Cent. Eur. J. Med. • 8(4) • 2013 • 517-522
DOI: 10.2478/s11536-012-0147-0

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Central European Journal of **Medicine**

Antiepileptic effects of short-wave radiation in hypogeomagnetic conditions

Research Article

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Received 13 May 2012; Accepted 4 October 2012

Abstract: The work was dedicated to investigation of dynamics of epileptic activity in conditions of extremely high frequency electromagnetic waves (EHF) radiation (7,1 mm, 0,1 mW/cm^2) upon focal epileptic activity. Epileptic activity was penicillin-induced (10,000 IU/ml) in the frontal region of the brain cortex in myorelaxed and artificially ventilated rats under acute experimental condition. Thermal effects were absent as far as absorbed dosage of energy did not exceed 0,1 J/ animal. It was established that preliminarily performed hypogeomagnetic period (3,0 h) with the inductivity of geomagnetic field at 5x10^{-8} Tesla caused the intensification of antiepileptic effects of EHF (15,0 min of exposure). It was pronounced in the form of decrease of foci intensity and significant reduction of life-span of foci – up to 115,3±13,4 minutes, which was both significant when compared with separate effects of hypogeomagnetic influence (187,3±12,5 min) and EHF (15,0 minutes of exposure) effect (164,2±12,5 minutes) ($P<0,05$). Besides, EHF (15,0 min) which was made after 3,0 h exposure to hypogeomagnetic influence suppressed generalized seizures in amygdalarly kindled rats.

Keywords: *Extremely high frequency electromagnetic waves (millimeter bandwith),* • *Hypogeomagnetic conditions* • *Focal epileptic activity* • *Kindled seizures*

1. Introduction

It was demonstrated that the influence of low-intensity electromagnetic radiation of extremely high frequency (EMR EHF) (wavelength 7,1 mm, power flux density 0,1 mW/cm^2) results in suppression of a focal form of epileptic activity (EpA), which was formed in brain cortex of cats [6,18]. Later special methods were developed for treatment of patients who suffered from epilepsy with the help of EHF influence [15].

The anti-epileptic effect may be based on the EHF radiation ability to increase the tone of serotonergic, opioid, noradregenic and neuromodulator systems [1,4], as well as histaminergic central mechanisms [5], the activation of which leads to suppression of convulsive activity of the brain [3,7,10]. EHF EMR provides significant anti-inflammatory effect, which manifests both in local [5] and systemic inflammation process [8]. Apparently EHF EMR-induced decrease production of phlogogenous compounds of endogenous origin may also cause suppression of convulsive activity, since cytokines such as alpha-factor of necrosis of tumors, interleukin-1-beta have a significant proepileptogenous effect [7,14].

The important feature in realization of effects of EHF EMR is their dependence on modulating the influence of static magnetic field [SMF], characteristics of which correspond to characteristics of Earth's geomagnetic field [5]. Also, while in independent use of EHF EMR the results mainly represented suppression of manifestation of inflammatory process, and on background of using SMF the effects of EHF had the opposite character [5]. These data allow the assumption that under conditions

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of weakened SMF the pronunciation of effects of EHF EMR might be modulated.

That is why the goal of this work was investigating of peculiarities of effects of EHF on models of focal epileptic activity under conditions of temporary containment of animals in hypogeomagnetic conditions.

2. Material and methods

2.1. Animals and surgery

76 Wistar rats in the age of 6-8 months were used. Animals were kept at standard conditions (constant temperature – 23°C, and relative humidity – 60%, 12 hrs dark/light cycles, standard diet and tap water were given ad libitum). Procedures involving animals and their care were conducted according to the university guidelines that are in compliance with international laws and policies [European Community Council Directive 86/609, OJ L 358, I, December 12, 1987; National Institute of Health *Guide for Care and Use of Laboratory Animals*, US National Research Council, 1996].

