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Extremely Low Frequency Magnetic Fields Modulate Bicuculline-Induced-Convulsion in Rats

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The effect of extremely low frequency (ELF, 60Hz) magnetic fields (MFs) on convulsions was investigated in rats. We determined the onset and duration of convulsions induced by bicuculline alone or by co-exposure to MFs and bicuculline. In addition, we measured the GABA concentrations in the rat brains using HPLC-ECD. MFs strengthened the convulsion induced by bicuculline (0.3, 1, and 3 μg, i.c.v.), with a shortening of the onset time, but lengthening of the duration time. Co-exposure to MFs and bicuculline decreased the GABA levels in the cortex, hippocampus and hypothalamus, whereas MFs alone reduced the level of GABA only in the hippocampus. These results suggest that the exposure to MFs may modulate bicuculline-induced convulsions due to GABA neurotransmissions in rat brains.

Key wods: Extremely low frequency magnetic fields, Convulsion, Bicuculline, GABA, Brain

INTRODUCTION

Extremely low frequency (ELF, <300 Hz) magnetic fields (MFs) have been reported to produce a variety of behavioral and physiological function in animals (Adey, 1981; Gould, 1984; Frey, 1993). Some reports have also presented "hypersensitivity" to electric or MFs, which has raised questions as to whether pains, depression, lethargy, sleeping disorders, and even epileptic seizures could be associated with electromagnetic field exposure, and suggested that convulsions and epileptic seizures could be related to electromagnetic field exposure (WHO, 1984, 1987, 1993).

There are considerable amounts of biochemical, electro-physiological and pharmacological data showing that GABA is a major inhibitory transmitter in the CNS (DeFeudis, 1977; Hosli and Hosli, 1978; Johnston, 1978). GABA receptor sites in the mammalian central nervous system (CNS) have been divided into two different and distinct sites, termed $GABA_A$ and $GABA_B$ receptors (Enna and Karbon, 1986; Bormann, 1988; Bowery, 1989). The $GABA_A$ receptor is of prime importance in the pathogenesis of convulsions because of its apparent role in the synchronization and desynchronization of thalamocortical circuitry. The direct blockade of $GABA_A$ receptors by bicuculline, a selective $GABA_A$ receptor antagonist, induces clonic-tonic convulsions in mammals (Piredda *et al.*, 1985; Sperber *et al.*, 1989). Also, a reduction in the inhibitory synaptic activity might be expected to trigger a convulsion. Most, if not all, of the known effects of benzodiazepines are mediated by the interaction of GABA and $GABA_A$ receptors (Costa and Guidotti, 1979; Tallman and Gallaher, 1985; Matsumoto, 1989).

Kim *et al.* (2004) recently reported the ELF-MF effects on the premorbid behaviors produced by cocaine in mice. We have previously reported that exposure to 20 G of MFs, acting on the benzodiazepine-GABA complex, induced a significant reduction of latency in normal mice. These reduction effects on latency were also strengthened by the administration of diazepam. Flumazenil, a benzodiazepine receptor antagonist, also inhibited the hyperalgesia of MFs, in a dose dependent manner (Jeong *et al.*, 2000). The object of the present studies was to investigate whether MFs exposure additionally contribute to the convulsions mediated by the benzodiazepine-GABA complex receptor.

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MATERIALS AND METHODS

Animals and magnetic fields

Male Sprague-Dawley rats (Hanlim, Korea), weighing 250-350 g, were used in all experiments. Animals were maintained in a temperature-controlled room (25 ± 2 °C), with a 12:12 light dark cycle (lights on at 08:00 h). Food and water were available *ad libitum*. MFs were generated using Helmholtz coils set parallel to each other in a wooden frame. The MFs were produced with a 20 G and 60 Hz time-varying field intensity and frequency, respectively.

