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Involvement of Nitric Oxide in the Mechanism of Analgesic Effect of ELF Magnetic Fields in Rats

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Taking into account the possible role of nitric oxide as a neurotransmitter the aim of the study was to estimate if nitric oxide is involved in ELF magnetic field-induced analgesia in male rats. The rats of the experimental group were exposed for 30 minutes to a sinusoidal magnetic field at a frequency of 40 Hz and a magnetic flux density 10 mT rms. The control rats were subjected to a sham-exposure. The analgesic effect was determined in both groups by means of a tail-immersion test before the exposure, and up to the 110th minute after the end of exposure. The experiments were also performed on N-Nitro-L-arginine methyl ester (l-NAME) and methylene-blue pretreated rats, in which both chemicals were administered into the right lateral brain ventricle. It was observed that exposure to an ELF magnetic field induced a significant analgesic effect in rats. This effect was prevented by icv. injection of l-NAME, an inhibitor of nitric oxide synthase, and of methylene-blue, an inhibitor of soluble guanylate cyclase. The results of the experiment suggest the involvement of nitric oxide in the mechanism of an ELF magnetic field-induced analgesia.

Key words: extremely-low-frequency magnetic field, tail immersion test, nitric oxide, N-nitro-L-arginine methyl ester — l NAME, methylene blue.

Introduction

The results of many experiments confirm that extremely-low-frequency (ELF) magnetic fields exert a strong and long-lasting analgesic effect in experimental animals. It seems that one of possible mechanisms of the analgesic effect of the magnetic field could be

related to the influence of the magnetic field on the synthesis and release of some neurotransmitters in the central and peripheral nervous system.

In previous studies [1, 2, 3] it was found that an antinociceptive effect of ELF magnetic fields is caused by a central opioid system stimulation, as it is blocked by prior i.p. injection of Naloxone (an opioid receptor antagonist).

It has been suggested that nitric oxide (NO), a novel and freely diffusable gaseous biological messenger molecule, is an interesting candidate for a potential retrograde neuronal messenger [4]. In the central nervous system NO is produced enzymatically by the conversion of L-arginine into L-citrulline catalysed by nitric oxide synthase, a calcium calmodulin-dependent enzyme. The glutamate-induced stimulation of N-methyl-D-aspartate receptors leads to calcium entry, which in turn activates nitric oxide synthase to produce NO. NO then diffuses freely across the cell membrane to reach target cells located postsynaptically, where it stimulates guanylate cyclase [4]. However, NO causes also modulation of the release of neurotransmitters at presynaptic nerve terminals [5, 6] and it has been shown to regulate the release of hormones and releasing factors [7]. Electrophysiological studies indicate that NO is involved in neuronal plasticity because it promotes long-term potentiation [8, 9] and long-term depression [10].

All these data suggest that NO may be an important endogenous molecule mediating fast and transient „alert" reactions to external stimuli. That is why the aim of the study was to evaluate in an animal model an analgesic effect of ELF magnetic fields by means of a tail immersion test, and to estimate the involvement of NO in the mechanism of the effect using inhibitors of nitric oxide synthase and soluble guanylate cyclase.

Material and Methods

The experiment was carried out on 42 male Wistar rats (age: 8 weeks, weight: 180-200 g) randomly divided into 7 groups of 6 rats each. All rats were housed in optimal environmental conditions (constant temperature and humidity of air), in a reverse 12h:12h light-dark cycle . They were fed with standard laboratory food and had free acces to water.

A week before the experiment animals from all groups were anaesthetized with Thiopental (administered intraperitonealy in a typical dose), and polyethylene cannules

were implanted into the right lateral brain ventricle and allowed to recover. On the day of the experiment, rats were placed into individual cages built of plexi-glass with a tail sticking out of the cage.

In the first group of rats under examination the whole body of the animal placed in specially designed cylindrical applicator was exposed for 30 minutes to a sinusoidal magnetic field at a frequency of 40 Hz and a magnetic flux density of 10 mT rms. The rats from the second — control group — were exposed to a sham-exposure, in which no magnetic field was produced in the applicator.

