Influence of Alternating Low Frequency Magnetic Fields on Reactivity of Central Dopamine Receptors in Neonatal 6-Hydroxydopamine Treated Rats

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The aim of this study was to evaluate the influence of extremely low frequency magnetic field (ELF MF) on the reactivity of the central dopamine D1 receptor in rats with dopamine neurons chemically damaged by 6-hydroxydopamine (6-OHDA), an animal model of human's Parkinson's disease. The experiment was carried out on male Wistar rats. On day 3 of postnatal life, a lasting and selective chemical damage of the central dopamine system was induced in the rats by infusion of 6-OHDA (133.4 μg intracerebroventricular. base form) given bilaterally into lateral ventricles of the brain. Control animals received similar treatments injecting only vehicle. At 2 months of age, both 6-OHDA treated and control rats were randomly divided into two groups. Rats from the first group were exposed to 10 Hz sinusoidal, 1.8–3.8 mT magnetic field one hour daily for 14 days. Rats of the second group were sham exposed, with the applicator solenoid turned off. On the day after the final exposure the evaluations were made of the rat’s spontaneous irritability, oral activity, and catalepsy. The MF exposed rat with chemically induced dopamine neurons damage exhibited a reduction of irritability and oral activity when stimulated with SKF 38393 (the agonist of central dopamine D1 receptor) and some increase of catalepsy after administration of SCH 23390 (the antagonist of central dopamine D1 receptor). These results indicate that ELF MF reduce the reactivity of central dopamine D1 receptors in rats. Bioelectromagnetics 22:479–486, 2001. © 2001 Wiley-Liss, Inc.

Key words: ELF magnetic field; SKF 38393 induced oral activity; SCH 23390 induced catalepsy

INTRODUCTION

One of many physical factors used in recent years in therapy are alternating extremely low frequency magnetic fields (ELF MF) with induction values from 1 pT to tens of mT and frequencies from several Hz to 60 Hz. At the present stage of knowledge, unequivocal explanation of mechanisms of action of those fields on living organisms is not fully possible.

As numerous experimental and clinical studies have shown, alternating low frequency magnetic fields influence the structure and function of the central and peripheral nervous systems of experimental animals and humans. For example, the following biological processes in the nervous system have been reported to be affected by low frequency magnetic fields: increase of activity of neuron oscillators of hypothalamic and intracerebral nuclei, activation of neurotransmitter synthesizing enzymes in nerve ganglia and endings, changes of nerve impulse conduction in axons, changes of structure and transmission of synaptic endings, and changes of structure and activity of cellular receptors of the nervous system [Warnke, 1980; Tenforde, 1991; Lai et al., 1993; Lyskov et al., 1993; Zhang et al., 1997].

It has been demonstrated that low frequency magnetic fields activate regenerative processes in nervous tissue, leading among other results, to an increase of nerve impulse conduction in damaged axons [McCaig and Rajniczak, 1991]. In other studies [Lyskov et al., 1993] a stimulation of alpha and beta wave activity and decrease of delta wave activity in the

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frontal area of brains of volunteers subjected to low frequency magnetic field exposure was observed. Changes in behavior of experimental animals exposed to low frequency magnetic fields have also been demonstrated. In most of the studies an increase in motor activity has been observed which is more pronounced in male animals [Ossenkopp and Ossenkopp, 1983; Rudolph et al., 1985]. However, the literature contains also reports that did not confirm the influence of low frequency magnetic fields on motor activity of animals [Davis et al., 1984].

Low frequency magnetic fields also influence the activity of endogenous opioid systems of animals and their responses to exogenous opiates. Results of many studies indicate that this effect is related to a change of the daily profile of endogenous opioid secretion and reduction of the effect of agonists of mu and kappa opiate receptors, as well as inhibition of delta receptor activity [Kavaliers and Ossenkopp, 1986, 1987].