Intracerebroventricular catheters

Following pentobarbital (50 mg/kg, intraperitoneal) anesthesia, the rat was placed in a stereotaxic frame. The skull was exposed, and a hole then bored at coordinates overlaying the left lateral ventricle, i.e., 1 mm posterior to the bregma and 1 mm left of the midline, according to the atlas of Paxinos and Watson (1986). The guide cannula was inserted 4 mm into the lateral ventricle, and fixed to the skull with dental acrylic and secured by stainless steel screws. Animals with normal motor functions and behaviors were used for the experiment 4-7 days later. Intracerebroventricular (i.c.v.) administration was performed using a Hamilton syringe. After the experiments, the rats were sacrificed 5 min after a methylene blue injection, followed by the rapid removal of the brain to confirm the accurate injection of the drug due to its deposition around the ventricular system (Tatsuo *et al.*, 1999).

Drugs and procedures

The bicuculline methobromide and phaclofen were bought from Sigma (St. Louis, MO, U.S.A.). The bicuculline was dissolved in saline and the phaclofen in 0.1 M NaOH. All drugs were administered intracerebroventricularly.

Seven to ten rats were used in each group. The experiments were all carried out between 16:00 and 17:00 h to avoid circadian rhythm. The exposure to MFs lasted for 6 h prior to the convulsion test. The drugs were administration i.c.v. after the MFs exposure or in the sham. The convulsion test was then conducted.

Convulsion test

The convulsions induced by bicuculline (0.3, 1, and 3 μg, i.c.v.) were evaluated for their onset and duration times. The onset time of the convulsions was represented by the time from the injection of bicuculline to the development of the first generalized convulsion. The duration of the convulsion was adjudged to be the period from the time of the initial onset to the appearance of the final tonic or clonic convulsion.

Measurement of brain GABA concentration

An HPLC-electrochemical detector system (Jasco, Japan), with a C_{18} reversed-phase column (Luna 5u C18, 4.6 mm I.D. ×250 mm length, 5 μm particle size, Phenomenex, U.S.A.) and an electrochemical detector with the working electrode set at +0.67 V, was used for the detection of the GABA concentration. The column was maintained at a constant 40°C using a column heater.

The mobile phase consisted of 0.1 M NaH_2PO_4, 0.5 mM EDTA with 25% methanol (v/v) water, adjusted to pH 4.5 with 1 M phosphoric acid. The buffer was allowed to sit overnight for the exothermic reaction to reach equilibrium. The buffer was filtered through a 0.45 μm membrane filter, degassed (Rowley *et al.*, 1995), and then used at a flow rate of 0.80 mL/min.

All standards, dry reagents and solvents were obtained from Sigma (St. Louis, Missouri, U.S.A.). All standards and samples were derivatized with *o*-Phthalaldehyde (OPA) prior to injection on to the HPLC system. OPA stock (22 mg OPA, 0.5 mL ethanol, 0.5 mL 0.1 M sodium sulfite, and 9 mL 0.1 M sodium tetraborate) was prepared freshly every 3 days.

Sample preparation

The rats were lightly anesthetized with pentobarbital (50 mg/kg, i.p.), the brain regions of interest rapidly removed, weighed to the nearest milligram, frozen rapidly in micro-centrifuge tubes and stored at -70°C. The brain regions were removed from the freezer, placed in an ice bath and an appropriate amount of deionized water, containing internal standards, added. The tissue samples were disrupted by sonication and centrifuged at 13500 rpm for 10 min at 4°C. Prior to injection onto the system, a 20 μL sample or standard was reacted with 20 μL OPA solution for 15 min, and then filtered through a syringe filter.

Statistical analysis

Data were presented as the mean ± SEM. Differences between groups were tested using Student's *t*-test. Differences between multiple groups were tested using an analysis of variance (ANOVA) for repeated measures. Differences were considered statistically significant when $P<0.05$.

RESULTS

Fig. 1 reveals the influence of MFs exposure to rats on bicuculline-induced convulsions. Bicuculline treatment with sham exposure produced convulsions in dose dependent manners at 0.3 to 3 μg. The convulsion occurred within 30 sec at the high dose, 3 μg bicuculline. The exposure to MFs (20 G, 6 h) prior to bicuculline decreased the convulsion onset time with all bicuculline doses tested.

