Alternating Extremely Low Frequency Magnetic Field Increases Turnover of Dopamine and Serotonin in Rat Frontal Cortex

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The aim of this study was to evaluate the influence of an extremely low frequency sinusoidal magnetic field (ELF MF) with frequency of 10 Hz and intensity of 1.8–3.8 mT on the levels of the biogenic amines dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxytyramine (3-MT), 5-hydroxytryptamine (5-HT), 5-hydroxyindolacetic acid (5-HIAA), and noradrenaline (NA), as well as on DA and 5-HT turnover in corpus striatum and frontal cortex of adult male Wistar rats. We found that ELF MF exposure for 14 days, 1 h daily, did not influence the level of the examined biogenic amines and metabolites, but increased the rate of synthesis (turnover) of DA and 5-HT in rat frontal cortex as compared to control, sham exposed rats. On the basis of the present results and our previous findings, extremely low frequency magnetic field (ELF MF) exposure has been found to alter both turnover and receptor reactivity of monoaminergic systems, as well as some behaviors induced by these systems or their agonists and antagonists. Bioelectromagnetics 00:1–5, 2004. © 2004 Wiley-Liss, Inc.

Key words: ELF magnetic field; brain; biogenic amine level; biogenic amine synthesis

INTRODUCTION

One of many physical factors used in recent years in therapy is an alternating extremely low frequency magnetic field (ELF MF), with induction values from 1 pT to tens of mT and frequencies from several hertzs to 60 Hz. At the present stage of knowledge, unequivocal effects of these electromagnetic fields on brain neurochemistry have not been realized.

As numerous experimental and clinical studies have shown, ELF MF influence the structures and function of the central and peripheral nervous systems of experimental animals and humans [Warnke, 1980; Tenforde, 1991; Lai et al., 1993; Lyskov et al., 1993; Zhang et al., 1997]. It has been demonstrated that ELF MF activates regenerative processes in nervous tissue, leading among other results, to an increase of nerve impulse conduction in damaged axons [McCaig and Rajniczek, 1991]. In other studies [Lyskov et al., 1993], a stimulation of alpha and beta waves activity and decrease of delta waves activity in electroencephalogram in the frontal area of brains of volunteers subjected to ELF MF exposure were observed.

In the literature, there are only sparse data on the effect of ELF MF on neurotransmitter systems in the brain or on behavioral alterations. Lai et al. [1993] indicated that low frequency magnetic fields increased activity of the parasympathetic nervous system, as indicated by increased choline uptake in the frontal cortex and hippocampus, along with enhanced vagal nerve activity [Gmitrova et al., 1988]. Zhang et al. [1997] found that ELF MF (60 Hz, 1 mT) promoted neurite varicosity formation in cultured rat chromafin cells and increased their catecholamine levels and release rates.

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Previously, in an animal model of Parkinson's disease produced by a neonatal 6-hydroxydopamine (6-OHDA) lesion of dopaminergic nigrostriatal neurons, we showed that ELF MF attenuated the reactivity of dopamine (DA) D_1 receptors in brain [Sieroń et al., 2001]. There are also some data showing positive therapeutic effects of ELF MF in patients with Parkinson's disease [Sandyk et al., 1992; Sandyk and Iacono, 1994; Sandyk, 1996]. Changes in the behavior of experimental animals exposed to ELF MF have also been demonstrated, mainly expressed as increased locomotor activity [Ossenkopp and Ossenkopp, 1983; Rudolf et al., 1985]. Also, in our team, we found that ELF MF influenced different parameters of rat behavior [Cieślar et al., 1994; Mrowiec et al., 1994], although others failed to confirm this influence of ELF MF on motor activity [Davis et al., 1984].

Central neurotransmitter systems, mainly dopaminergic, serotoninergic, and noradrenergic neurons, play an important regulatory role in behavioral expression. Therefore, the aim of this study was to evaluate the effect of ELF MF on endogenous levels of monoamines in brain, as well turnover (synthesis rate) of DA and 5-hydroxytryptamine (5-HT), neurotransmitters known to modulate motor activity.

MATERIALS AND METHODS

Twenty-four adult Wistar albino male rats, mean weight about 220 g, were housed in the university's Animals Department, three animals per cage, at a temperature of 22 ± 1 °C under a 12 h light-dark cycle (light on at 07:00 h) with free access to tap water and pellet food [Murigran, Motycz, Poland]. The local Bioethic Committee of Medical University of Silesia for Animals approved the experiment (permission no. 06/2001 issued on May 15, 2001). All animal testing was conducted according to NIH regulations on animal care, as described in "Guide for the Care and Use of Laboratory Animals" [National Academy of Sciences, 1996].

