auto-Gamma 5530 Gamma Counting System, Packard Instrument Co. Ltd.).

Statistical Analysis

Results were expressed as mean ± standard error of the mean (SE) or the data set, as median, 25th percentile, 75th percentile, minimum, and maximum values. Statistical significance of difference between paired groups was calculated by Student's *t* test or one way ANOVA, and the significance was defined as $P < .05$. All computations for the statistical analysis were carried out in MS-EXCEL® Japanese Edition (Microsoft Office software: Ver. 9.0.1, Microsoft Japan, Inc., Tokyo, Japan).

RESULTS

Effect of Restraint on Plasma ACTH, Glucose, Lactate, and Pyruvate Levels

In order to know how restraint stress affects plasma levels of ACTH, glucose lactate, and pyruvate, those plasma factors were measured before and after restraint. Figure 3 shows the changes of ACTH level in individual animal before and after restraint. In the restrained rats, plasma ACTH level increased from 231 ± 94.3 to 1177 ± 325 pg/ml (mean ± SE) after restraint. The plasma ACTH levels in restrained and diazepam injected rats increased from 358 ± 73 to 810 ± 121 pg/ml (mean ± SE). Comparing the ACTH levels of before with after restraint stress, the 30 min restraint increased the plasma ACTH levels 5.1- and 2.3-fold in restrained or restrained and diazepam treated rats, respectively. Plasma levels of glucose and lactate significantly increased after the restraint period ($P < .05$: Student's *t* test). However, those levels were restored to their normal levels after the end of the restraint period (Fig. 4A,B). The level of pyruvate did not change during the experiment (Fig. 4C).

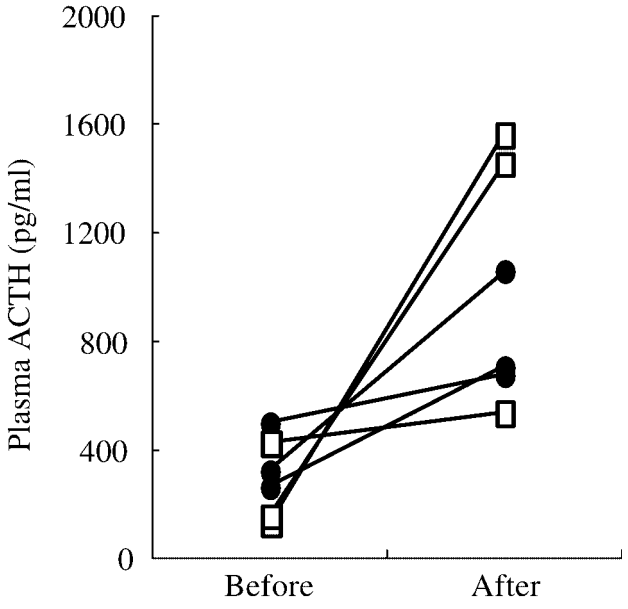

Fig. 3. Effect of restraint stress on plasma ACTH levels on each of six rats that were intraperitoneally injected with 1 mg/kg BW of diazepam (filled circle) or saline (open square) at 30 min before restraint and were restrained for 30 min.

Effect of the 50 Hz EF Exposure on Plasma ACTH and Glucose Levels

The 50 Hz EF exposure did not affect plasma ACTH level (data not shown). However, plasma ACTH level in the restrained group (Fig. 5A) was significantly higher than that of control group. Both the EF exposure and diazepam treatment decreased plasma ACTH level (Fig. 5A), and the difference of the ACTH level among three restrained groups, namely, restrained, restrained under the EF exposure, and restrained with diazepam treatment, was significant ($P < .05$: one way ANOVA). While both the EF exposure and diazepam treatment tended to decrease