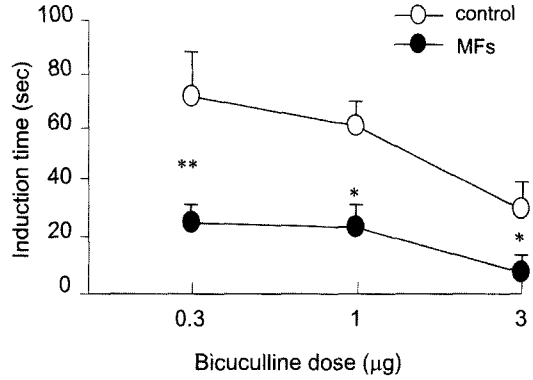

Fig. 1. The influence of MFs on the convulsion onset time induced by bicuculline. Rats received bicuculline (0.3, 1 and 3 μg, i.c.v.) after exposure of MFs (20 G) for 6 h or to a sham. The convulsion onset time was measured. The rats exposed to MFs were more sensitive to bicuculline, as shown by the earlier onset, compared to the sham control. Results are expressed as the means ± SEM. Significant differences were found between the MFs and sham control groups (P<0.05, ANOVA).

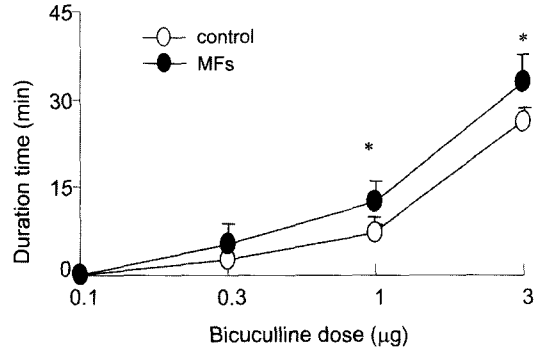

Fig. 2. The influence of MFs on the convulsion duration time induced by bicuculline. Rats received bicuculline (0.3, 1 and 3 μg, i.c.v.) after exposure to MFs (20 G) for 6 h or to a sham. The convulsion duration time was measured. An increase in the convulsion duration was observed in rats exposed to MFs compared to those in the sham control group. Results are expressed as the means ± SEM. Significant differences were found between the MFs and sham control groups (P<0.05, ANOVA).

The decrease in onset time due to MFs was statistically significant (P<0.05, ANOVA) compared to bicuculline only groups.

In contrast to the onset time, the convulsion duration time induced by bicuculline increased in dose dependent manners. The convulsion durations at low and high doses of bicuculline were 3 and 26 sec, respectively. The environmental exposure of rats to MFs (20 G, 6 h) increased the duration of the convulsion time induced by bicuculline. The dose dependent increase in the convulsion duration was also observed in MFs exposure groups. Statistical analysis indicated the increase induced by the MFs on the convulsion duration was significant (P<0.05, ANOVA).

Table I summarizes the GABA concentrations determined in the cortex, hippocampus, cerebellum, striatum and hypothalamus of the four groups. The first group was exposed to the sham as a control, the second with exposure to MFs (20 G, 6 h) alone, the third with bicuculline (1 μg) alone, and the last group with both MFs and bicuculline.

Compared to the control, the bicuculline-treated rats showed a significant decrease in the GABA concentration in the hypothalamus. MFs-exposure produced a significant decrease in the GABA concentration in the hippocampus compared to control. In addition, the administration of bicuculline after MFs exposure significantly decreased the GABA concentrations in the cortex, hippocampus and hypothalamus compared to control. Also, the concentration of GABA decreased in the cortex with exposure to MFs and bicuculline compared to MFs exposure alone.

DISCUSSION

There have been some reports by WHO suggesting convulsions and epileptic seizures could be associated with exposure to electromagnetic field exposure (WHO, 1984; 1987; 1993). Since the synapse plays a pivotal role in mediating communication between the neurons in mammalian brains, a defective synaptic function leads to convulsions. More precisely, a reduction of the inhibitory synaptic activity or an enhancement of the excitatory

Table I. GABA concentrations (mmol/g tissue) in rat brains exposed to sham, MFs and/or bicuculline