In order to estimate the involvement of nitric oxide in the mechanism of the analysed analgesic effect, the animals of the next two groups were pretreated with L-NAME (N-nitro-L-arginine methyl ester) and methylene blue, respectively. L-NAME is considered as an inhibitor of nitric oxide synthase, and methylene blue is an inhibitor of soluble guanylate cyclase. These enzymes are involved in the formation of physiological and regulatory function of nitric oxide. The removal of the analgesic effect of the magnetic field by inhibitors of both enzymes could confirm the involvement of nitric oxide in the mechanism of magnetic field-induced analgesia [11].

In the third group of rats, a solution of L-NAME (100 nmol in 5 μl of 0.9% solution of natrium chloride) was administered through a cannule to the right lateral brain ventricle and then immediately after the administration the same exposure as before was made. In the fourth group of rats, a solution of methylene blue (100 nmol in 5 μl of 0.9 % solution of natrium chloride) was administered through a cannule to the right lateral brain ventricle and then immediately after the administration the same exposure as before was made. In both cases, the control groups consisted of rats in which a sham-exposure was made.

The last, seventh group of animals made up a control one The rats of last, control group were pretreated with 5 μl of 0.9% solution of natrium chloride and then immediately exposed to magnetic field in order to evaluate the influence of solvent on the course of the observed reaction.

In all rats the analgesic effect was determined by means of tail immersion test [12], before and at 1, 2, 3, 4, 5 and 10 minute of exposure and at 5, 10, 15, 20, 35, 50, 80 and 110 minute after the end of exposure. The tail of animals was placed in hot water (56°C) and then time till the appearance of painful reaction (tail-flick reflex) was measured. The maximal time of exposure in hot water was 15 s. The tail-flick reflex latency time was considered as the index of analgesia. On the basis of measured values of time latency the

index expressing the percentage of maximal analgesic effect was calculated according to the equation: $T_{\%} = (T_x - T_0) : (15 - T_0) \times 100$.

The data obtained in particular groups were compared to each other and elaborated statistically according to ANOVA rang Kruskal-Wallis test, followed by post-hoc U Mann-Whitney test.

Results

The mean values of tail-flick reflex latency time and index $T_{\%}$ in consecutive time intervals of experiment in a group of rats exposed to magnetic field and in a group pretreated with 0.9% NaCl icv and subsequently exposed to magnetic field as compared to the group in which sham-exposure was made are presented in Fig. 1 and 2 respectively. A significant increase of tail-flick reflex latency time and index $T_{\%}$ values in both analysed groups was observed till the end of experiment as compared to the sham-exposed group.