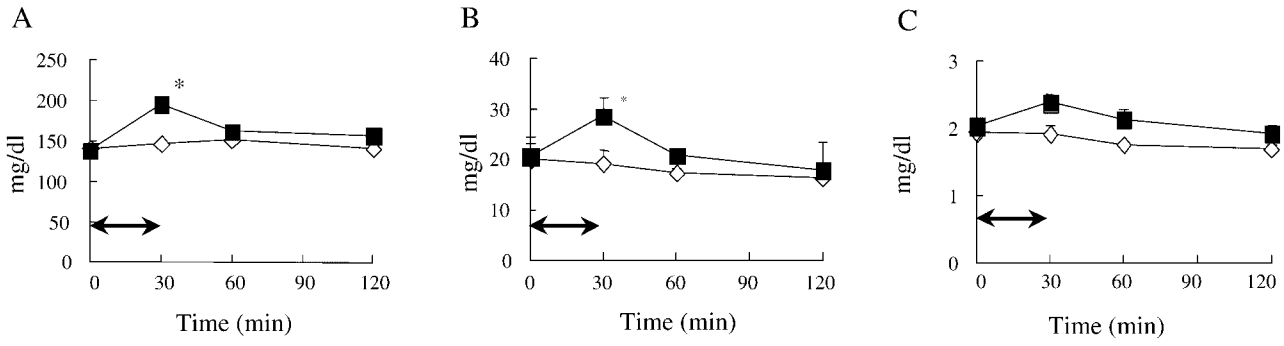

Fig. 4. Effect of restraint stress on plasma glucose (**A**), lactate (**B**), and pyruvate (**C**) levels. Rats were restrained for 30 min (double-headed arrow). Rats were intraperitoneally injected with 1 mg/kg BW of diazepam (filled square) or saline (open diamond) at 30 min before restraint and were restrained for 30 min. Blood sample were collected from subclavian vein every 30 min. The values show mean ± SE (n = 5). *Significantly higher than the initial value ($P < .05$).

plasma glucose level, but the difference was not significant (Fig. 5B).

Effect of the 50 Hz EF Exposure on Plasma Lactate and Pyruvate Level

Plasma lactate levels in both the EF exposure and diazepam treatment groups were significantly lower than that of restrained group ($P < .05$: Student's t test) (Fig. 6A). Likewise, plasma pyruvate levels in groups of the EF exposure and diazepam treatment tended to be low compared to those of restraint alone group (Fig. 6B).

DISCUSSION

The effect of the 50 Hz EF on stress induced changes of plasma ACTH, glucose, lactate, and pyruvate levels was examined in rats. In preliminary experiments, we had observed that plasma levels of ACTH

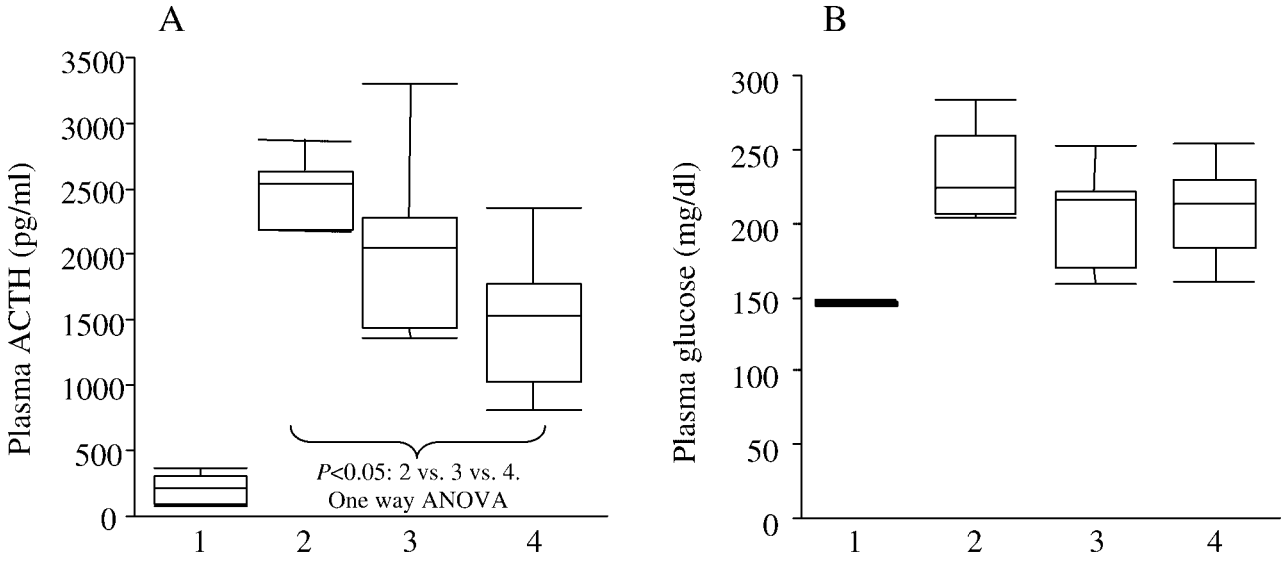

Fig. 5. Effect of exposure to the 50 Hz EF on plasma levels of ACTH (**A**) and glucose (**B**). Rats were restrained for the latter half (30 min) of the EF exposure period (60 min). Plasma ACTH and glucose levels just after the EF exposure were measured in control rats (1), restrained rats (2), rats restrained with EF (3), and rats restrained with diazepam treatment (4). Diazepam treatment was performed 30 min before the EF exposure. Data was expressed as a median, 25th percentile, 75th percentile, minimum, and maximum value (n = 6).