	Brain area				
	cortex	hippocampus	cerebellum	striatum	hypothalamus
Sham control	1.13 ± 0.06	1.34 ± 0.15	0.89 ± 0.06	1.37 ± 0.21	1.79 ± 0.07
MFs	0.91 ± 0.11	0.91 ± 0.03*	0.83 ± 0.03	1.09 ± 0.15	1.74 ± 0.08
Bicuculline	1.10 ± 0.08	0.94 ± 0.03	1.04 ± 0.11	1.14 ± 0.18	1.35 ± 0.16*
MFs and bicuculline	0.69 ± 0.10** #	0.85 ± 0.13*	0.73 ± 0.18	1.04 ± 0.16	1.19 ±0.13**

GABA concentrations in the cortex, hippocampus, cerebellum, striatum and hypothalamus were measured. The administered dose of bicuculline was 1 μg, i.c.v. Results are expressed as the means SEM of the GABA concentration. * and # designate significant differences (*P<0.05, **P<0.01) compared with the sham control or (#P<0.05) MFs alone, respectively.

synaptic activity would be expected to trigger a convulsion. Considerable amounts of biochemical, electrophysiological and pharmacological data show that GABA is a major inhibitory transmitter in the CNS. The GABA hypothesis of convulsions states; "one of the major factors in epileptogenesis is a decreased activation of $GABA_A$ receptors". The direct blockade of $GABA_A$ receptors by bicuculline, a selective $GABA_A$ receptor antagonist, induced clonic-tonic convulsions in mammals (Piredda *et al.*, 1985; Sperber *et al.*, 1989).

The present results indicate that exposure to MFs affects the pharmacological action of bicuculline, altering the level of GABA in brain areas. First, the decrease in the convulsion onset time induced by MFs may suggest that rats become more sensitive to bicuculline after exposure to MFs. A recent report suggested that ELF-MFs (4 Hz) affected tonic-clonic convulsions (Kim *et al.*, 2004); convulsion were induced with cocaine, and the effects of MFs on the premorbid behaviors investigated. The results from the present study are, at least in part, consistent with those stated above. MFs exposure also prolonged the duration of convulsions induced by bicuculline, suggesting that the recovery mechanism of neurons, from a convulsion to the normal state, may be affected by MFs. Bicuculline is known to induce convulsions by blocking the binding of GABA to its receptor. GABA/receptor binding opens chloride channels, which produce an influx of chloride into the intracellular cytosol of neurons. The inhibition induced by bicuculline on the chloride influx is responsible for the mechanism of a lasting convulsion. The prolonged duration induced by MFs indicate the pharmacodynamic action of bicuculline in nervous cells may be modulated, and result in the increase in the bicuculline convulsive effects.

Conversely, a decrease of the GABA concentration could induce a convulsion or epilepsy in the brain (Moshe, 2000). The hippocampus is also a region generally considered to be involved in the etiology of some seizure disorders, as temporal lobe epilepsy can result from pathological changes in this region (Scheibel, 1980). As shown in Table I, MFs decreased the concentration of GABA in the hippocampus compared to the control. The administration of bicuculline, after MFs exposure, also significantly decreased the GABA concentrations in the cortex, hippocampus and hypothalamus compared to the control. Moreover, convulsions are also thought to arise from the cerebral cortex, but not from other CNS structures, such as the thalamus, brainstem or cerebellum. From our results, the concentration of GABA was found to be decreased in the cortex after exposure to MFs and bicuculline compared with MFs alone. Therefore, these results indicate that MFs inhibit the release of GABA, which may result in the suppression of $GABA_A$ergic synaptic transmission in some brain regions.

In our previous study, it was suggested that MFs induce hyperalgesia through activation of benzodiazepine receptor (Jeong *et al.*, 2000). This result gave a clue of the potential involvement of MFs in the action of GABA in animals, since GABA and benzodiazepine receptors exists as a complex, and interacts with each other. The present study found that exposure to MFs might also alter the $GABA_A$ ergic synaptic transmission. The finding that MFs are able to change the pharmacological actions of bicuculline, including the convulsion onset and duration, may give us a new clinical implication. Moreover, we should not neglect the action of MFs leading to GABA level modulation, as chronic exposure to MFs may potentially act as invisible xenobiotics. However, in order to confirm MFs as harmful substances that induce convulsions, more attention should be paid and further studies undertaken on the mode of involvement.

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