3. Experimental procedures

3.1. Model of focal epileptogenesis and EEG registration

Animals were anaesthetized with ether narcosis and tracheostomy, trepanation of the skull. Frontal and parietal areas of brain cortex were exposed as a result. Electrodes were fixed in nasal bones. Registering nichrome electrode (0,25 mm in diameter) were fixated to cranial bones with the help of fast-hardening dental cement. Animals were maintained on artificial breathing after injection of d-tubocurarin chloride (0,25 mg/kg, i.p.) (“Orion”, Finland). Rats have been fixed in stereotaxic frame and all points of pressure were infiltrated with 0,25% novocaine solution (“Darnitsa”, Ukraine) every 1,0 h. Observation started in 2,5 hours after ether narcosis effect ceased.

After transection of dura mater a focus of EpA was created, using application of filter paper (2x2 mm) soaked with fresh solution of sodium salt of benzylpenicillin (10.000 IU/ml) on frontal areas of brain cortex. Focal EpA was registered monopolarily, using computer electroencephalographic system (“DX-5000”, Ukraine).

Intensity of EpA was expressed in relative units, 1 unit being the medium spike amplitude of 1,0 mV with discharge frequency of 1 per minute. To estimate the average level of EpA an epoch of generating discharges with duration of 1 minute was taken. Indices of EpA power have been recalculated dynamically in control group and have been taken as 100% pertained to corresponded data in experimental groups of animals. Duration of existence of foci was determined starting from the first till the last spike [7].

3.1.1. *EMR EHF and hypogeomagnetic influences*

“Ramed-Expert” (Ukraine) was used to generate EMR EHF with working wavelength 7,1 mm, radiation frequency 42,3 GHz, power flux density 0,1 mW/cm^2, modulation frequency 10±0,1 Hz. Taking into consideration the experimental conditions, absorbed dosage of emitted energy did not exceed 0,05-0,1 J per animal, when 15,0 min exposition of EMR was performed. Hence, EMR did not induce thermal effect, which is quite characteristic for an action of such type of irradiation [1,5,6]. Hence, under such conditions the absence of thermal effects of such influence was expected [18].

Influence on zone of localization of the foci was performed just before the application of epileptogen. Duration of the influence was 5,0 and 15,0 min. Hypogeomagnetic conditions were created by using two-layered permalloy chamber (thickness of plates being 1,5 mm), with 1,5 mm thick copper plates placed between the layers [16]. The remaining magnetization of the chamber was 5x10 $^{-8}$ Tesla, which resulted in decreasing geomagnetic field inductivity at least 100 times. Rats were kept in the chamber for 3 hours prior to the observation. All influences have been performed from 9.00 till 12.30 AM.

The animals of control group were kept in analogous chambers, which had plastic walls, and later the EMR EHF source was projected on focal area without affecting the foci.

3.1.2. *Experimental groups*

Experimental observation with focal epileptogenesis modeling was performed in the following groups:

1. Control group – application of penicillin solution on brain cortex after i.p. injection of 0,5 ml of 0,9% NaCl (6 rats);
2. Two groups of animals with the use of EMR EHF, performed during 5,0 min (6 rats) and 15,0 min (6 rats) before epileptogen application (6 rats);
3. Group of rats that were contained in hypogeomagnetic conditions with subsequent modeling of EpA foci (6 rats)
4. Two group of rats with creation of foci after preceding containment of the animals in hypogeomagnetic conditions (7 rats in each group). Subsequent exposure to EMR EHF with duration of 5,0 and 15,0 min was performed.

3.2. Kindled seizures

Animals were anesthetized with i.p. Nembutal (35,0 mg/kg), and bipolar constantan electrodes (distance between electrodes 0,3 mm) were implanted stereotaxically into the left basolateral amygdala, according to the coordinates of rat brain atlas [11]: AP=-2.2; L=4.7; H=8.5. Electrodes were fixed to the skull using dental cement.