At the same time, results of other studies have indicated the increase of choline uptake in the brains frontal cortex and hippocampus [Lai et al., 1993] and stimulation of activity of the vagus nerve [Gmitrova et al., 1988] in animals exposed to low frequency magnetic fields, pointing to an effect on the parasympathetic nervous system.

Results of many other experimental studies have shown that exposure of animals to low frequency magnetic fields causes a reduction in the activity of enzymes that synthesize melatonin, leading to a reduction of melatonin contents and a secondary increase in serotonin contents in the pineal gland and blood [Reiter, 1993; Jacobson, 1994]. This implies a participation of serotonergic fibers in the nervous system in response to the action of low frequency magnetic fields.

There are also some data showing positive therapeutic effects consisting of subsidence of disease symptoms caused by dopamine and serotonin depletion, for instance, in patients with Parkinson's disease being treated with low frequency and very low intensity magnetic fields [Sandyk et al., 1992; Jacobson, 1994; Sandyk and Iacono, 1994a; Sandyk 1996].

Taking the above into account, the aim of this study was to evaluate the influence of low frequency magnetic fields on the reactivity of central dopamine (DA) D1 receptors in neonatal 6-hydroxydopamine-treated rats (an animal model of Parkinson's disease).

**MATERIALS AND METHODS**

Wistar Albino female rats were bred and housed in the university's animal breeding facility (three animals in each cage) at 22 ± 1 °C under a 12 h light-dark cycle and with free access to tap water and pellet food (Murigran, Moriycz, Poland). Three days after birth, male pups were selected and were pretreated with desipramine HCl 20 mg/Kg intraperitoneal (ip) (Sigma Chemicals, St. Louis, MO) to prevent uptake of the neurotoxin 6-OHDA to noradrenergic neurons. Thus DA neurons would be destroyed. An hour later the rats were given bilateral intracerebroventricular (icv) injection of 6-hydroxydopamine-HBr (6-OHDA), 66.7 μg of the base (Regis Chemicals, Chicago, IL) to each side of the brain in a volume of 5 μl of vehicle (0.9% NaCl saline containing 0.1% ascorbic acid) to damage dopamine neurons. Control male rats were administered the desipramine ip and then i.c.v. vehicle under the same procedure [for details see Brus et al., 1994]. Rats were weaned at 21 days. Experiments were conducted when the animals were about 2 months old. By this procedure we obtain an animal model of Parkinson's disease.

The 6-OHDA lesioned rats were randomly divided into two groups. Rats from the first group 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. Magnetic field intensity was not uniform; magnetic field induction values within the applicator measured by a "Magnet Physik" FH 35 (Germany) meter with a Hall effect probe were in the range of 1.8-3.8 mT, depending on the position of measurement points. The applicator was laid horizontally. The diameter of the solenoid was 51 cm and its height was 16 cm (Fig. 1). Positions of magnetic induction measurement points inside of applicator are shown in Figure 1 and spatial arrangement of magnetic induction values inside the applicator is shown in Figure 2.

Rats were placed in a specially designed plastic chamber which fits tightly inside the applicator, and the whole body of the animals was exposed. The 1 h exposures were made on 14 successive days, beginning at 10 am. Every time, five animals were exposed in the chamber simultaneously. An efficient ventilating system was used in order to keep stable temperature inside the chamber during exposure. No significant changes of body temperature before and after exposure were observed.

Rats from the second group were subjected to sham exposure in which applicator connectors received no voltage, and therefore the applicator solenoid did not generate magnetic field. No significant difference of body temperature was observed between animals exposed to magnetic field and the sham-exposed ones.

The control rats were also randomly divided into two groups. The first group was exposed to the
magnetic field in the same scheme as above, and the second group was subjected to sham exposure.

Before the 14 daily exposure sessions and on the day immediately after the end of the exposure sessions the following behaviors were studied on the rats:

Spontaneous irritability, oral activity, and Catalepsy.