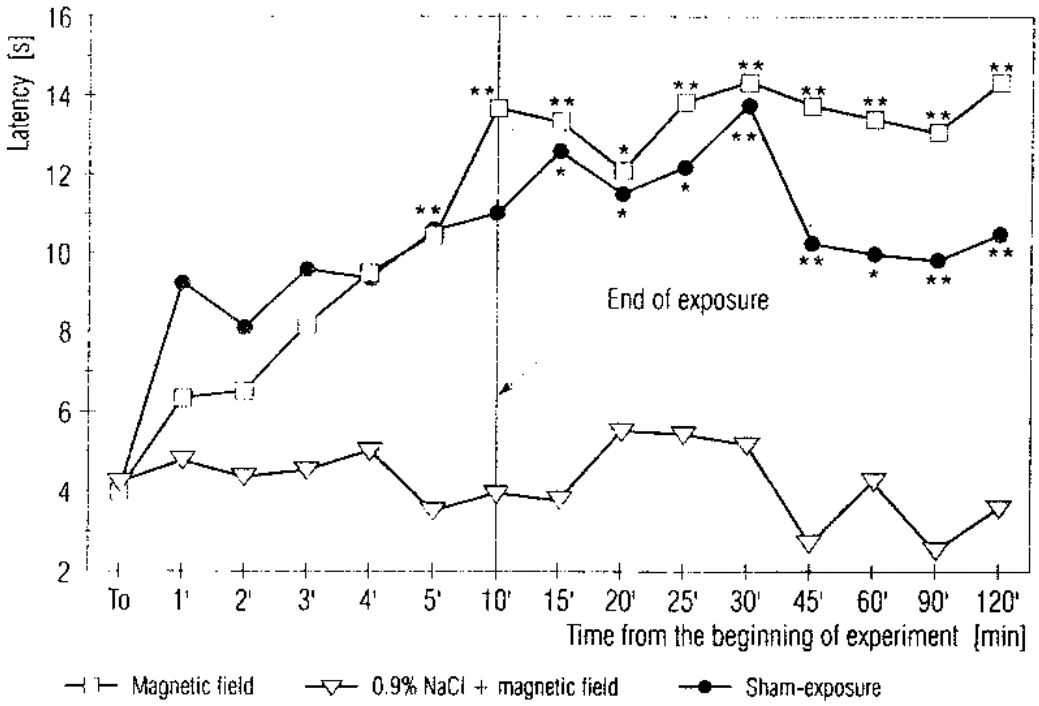

Figure 1. The values of tail-flick reflex latency time before the experiment (T_0) and in consecutive time intervals of experiment in group exposed to a magnetic field and 0.9% NaCl pretreated, subsequently exposed to a magnetic field group as compared to the sham-exposed group with statistical elaboration (* — $p < 0.05$, ** — $p < 0.01$)

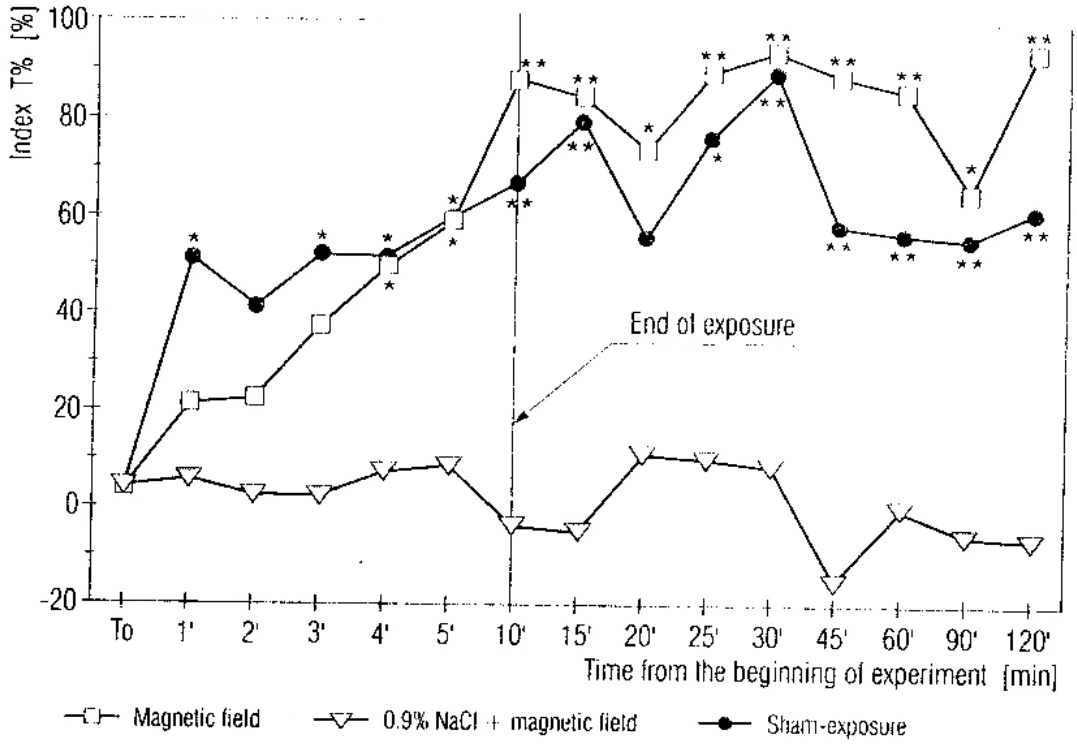

Figure 2. The values of index $T_{\%}$ in consecutive time intervals of experiment in group exposed to a magnetic field and 0.9% NaCl pretreated, subsequently exposed to a magnetic field group as compared to the sham-exposed group with statistical elaboration (* — $p < 0.05$, *** — $p < 0.01$)