Kindling started 10-14 days after the surgery. Electrical stimulation (ES) of the amygdala was performed by means of universal electrostimulator ESU-2 (FSU). Rectungle electrical stimuli (one-phase) were applied with the frequency 60 Hz, duration 1 ms. The duration of ES was 1 s. For kindling procedure those intensities were used for each animal individually, followed by appearance of after discharges in the EEG. On average the index mentioned was 20-50 mcA. Animals received 15-42 trials daily (once per day) to reach stage 4-5 of the convulsions (see below). Severity of convulsions was evaluated according to the scale of [12] immediately after ESs: stage 0 - behavioral arrest: animal stops normal exploratory or grooming behavior for a few seconds poststimulation. Stage 1 - facial and jaw clonus: rhythmic mouth movements or facial twitches. Stage 2 - head nodding: clonic contractions of neck and shoulder muscles; rearing without forelimb movements. Stage 3 - unilateral forelimb clonus: rhythmic ipsilateral or contralateral forelimb movements. Stage 4 - bilateral forelimb clonus: rhythmic movements of both forelimbs with rearing. Stage 5 - rearing and falling seizure: animal loses control of postural reflexes, e.g. righting reflex.

Animals which responded with 3-5 stage convulsions on two subsequent ES were used for observation of the effects of the compounds. Influence with EMR EHF was made during 15,0 min upon rostral part of head timely immobilized animals in plastic cages. The same immobilization was made in control group of animals. Testing ES was made in 0,5 h from the moment of influence with EMR. Similar hypogeomagnetic influences along with EHF EMR have been performed from 9.00 till 12.30 AM.

Thereby next experimental groups have been used in this chapter of investigations:

1. Control one – kindled rats with 15,0 min immobilization (10 rats);
2. kindled rats which have been kept under hypogeomagnetic conditions (10 rats);
3. kindled rats which have been influenced with EMR EHF (9 rats)
4. kindled rats which have been influenced with EMR EHF after hypogeomagnetic influence (9 rats).

4. Statistics

Intensity of foci of EpA was estimated using one-way ANOVA method (Analysis of Variance) with consequent processing using Neuman-Keuls criteria. Ratio of $P<0,05$ was taken as reliable differences. Estimating duration of existence of the foci was done using criteria of Wilcoxon-Whitney. Seizure severity in kindled rats was estimated using Kruscal-Wallis test.

5. Results

5.1. Focal epileptogensis

5.1.1. Control group

First spike discharges appeared in 1,5-6,0 minutes after application of the benzylpenicillin solution (10,000 IU/ml) in rats of control group, and were registered in the application zone. The amplitude and frequency of discharges were growing during next 10-15 minutes of observation, reaching up to 1,2-1,8 mV and 23-45 discharges per minute correspondingly. The power of EpA was 56,4±8,7 relative units in 20 minutes from the moment of starting of epileptogen application. Stable level of EpA was registered in the control group during next 20-45 min, after which during 30-60 min frequency and amplitude of discharges decreased, and they disappeared. The lifespan of focal EpA was 211,2±22,3 min.

5.1.2. Groups exposed to EHF EMR

Preliminarily 5,0-min exposure to EMR EHF was followed by appearance of first spike discharge with latency of 2,0 – 7,5 min, and in 15,0 min from the starting of epileptogen application spikes were observed with amplitude of 0,7-1,3 mV and frequency of discharges of 17-36 per min. The power of focal EpA was 32,1± 5,4 relative units at this period of time ($P<0,05$) (Figure 1). The stable decreasing of amplitude and frequency of discharges was characteristic for the next period of observation, and significant differences with the corresponded data in control group of animals persisted till the end of observation (Figure 1). The lifespan of focal EpA was 182,4± 14,6 min, and it had no differences from the analogous index in control group ($P>0,05$).