Animals were divided into two groups. Rats of the first group (12 animals) were exposed to a 10 Hz alternating sinusoidal magnetic field generated inside the cylindrical applicator of the magnetic therapy device "Ambit 2000" manufactured in Poland. Exposure conditions (frequency, magnitude, and exposure time) chosen for this study are typical for therapeutic procedures of magnetotherapy in the treatment of some nervous system diseases in humans. Magnetic field intensity was not uniform; magnetic field induction values within the cylindrical applicator, measured by a meter with a Hall effect probe "Magnet Physik" FH 35 (Germany), were in the range of 1.8–3.8 mT RMS depending on the position of magnetic induction measurement points. The measurements were taken in 9 points placed regularly along diameter of the solenoid. The highest values of magnetic field induction (3.8 mT RMS) were found near the edge of the cylindrical applicator and the lowest ones (1.8 mT RMS), in the center of solenoid. The applicator was laid horizontally on the ground and the direction of magnetic field axis was vertical. The diameter of the solenoid was 51 cm and its height was 16 cm. Magnetic field was monitored by a computer system.

Rats were placed in a specially designed plastic chamber that fit tightly inside the applicator, and the whole body of the animals was exposed for 1 h during 14 successive days, beginning at 10 AM. In each session, six animals were simultaneously exposed in the chamber. An efficient ventilating system was used to maintain a stable temperature of 22 °C inside the chamber during exposure. No significant difference of mean body temperature measured in animal anus was observed between both groups of rats.

Rats from the second group were subjected to a sham exposure in which applicator connectors received no voltage, and therefore the applicator solenoid did not generate a magnetic field. For details of exposure procedure, see Sieroń et al. [2001].

In order to estimate the persistent changes in biogenic amine levels and turnover as a result of long term, repeated exposure to ELF MF, on the 15th day, 24 h after the last ELF MF or sham exposure, rats were used for the biochemical studies described below.

Catecholamine Assay

In first part of the experiment, 12 rats (6 ELF MF exposed and 6 sham exposed) were sacrificed by guillotine, and their brains were immediately excised and placed on ice. The corpus striatum and frontal cortex were separated, placed on dry ice, weighed, and stored at −70 °C until the assay. 5-Hydroxytryptamine (5-HT) and its major metabolite, 5-hydroxyindolacetic acid (5-HIAA), as well as noradrenaline (NA), DA and their metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 3-methoxytyramine (3-MT), were assayed by an HPLC/ED technique [Magnusson et al., 1980; Wagner et al., 1982].

DA and 5-HT Turnover

In second part of experiment, the other 12 rats (6 ELF MF exposed and 6 sham exposed) were used to determine turnover rates of DA and 5-HT. The aromatic amino acid decarboxylase inhibitor *m*-hydroxybenzylhydrazine dichloride (NSD-1015) (100 mg/kg IP) was

administered, and increased levels of L-dihydroxyphenylalanine (L-DOPA) and 5-hydroxytryptophan (5-HTP), due to less conversion to DA and 5-HT, respectively, were assessed 30 min later. At this time, rats were decapitated, the skull was opened, and the brain was excised for removal of corpus striatum and frontal cortex. Tissues were stored at −70 °C until the assay. L-DOPA and 5-HTP were assayed by a standard HPLC/ED method [Magnusson et al., 1980; Wagner et al., 1982]. The levels of amino acids were expressed in ng/g of wet tissue, and synthesis rate (turnover) of DA and 5-HT, were calculated in nmol/g/h, according to [Carlson et al., 1972]. To calculate the "turnover" of DA and 5-HT, we applied the formula of Carlson et al.: $n = (m/M) \times 2$, where n is synthesis rate (turnover) [nmol/g/h], m is the level of L-DOPA or 5-HT in rat brain [ng/g of tissue], and M is mol mass of L-DOPA or 5-HT [g/mol].

Statistical Analysis

For statistical analysis of obtained results, we applied a STATISTICA program. An analysis of variance (ANOVA) and the post-ANOVA test of Neuman–Keuls were used to test the differences between groups for significance. A P value of .05 or less was used to indicate a significant difference between groups.

RESULTS

The 14 day exposure of rats to ELF MF did not influence the endogenous level of DA, 5-HT or their respective metabolites in corpus striatum and frontal cortex, compared to sham exposure (Table 1). In the corpus striatum, in ELF MF exposed rats, the mean values of DA, 3-MT, and 5-HIAA were insignificantly higher (0.6%, 19.4%, and 4.6%, respectively) and the mean values of DOPAC, HVA, 5-HT, and NA were insignificantly lower (9.4%, 5.6%, 2.4%, and 19.6%, respectively), compared to sham exposed rats. In the frontal cortex, in ELF MF exposed rats, the mean values of DA, DOPAC, HVA, and 5-HT were insignificantly higher (6.7%, 18.8%, 3.0%, and 2.0%, respectively) and the mean values of 5-HIAA and NA were insignificantly lower (1.4% and 26.9%, respectively), compared to sham exposed rats.