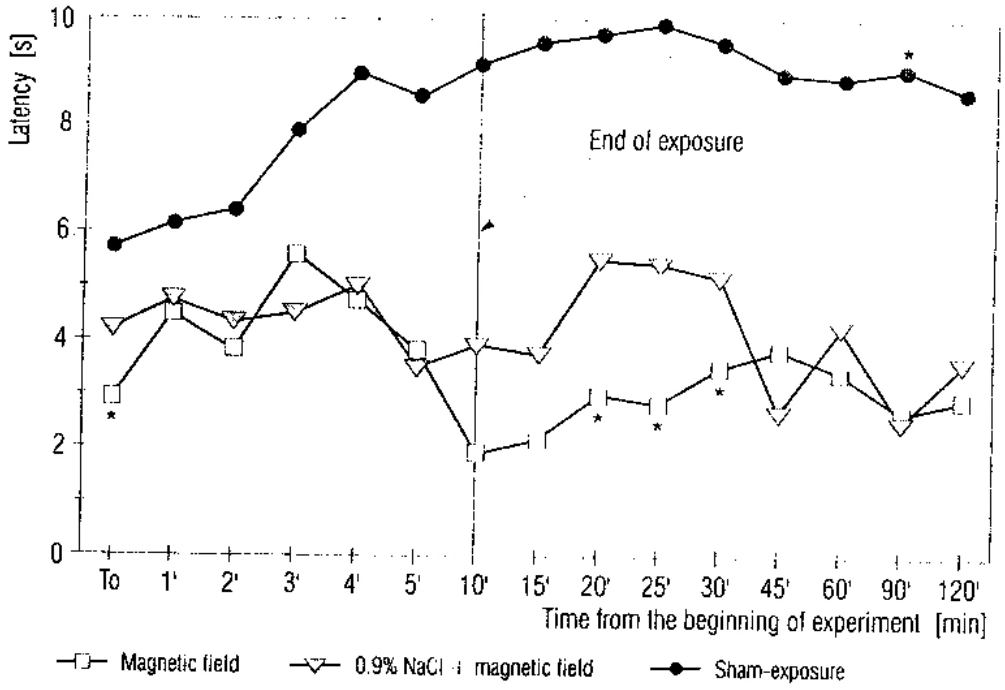

Figure 3. The values of tail-flick reflex latency time before the experiment (T_0) and in consecutive time intervals of experiment in NAME pretreated subsequently exposed to a magnetic field group and methylene blue pretreated subsequently exposed to a magnetic field group as compared to the sham-exposed group with statistical elaboration