Spontaneous irritability was evaluated with the Nakamura-Thoenen [1972] score scale test. In this test reactivities of animals to four different exogenous stimuli were estimated (Table 1). The scores were added for each rat and then averaged for different groups.

Oral activity was evaluated with the method described by Kostrzewa and Gong [1991]. Rats were placed in individual clear glass cages (48 × 26 × 26 cm) in a quiet, well-ventilated, and well-lighted room. Rats were allowed to adapt to the new environment for at least 30 min. Tests were performed between 9 am and 3 pm as follows: On the first day of the experiment, rats of all groups received saline (0.9% NaCl) (1.0 ml/kg ip). Each rat was then observed one at a time for 1 min every 10 min, over a 60 min period, beginning 10 min after injection. The number of oral movements was counted by an experienced observer.

After completing the observation, rats were on the following four days injected ip with dopamine D1 agonist SKF 38393 · HCl (RBI Chemicals Co., Natick, MA) in an escalating dosage (day 1.0.03; day 2.0.1; day 3.0.3; and day 4.1.0 mg base/kg ip) and the observations were repeated as above. The same schedule was repeated after the end of 14 daily exposure sessions. Results were expressed as dose response curves [Brus et al., 1994]. In these experiments the oral activity has been described as spontaneous episodes of chewing movements not connected with any material in the mouth (food litter, excrements, etc.) [Waddington, 1990].

Catalepsy in rats was evaluated using the method described by Kostrzewa and Kastin [1993]. Rats were administered an antagonist of central dopamine D1 receptor SCH 23390 (RBI Chemical Co., Natick, MA) (0.5 mg base/kg ip). Thirty minutes after the injection, animals were placed individually on a framed wire screen with a 10 × 10 mm mesh inclined at a 60° angle from the horizontal plane. Time duration from the moment of placing the rat on the screen to the moment when the animal lost contact with the screen with at least one paw was measured. Maximum time allowed was 60 s. The test was repeated after 15, 30, 60, and 120 min, results were added and averaged for each group. The intensity of catalepsy was expressed in seconds.

At the end of the experiments, animals were decapitated and their striata were dissected from the brains. In the striatum the contents of dopamine (DA) and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) were determined, by HPLC technique [Magnusson et al., 1980].

Particular experimental groups consisted of 7–10 rats. The results in different groups were presented as mean values and SEM and evaluated statistically with the Statistica programme. ANOVA rank Kruskal-Wallis test was used for catalepsy and irritability analysis. Since variances in different groups estimated by the Levene test were inhomogenous, ANOVA was followed by post hoc nonparametric U Mann-Whitney test. For oral activity, the nonparametric U Mann-Whitney test was used.

RESULTS

After the administration of 6-OHDA in the neonatal period, adult animals showed a significant lowering of striatal content of DA and its metabolite DOPAC as compared with control animals. Average (±SEM) DA contents in a group of 6-OHDA treated
rats \( n = 8 \) was \( 3.3 \pm 0.4 \) nmol/g of fresh tissue and was significantly lower \( P < 0.001 \) compared to control rats \( 71.1 \pm 4.1 \) nmol/g of fresh tissue; \( n = 7 \). Striatal contents of DOPAC in 6-OHDA treated rats was \( 0.52 \pm 0.1 \) nmol/g of fresh tissue. It was also significantly lower \( P < 0.001; \ n = 8 \) than that of the controls \( 24.7 \pm 1.9 \) nmol/g of fresh tissue; \( n = 7 \).

Irritability differed significantly between the different treatment groups of animals \( H = 26.58, P < 0.001 \) in exposed rats and \( H = 30.11, P < 0.001 \) in sham-exposed rats) (Fig. 3). In rats treated with 6-OHDA there was a significant increase in spontaneous irritability, compared to the control \( U = 1, P = 0.0002 \). At the same time, 14 days of exposure to alternating magnetic field caused in 6-OHDA treated rats a significant \( U = 13, P = 0.0052 \) lowering of irritability, compared to that of the same animals before the beginning of exposure sessions. Sham exposure did not significantly influence irritability in 6-OHDA treated rats \( U = 48, P = 0.8798 \).