However, this length of ELF MF treatment significantly increased turnover of both DA and 5-HT in frontal cortex, but not in rat corpus striatum (Table 2). In the frontal cortex, in ELF MF exposed rats, the mean values of both DA and 5-HT synthesis rate were significantly higher (18.9 and 20.9%, respectively), compared to sham exposed rats. On the other hand in the corpus striatum, in ELF-MF exposed rats, the mean values of DA and 5-HT synthesis rate were insignificantly lower (4.2% and 17.3%, respectively), compared to sham exposed rats.

DISCUSSION

ELF MF alters behavior, mainly by increasing locomotor and exploratory activity [Smith and Justesen, 1977; Ossenkopp and Ossenkopp, 1983; Rudolf et al., 1985; Dura and Csorba, 1988]. Others, using magnetic fields with different parameters (50 Hz, 46 mT) reported decreased irritability with reduced performance in learning and memory tasks [Trzeciak et al., 1993]. In another study performed by our team, ELF MF exposure (40 Hz, 10 mT) reduced locomotor activity and improved spatial memory in a water maze [Mrowiec et al., 1994]. It must be added that mechanisms of these effects of ELF MF are not eluciated.

ELF MF induced an analgesic effect, examined by different means [Kavaliers et al., 1983; Kavaliers and Ossenkopp, 1986a,b; Ossenkopp and Kavaliers, 1987]. These authors have proposed that ELF MF influenced the central opioid system and altered the reactivity of

TABLE 1. Effect of Extremely Low Frequency Magnetic Field on Biogenic Amine Content in the Corpus Striatum and Frontal Cortex of Rat Brain

	Corpus striatum		Frontal cortex	
Examined amine	Sham exposure	ELF MF	Sham exposure	ELF MF
DA	66490 ± 630[a]	6650 ± 430	116 ± 10	125 ± 19
DOPAC	729 ± 72	660 ± 46	41 ± 2	50 ± 10
HVA	518 ± 49	489 ± 62	58[Q1] ± 6	60[Q2] ± 3
3-MT	174 ± 20	215 ± 16	—	—
5-HT	369 ± 37	361 ± 1.5	182 ± 19	186 ± 32
5-HIAA	280 ± 17	293 ± 24	119 ± 17	117 ± 18
NA	140 ± 15	110 ± 22	270 ± 25	198 ± 29

[a]ng/g of wet tissue.
n = 6; x ± SEM; no diferences are statistically significant.

TABLE 2. Effect of Extremely Low Frequency Magnetic Field on the Synthesis Rate (Turnover) of Dopamine (DA) and 5-Hydroxytryptamine (5-HT) in the Corpus Striatum and Frontal Cortex

		Turnover (nmol/g/h)		
Examined amine	Part of the Brain	Sham exposure	ELF MF	F
DA	Corpus striatum	8.17 ± 0.38	7.83 ± 0.42	0.42
	Frontal cortex	0.99 ± 0.04	1.22* ± 0.09	6.056
5-HT	Corpus striatum	1.39 ± 0.16	1.15 ± 0.08	2.238
	Frontal cortex	0.91 ± 0.05	1.15* ± 0.05	14.079

n = 6; x ± SEM.
*$P < .05$ vs. control.

opioid receptors. In our team, we confirmed the analgesic effects of ELF MF (40 Hz, 10 mT) in rats and noticed that pretreatment of animals with naloxone abolished the effect of ELF MF [Cieślar et al., 1994].

In our previous study, we evaluated the influence of ELF MF on the reactivity of central DA D_1 receptors in rats in which nigrostriatal dopaminergic nerves were largely destroyed (>95% long-term depletion of endogenous DA level in striatum) by neonatal 6-OHDA intracerebroventricular treatment (134 μg), a recognized animal model of Parkinson's disease [Sieroń et al., 2001]. At 2 months of age, in 6-OHDA lesioned rats exposed daily at 2–3 months of age for 14 consecutive days to a 10 Hz sinusoidal, 1.8–3.8 mT magnetic field, there was a reduction in both oral activity and irritability induced by the DA D_1 agonist SKF 38393 (1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol), versus controls. Also, in the 6-OHDA lesioned rats exposed to the magnetic field, there was an increase in catalepsy induced by the DA D_1 receptor antagonist SCH 23390 [(R)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine-7,8-diol] [Sieroń et al., 2001].

In the present study, we have specifically shown that low frequency magnetic field exposure increased both DA and 5-HT turnover in rat frontal cortex. This effect could be an important element in the previously observed behavioral changes following ELF MF exposure [Cieślar et al., 1994; Mrowiec et al., 1994]. In summary, low frequency magnetic field exposure has been found to alter both turnover and receptor reactivity of monoaminergic systems, and some behaviors induced by these systems or their agonists and antagonists. We propose that ELF MF may influence the activity of many other neurotransmitter systems, such as parasympathetic, cholinergic neurons, and that the overall altered firing patterns in these associated neurotransmitter specific neural circuits can account for a variety of behavioral alterations produced by ELF MF.

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