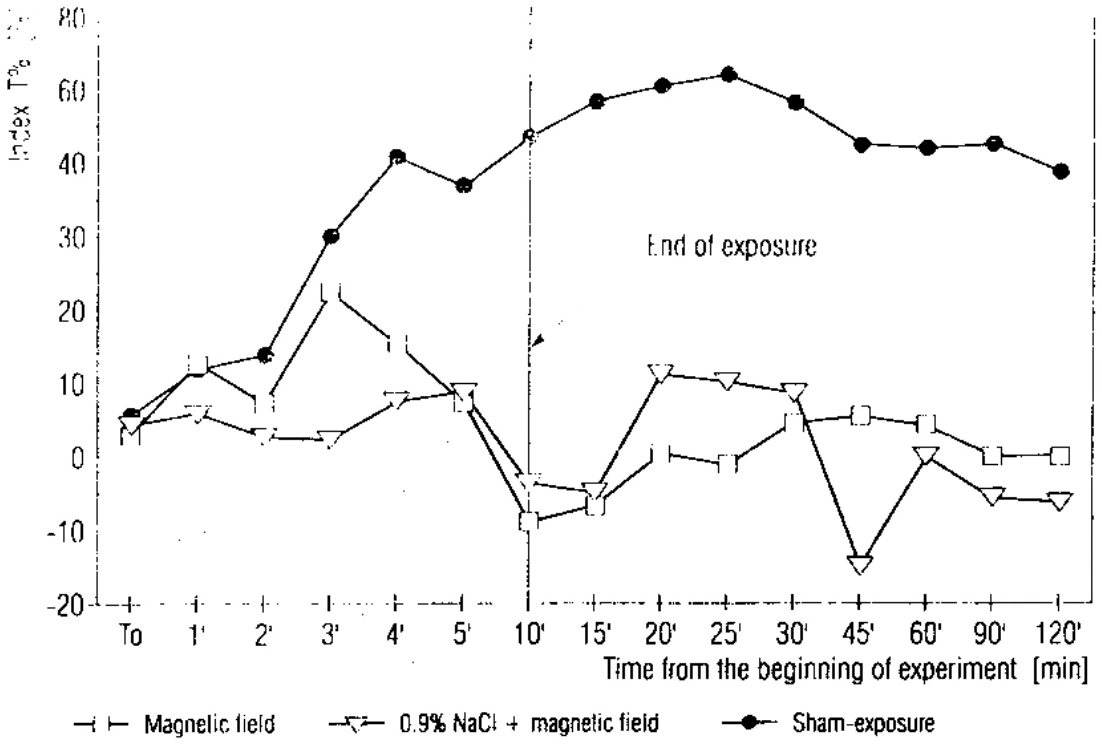

Figure 4. The values of index $T_{\%}$ in consecutive time intervals of experiment in NAME pretreated, subsequently exposed to a magnetic field group and methylene blue pretreated, subsequently exposed to a magnetic field group as compared to the sham-exposed group with statistical elaboration (* — $p < 0.05$)

The pretreatment of animals with NAME and methylene blue administered to the right lateral brain ventricle before the exposure blocked the observed analgesic effect of magnetic field as it is shown in Fig. 3 and 4 respectively. There were no significant differences in tail-flick reflex latency time and index $T_{\%}$ values between both pretreated and subsequently exposed groups comparing to sham-exposed group.

Discussion

The results of this experiment confirm our earlier observations that exposure of experimental animals to ELF magnetic field exerts a strong analgesic effect in rats. This effect is evident in different experimental models as in our previous study [1] analgesic effect was determined by means of a hot-plate test while in a present study a tail immersion test was used.

The results of this study revealed that magnetic field-induced analgesia was blocked by L-NAME (nitic oxide synthase inhibitor) and by methylene blue (soluble guanylate

cyclase inhibitor) pretreatment. Therefore we suggest that exposure of rat brain to magnetic field induces either increase in NO enzymatical production in nitric oxide synthase positive neurons in several regions of central nervous system or activates its messenger action by stimulation of guanylate cyclase in target cells located postsynaptically resulting in decrease of pain perception.

The involvement of nitric oxide and other oxygen reactive species in the mechanism of neural transmission resulting in modification of pain perception was confirmed in some experimental studies. In the study [13] the modulatory effect of 60 Hz magnetic field on opioid-induced antinociception in the land snail was significantly reduced by l-NAME, and significantly enhanced by the NO releasing agent S-nitro-N-acetylpenicillamide (SNP). It was observed [14] that intracerebroventricular or pretectal injection of L-Arginine (NO donor) induced in rats significant analgesia, which was blocked by L-NAME. The results of other authors suggest that NO and other free radicals may modulate both excitatory and inhibitory synaptic processes [15]. There are also evidences [16] that they disrupt long-term potentiation.