Alternating magnetic field or sham exposure does not significantly influence irritability of control animals \( U = 47.5, P = 0.8501 \) and \( U = 49.5, P = 0.9699 \), respectively (Fig. 3).

Fourteen days of exposure to alternating magnetic fields did not significantly influence spontaneous oral activity of 6-OHDA treated or control rats. It, however, significantly lowered the effect of the central dopamine \( D_1 \) receptor agonist SKF38393, with a decrease in number of chewing episodes both in 6-OHDA treated and control rats (Figs. 4 and 5).

In the group of 6-OHDA treated rats, changes of oral activity were more pronounced, compared to the control group, and already were evident at the lower concentrations of SKF 38393. A significant decrease in the number of episodes of chewing compared to values observed before the beginning of exposure sessions was noted in 6-OHDA treated rats after administration of 0.1 mg/kg ip of SKF 38393 \( U = 1, P = 0.0005 \) and 1.0 mg/kg ip of SKF 38393 \( U = 18, P = 0.047 \), whereas in control rats such significant reaction was observed only after administration of 0.3 mg/kg ip of SKF 38393 \( U = 5.5, P = 0.002 \) (Figs. 4 and 5).

Sham exposure did not cause significant changes of spontaneous oral activity or oral activity induced by SKF 38393 both in 6-OHDA treated and control rats (Fig. 6 and 7).

The intensity of catalepsy after administration of the specific dopamine \( D_1 \) receptor antagonist SCH

### TABLE 1. Manifestations of increased irritability in rats according to the Nakamura and Thoenen [1972] Score Scale Test

<table>
<thead>
<tr>
<th>Exogenous stimuli</th>
<th>Reactivities</th>
<th>Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blowing air on the back</td>
<td>No response</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Stiffening or tolerating</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Crying, jumping or running</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Panic jumping and running</td>
<td>3</td>
</tr>
<tr>
<td>Touching whiskers with a glass rod</td>
<td>No response</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Avoiding</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Stiffening, biting</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Severe attack, panic or menacing upright posture</td>
<td>3</td>
</tr>
<tr>
<td>Touching the back with a glass rod</td>
<td>No response</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Avoiding or escaping</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Crying, biting</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Severe attack, panic jumping</td>
<td>3</td>
</tr>
<tr>
<td>Holding the animal by the hand</td>
<td>No response</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Crying or escaping</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Upright posture or trembling</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Biting, difficult to hold, panic</td>
<td>3</td>
</tr>
</tbody>
</table>
23390 was significantly (H = 24.57, P < 0.001) lower in 6-OHDA treated rats, compared to control rats, both before and after exposure to alternating magnetic field (Fig. 8). The same tendency was observed in sham-exposed rats (H = 24.97, P < 0.001) (Fig. 9).

Fourteen days of exposure to alternating magnetic fields significantly increased (U = 11, P = 0.0274) catalepsy in 6-OHDA treated rats, but did not significantly influence catalepsy of control rats (U = 28, P = 0.6744) (Fig. 8).

After a cycle of repeated sham exposures, no significant changes of catalepsy intensity were observed in 6-OHDA treated rats (U = 18.5, P = 0.1563), whereas significant (U = 9, P = 0.0157) decrease in catalepsy intensity was observed in control rats (Fig. 9).

**DISCUSSION**

Results from this experiment showed significantly lowered striatal contents of DA and DOPAC in rats treated in neonatal period with 6-OHDA, as compared with control rats, and confirmed that their striatal dopamine system was destroyed. By this permanent chemical destruction of the dopamine system, we obtained an animal model of Parkinson's disease [Zigmond and Keefe, 1998]. The results also demonstrate the usefulness of the experimental model to evaluate the influence of alternating magnetic field on the reactivity of central dopamine D1 receptor.