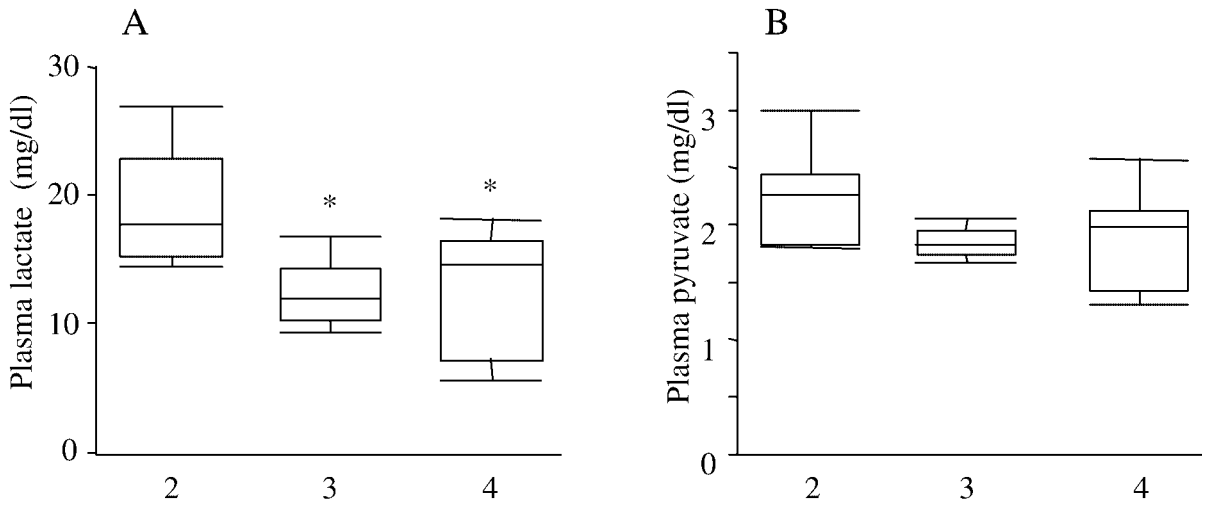

Fig. 6. Effect of exposure to the 50 Hz EF on plasma levels of lactate (**A**) and pyruvate (**B**). Rats were restrained for the latter half (30 min) of the EF exposure period (60 min). Plasma lactate and pyruvate levels just after the EF exposure were measured in restrained rats (2), rats restrained with EF (3), and rats restrained with diazepam treatment (4). Diazepam treatment was performed 30 min before the EF exposure. Data was expressed as a median, 25th percentile, 75th percentile, minimum, and maximum value (n = 6). *Plasma lactate level is significantly lower than that of group (2) ($P < .05$).

were affected by an estrous cycle, so that all rats were ovariectomized 4 weeks before experiments (data not shown). Rats were stressed by wrapping with a thin polyethylene sheet for 30 min. Plasma levels of ACTH increased up to 5.1-fold after the restraint, and diazepam treatment suppressed the increase in the levels of ACTH. These findings indicate that restrained rats were stressed, and the restraint stress affected the endocrine system such as pituitary–adrenocortical axis and sympathetic–adrenomedullary system [Kvetnansky et al., 1979; Sudo and Miki, 1993; Yamada et al., 1996; Arakawa et al., 1997].

The results showed that the EF exposure suppressed increase of ACTH in stressed rats. This finding indicates the EF has an anti-stress effect and/or suppressive effect on ACTH secretion. It was known that stress dependent increase of plasma ACTH occurs simultaneously with increase of plasma glucose [Arakawa et al., 1997]. Glucose levels significantly increase after restraint. But EF did not suppress this increase. Therefore, the suppressive effect of the EF on ACTH levels might be not an anti-stress effect but a specific suppression of ACTH secretion.

According to our previous report [Harakawa et al., 2002], the 50 Hz EF exposure had a curative effect on several pains, particularly on skeletal muscle related pains. Therefore, we also examined effects of the EF exposure on plasma levels of lactate and pyruvate in stressed rats. Plasma levels of lactate were significantly suppressed by the EF exposure, but that of pyruvate was not. Since the EF exposure does not cause any pathological changes (data not shown), the suppression of lactate levels will result from altered energy metabolisms.

The stress accompanied by locomotion, such as an enforced running exercise, causes accumulation of cellular or plasma lactate. In glycolysis, LDH participates in regulation of a lactate level, resulting in lactate being metabolized into pyruvate or amino acid. However, it was unknown whether the stress given to the animal by the restraint induces an emotional stress alone or adds a physical stress. Our results showed that stress without movement also promotes glycolysis and peripheral accumulation of plasma lactate. Moreover, although EF exposure caused suppression of the plasma lactate level, an EF induced change was not observed in the pyruvate level. These findings may indicate that the effect of EF is not caused by acceleration of catabolic of lactate, but suppression of anabolism of lactate. The mechanism of clinical effect of EF to the pain of a skeletal muscle mentioned above [Harakawa et al., 2002] may be associated with the living body response against stress via the endocrine system of pituitary and with the stress related peripheral energy metabolisms.

In conclusion, the EF exposure suppressed stress dependent ACTH secretion and lactate production, although mechanisms of the suppression were not clear. The present study shows evidence that the 50 Hz EF can modulate endocrine and energy metabolic systems in the stressed rats. In this regard, in vitro studies are also

required to clarify which levels of metabolic pathways are in charge of effects of the EF.

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