The mechanisms by which NO mediates long-term potentiation, long-term depression and neurotransmitter release are still unknown. That is why the potential explanation of the influence of magnetic field on these processes needs further research work.

Conclusions

1. Sinusoidal extremely low frequency magnetic field induces a significant, long-lasting analgesic effect in rats, confirmed by means of tail immersion test.
2. Nitric oxide is involved in the mechanism of magnetic field-induced analgesia.

References

[1] Cieślar G, Mrowiec J, Sieroń A, Plech A, Biniszkiewicz T. Zmiana reaktywności na termiczny bodziec bólowy pod wpływem zmiennego pola magnetycznego. Baln Pol 1994; 36(3-4): 24-8.

[2] Ossenkopp KP, Kavaliers M. Morphine-induced analgesia and exposure to low-intensity 60-Hz magnetic fields: inhibition of nocturnal analgesia in mice is a function of magnetic field intensity. Brain Res 1987; 418: 356-60.

[3] Prato FS, Carson JJ, Ossenkopp KP, Kavaliers M. Possible mechanisms by which extremely low frequency magnetic fields affect opioid function. FASEB J 1995 Jun; 9(9): 807-14.

[4] Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: Physiology, pathophysiology and pharmacology. Pharmacol Rev 1991; 43(2): 109-42.

[5] Lonart G, Wang J, Johnson KM. Nitric oxide induces neurotransmitter release from hippocampal slices. Eur J Pharmacol 1992; 220(2-3): 271-2.

[6] Pogun S, Kuhar MJ. Regulation of neurotransmitter reuptake by nitric oxide. Ann N Y Acad Sci 1994; 738: 305-15.

[7] Karanth S, Lyson K, McCaan SM. Role of nitric oxide in interleukin-2 induced corticotropin-releasing factor release from incubated hypothalami. Proc Natl Acad Sci USA 1993; 88: 3383-5.

[8] Bohme GA, Bon C, Stutzmann JM, Doble A, Blanchard JC. Possible involvement of nitric oxide in long-term potentiation. Eur J Pharmacol 1991; 199: 379-81.

[9] Haley JE, Wilcox GL, Chapman PF. The role of nitric oxide in hippocampal long-term potentiation. Neuron 1992; 8: 211-16.

[10] Shibuki K, Okada D. (1991) Endogenous nitric oxide release required for long-term synaptic depression in the cerebellum. Nature 1991; 349: 326-328.

[11] Meller ST, Dykstra C, Gebhart GF. Production of endogenous nitric oxide and activation of soluble guanylate cyclase are required for N-methyl-D-aspartate produced facilitation of the nociceptive tail-flick reflex. Eur J Pharmacol 1992; 214: 93-6.

[12] Janssen PAJ, Hiemegeers CJE, Donry JGH. The inhibitory effect of fentanyl and other morphine-like analgesics on the warm induced tail withdrawal reflex in rats. Arzneimittel Forsch 1963; 13: 502-7.

[13] Kavaliers M, Choleris E, Prato FS, Ossenkopp K. Evidence for the involvement of nitric oxide and nitric oxide synthase in the modulation of opioid-induced antinociception and the inhibitory effects of exposure to 60 Hz magnetic fields in the land snail. Brain Res 1998 Oct 26; 809(1): 50-7.

[14] Kumar A, Raghubir R, Srimal RC, Dhawan BN. Evidence for involvement of nitric oxide in pretectal analgesia in rat. Neuro Report 1993; 4: 706-8.

[15] Pellmar TC, Gilman SC, Keyser DO, Lee KH, Lepiński DZ, Livengood D, et al. Reactive oxygen species in neural transmission. Ann N Y Acad Sci 1994; 738: 121-9.

[16] Pellmar TC, Hollinden GE, Sarvey JM. Free radicals accelerate the decay of the long-term potentiation in field CA1 of guinea pig hippocampus. Neuroscience 1991; 44: 353-9.