A significantly higher number of chewing episodes, both spontaneous and after stimulation of SKF 38393, was observed before the beginning of experiment in the group of 6-OHDA treated rats as compared with control rats. This was probably due to an increased reactivity of central dopamine receptor D1 in the 6-OHDA treated animals, resulting from adaptation to lowered dopamine activity in the brain tissue of these animals.

Some indirect indicators of the increased reactivity of dopamine D1 receptors in 6-OHDA treated rats are the markedly increased irritability and much lower intensity of catalepsy after SCH 23390 treatment, observed before the beginning of the exposure in...
the group of 6-OHDA treated rats as compared with control rats.

The symptoms of increased reactivity of central dopamine receptor after 6-OHDA treatment observed in the experimental model used, may correspond to certain pathologic clinical symptoms (increased motor activity and involuntary movements) present in patients suffering from illnesses with etiology related to damage of central dopamine system, e.g., Parkinson's disease. Therefore evaluation of the influence of alternating low frequency magnetic fields on the reactivity of central dopamine receptors of experimental animals may indirectly explain the positive therapeutic effect observed in some clinical studies of Parkinsonian patients treated with very low intensity magnetic fields [Sandyk et al., 1992; Sandyk, 1994; Sandyk and Lacono, 1994a; Sandyk, 1995, 1996].

The significant reduction of the number of chewing episodes after stimulation with the D₁ receptor agonist SKF 38393, observed after completion of 14 days of exposure to alternating low frequency magnetic fields both in the groups of 6-OHDA treated and control animals, combined with an absence of such effect in sham exposure, indicates a distinct decrease in central dopamine receptor D₁ reactivity under the influence of the field.

Confirmation of the influence of low frequency magnetic fields on the reactivity of central dopamine
irritability of 6-OHDA treated animals after the completion of the set of 14 daily exposures to magnetic field, with absence of such reaction in control and sham exposed rats, may be another confirmation of the reduction of D1 receptor reactivity after exposure to low frequency magnetic field.

As has been mentioned before, a confirmation of the results of the present experiments may be seen in results of clinical studies, in which patients with damage of central dopamine and serotonin systems as a result of Parkinson’s disease were treated with alternating low frequency, very low intensity magnetic fields. It has been suggested that the therapeutic action of low frequency magnetic fields, especially on motor dysfunctions, was due to stimulation of nerve conduction in dopaminergic system in the frontal lobes, thalamus, and spinal cord (Sandvik and Iacono, 1994a; Sandvik, 1996). On the other hand, regression under the influence of low frequency magnetic fields of some clinical symptoms, that usually do not yield to the therapy with standard dopaminergic drugs such as micrographia and disorders of visual orientation, may indicate a share of other factors not directly connected with dopaminergic systems (Sandvik and Iacono, 1994b; Sandvik 1995).

In the opinion of some investigators (Reiter, 1993; Jacobson, 1994; Sandvik, 1996), such actions of low frequency magnetic fields may involve hormonal activity of pineal gland and serotonergic system, which strongly influence the activity of hypothalamic dopaminergic system and endogenous opioid system.

The complete understanding of the mechanisms of action of alternating low frequency magnetic fields on the activity of central dopaminergic system needs further detailed experimental and clinical research. Nevertheless, the present work is one of the first to present experimentally the influence of alternating low frequency magnetic fields on the reactivity of central dopaminergic systems.

CONCLUSIONS

1. Fourteen daily sessions of exposures to alternating low frequency magnetic field caused in adult male rats with neonatal 6-OHDA lesion of central dopamine system, a reduction of irritability and oral activity induced with SKF 38393 (a central dopamine D1 receptor agonist) and an increase of catalepsy induced by SCH 23390 (a central dopamine D1 receptor antagonist).

2. These results suggest that exposure to alternating low frequency magnetic field reduces the reactivity of central dopamine D1 receptors in rats.
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REFERENCES