Preliminarily performed 15,0 – minutes EMR EHF influence was followed by the increasing of the latency of first spike discharges up to 3,0 – 8,0 min and the net decreasing of EpA power by 44,7% pertained to similar index in control group was registered on 5th min of observation ($P<0,05$) (Figure 1). Significant differences between experimental and control groups have been

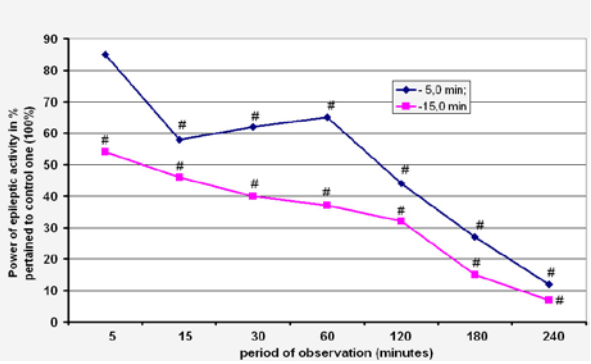

Figure 1. Effects of EMR EHF on foci of EpA, created by application of penicillin solution (10,000 IU/ml) on brain cortex of rats.
Abscissa: time from the moment of appearance of epileptic discharges in the focus; ordinate: intensity of foci in % pertained to corresponded in control group of rats (intra-abdominal injection of 0,5 ml of NaCl solution), which was taken as 100%.
#- P<0,05 vs control group (ANOVA+Neuman-Keuls test).

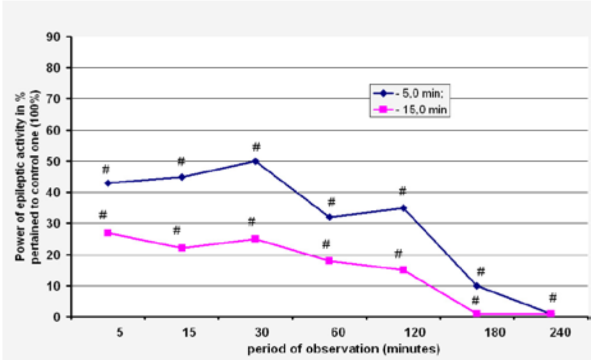

Figure 2. Effects of EMR EHF on penicillin-induced (10,000 IU/ml) foci of EpA in brain cortex of rats which have been affected by hypogeomagnetic factor.
Notes: those groups of rats, which have been influenced by EMR EHF without hypogeomagnetic influence have been taken as corresponded control groups. All the rest notes are the same as on Figure 1.
#- P<0,05 compared to corresponding indicator in the control group (ANOVA+Neuman-Keuls).

registered till the end of observation and focal life-span was 164,2± 12,5 min (P>0,05).

5.1.3. Group exposed to hypogeomagnetic condition

Application of benzylpenicillin solution (10,000 IU/ml) upon the brain cortex of rats was followed by appearance of the first spike potentials in 5-16 min after introduction of the epileptogen. During next 10-17 min steady increase of EpA power was registered, which reached 45,1±6,3 relative units in 15 min after appearance of the first potentials. Steady character of EpA was observed during next 25-45 min, with the consequent decrease of frequency and amplitudes of spike potentials. The life-span of focal EpA was 187,3±16,4 min, which wasn't differ from the corresponding data in the group of control rats (211,6±22,3 min) (P>0,05).

5.1.4. Group exposed to EHF EMR-hypogeomagnetic conditions

The latent period of first penicillin-induced discharges registered in rats that had been influenced with 5,0 minutes EMR EHF was 3,5-9,0 min. The power of EpA was reduced by 55,6% when compared with the analogous index determined on 5th minute from the start of epileptogen application in the group of rats which have been exposed to 5,0 min EMR EHF, but without hypogeomagnetic influence (P<0,05) (Figure 2). Significant differences between groups were registered till the end of observation (Figure 2). The lifespan of focal EpA was 165,1± 14,2 min, which had no difference from the data registered in control group (P>0,05). 15,0 min duration of EMR EHF influence was followed by 3,7 times fold falling down of power of EpA when compared with the similar data in the rats exposed to EMR EHF during 15,0 min, but without hypogeomagnetic influence, and this effect was registered on the 5th min from the moment of epileptogen application (P<0,05) (Figure 2). Significant differences between groups were noted till the end of observation, and life-span of focal EpA was 115,3±13,4 min, which was significantly less than in rats which were affected by EMR EHF during 15,0 min (P<0,05).

5.2. Kindled seizures

Investigation of severity of kindled seizures revealed the absence of effects, which were caused by both hypogeomagnetic influence and EMR EHF (15,0 min) (Table 1). Similar action of EMR EHF which was made after hypogeomagnetic influence was followed by the net reduction of seizure severity (P<0,01).

6. Discussion

Acquired data demonstrated that generating interictal (spike) activity influence with EMR EHF in the model

Table 1. The effects of hypogeomagnetic influence and EHF EMR on kindled seizures in rats.

Fractions	Number of rats with the seizures of score						P vs the control for seizure score
	0	1	2	3	4	5	
1.Control	0	0	0	2	5	3	-
2.Hypogeomagnetic influence	0	0	1	0	6	3	<0,01
3.EHF EMR	0	0	1	2	5	1	<0,01
4.Hypogeomagnetic influence+ EHF EMR	0	2	3	2	2	0	< 0,01 (P vs 3 <0,05)

of penicillin-induced cortical focal EpA provides anti-epileptic effect, which depends on duration of the EMR influence. Under the influence of EMR EHF frequency and amplitude of spike discharges decreased, and there was tendency towards decrease of duration of foci EpA life-span. That fact is in congruence with earlier presented data, which were gained during experimental observations performed on cats [6].

On kindled model of epilepsy, which was induced by amygdalar ES exposure to EHF EMR was followed by the net reduction of seizures manifestations induced via testing ES.

Deprivation of rats from the influence of geomagnetic field hampered the development of penicillin-induced foci of EpA, and this fact proves the ability of hypogeo-magnetic factor to affect brain activity. Consequent use of EMR EHF precipitated significant antiepileptic effect, which was more pronounced than one observed under conditions of separate EMR EHF influence. Kindled seizures have not been affected by hypogeomagnetic influence, but consequent EMR EHF was followed by rising of seizure – protective effects of EMR.

Hence, two main facts have been established:

- antiepileptic action of EHF EMR;
- heightening of antiepileptic effects of EHF EMR by arts exposure to hypogeomagnetic influence.

Both effects have been registered both on focal and kindled seizures models and apparently were not de-pended upon specific targets being involved in primarily accepting of EMR.

Considering possible mechanisms, which are in charge for EMR SHF antiepileptic effects, it should be mentioned that involvement of serotoninergic and histaminergic receptor-depended mechanisms in the development of antinociceptive effects of EMR EHF has been established [1,5]. Besides, transcutaneus action of EMR EHF activated endogenous opioid system and precipitated analgesic action [2]. Thus, similar systems might be of importance for the suppression of focal and kindled seizures.

It might be also assumed that deprivation of magnetic field or "hypogeomagnetic influence" which was resulted in the net increasing of antiepileptic EHF EMR action re-sembles well known phenomena of receptor hypersen-sitivity induced by deafferentation of neuronal structures [7]. Just to explain the increased EHF EMR antiepileptic action it is of worth to mention that hypogeomagnetic influence (below 12 microTesla, during two months) re-sulted in decreasing of NO- induced peroxidation [9,13]. This effect might be helpful for promoting antiepileptic action of EMR EHF as far as oxidative stress is followed by facilitation of epileptogenesis [17].

Established phenomena of increasing of antiepilep-tic action of EHF EMR by hypogeomagnetic influence which is realized at level of receptor sensitivity control needs further development. Namely, low penetration of EHF EMR through biological tissues [4,5] needs expla-nation. Most probable hypothesis is confined to the role played by magnetic field – sensitive molecules, which are involved in the receptor sensitivity control of those neuromodulative systems which are involved in the con-trol of neuronal brain excitability. Those fur-free parts of skin along with the eyeball surface which are exposed to EMR might be on the first place with absorption of EHF EMR and consequent realization of it's action [18].

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