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Original article

Suppressive effect of electromagnetic field on analgesic activity of tramadol in rats

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Abstract

The electromagnetic fields (EMFs) have been shown to alter animal and human behavior, such as directional orientation, learning, pain perception (nociception or analgesia) and anxiety-related behaviors. The aim of this study was to evaluate the influence of electromagnetic fields of high-frequency microwaves on pain perception and anti-nociceptive activity of tramadol (TRAM) – analgetic effective in the treatment of moderate to severe acute and chronic pain states.

Electromagnetic fields exposures of a)1500 MHz frequency and b) modulated, 1800 MHz (which is identical to that generated by mobile phones) were applied. Paw withdrawal latency (PWL) to thermal stimulus was measured in vehicle or tramadol (TRAM) treated animals before and after 30, 60 and 90 minutes from injections.

The differences in the level of pain (PWL) between control group and rats exposed to EMF alone in three measurements, were not observed. Tramadol alone significantly increased PWLs to thermal stimulus in comparison to vehicle results at 30 ($p < 0.001$) and 60 minutes ($p < 0.05$) after drug injection. EMF exposure of both frequencies transiently suppressed analgesic effect of tramadol, significantly reducing paw withdrawal latency in animals treated with this drug at 30 minutes from the drug injection.

Key words: rats, electromagnetic field, pain perception, tramadol

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Introduction

We began a series of studies on the effects of electromagnetic fields on the analgesic activity of drugs with affinity to opioid receptors. Currently, the potential impact of high-frequency microwaves; effects on drugs acting in the central nervous system is still underestimated. Bearing in mind the absence of appropriate legislation, there is a threat to public health, especially among patients undergoing treatment with opioid analgetics.

Tramadol hydrochloride is synthetic analgetic broadly used in the treatment of moderate to severe pain which possesses weak opioid agonist properties and effects on monoaminergic transmission, but its mechanisms of action have not been fully elucidated (Minami et al. 2007).

The opioid drugs have affinity to μ-opioid receptors by the indirect influence on some ionotropic receptors eg. calcium channels. There is no assertion in the available literature that the electromagnetic field (EMF) can directly influence the molecular equilibrium among ionotropic transmembrane receptors.

The drug tramadol (synthetic opioid derivative) has been chosen due to its extensive worldwide availability and popularity in the paint treatment.

Tramadol binds to μ-opioid receptors with low affinity and inhibits reuptake of monoamines in the central nervous system.

These actions are believed to primarily contribute to its antinociceptive effects. Therefore, it is conceivable that there might be other action site(s) of tramadol, but little is known about other antinociceptive mechanisms or pharmacological actions of tramadol or M1 metabolite. The effects of tramadol and its M1 metabolite (0.1-100 μM) was tested on human recombinant neurotransmitter-gated ion channels, including glycine, γ-aminobutyric acidA (GABAA), and N-methyl-d-aspartate (NMDA) receptors, expressed in Xenopus oocytes (Hara et al. 2005). Tramadol and M1 metabolite did not have any effects on glycine receptors.

GABAA receptors were significantly inhibited only at large concentrations (100 μM).

NMDA receptors were inhibited in a concentration-dependent manner. Tramadol and M1 metabolite inhibited the glutamate-concentration response curve without changing the half-maximal effective concentration indicating a noncompetitive inhibition. This study suggests that glycine receptors do not provide the antinociceptive effect of tramadol and that the inhibition of GABAA receptors at large concentration might correlate with convulsions.

The inhibitory effect on NMDA receptors may contribute to the antinociceptive effect of tramadol at relatively large concentrations.

Tramadol, (1RS, 2RS)-2-dimethylaminomethyl-1--(3-methoxyphenyl)cyclohexanol, is a centrally-acting analgetic used clinically for the treatment of postoperative and cancer pain. Tramadol binds μ-opioid receptors with low affinity and inhibits reuptake of monoamines such as norepinephrine and serotonin in the central nervous system (CNS), resulting in the activation of the descending inhibitory system (Raffa et al. 1992, Driessen et al. 1993).

These actions are believed to primarily contribute to tramadol's antinociceptive effect.

Its major active metabolite, O-desmethyltramadol (M1 metabolite), also has analgesic potency. M1 metabolite is a demethylated compound of tramadol. Similar to the parent molecule, M1 metabolite has an agonistic effect on the μ-opioid receptor but with a higher affinity than tramadol and inhibits monoamine reuptake (Goeringer et al. 1997). Although the administration of opioids is accompanied by several adverse effects, including respiratory suppression, hypnosis, dependence, and abuse potential, these are uncommon with the administration of tramadol at equipotent doses (Lehmann 1997). Additionally, tramadol is effective in treating acute pain alone, whereas tricyclic antidepressants which are classic monoamine reuptake inhibitors, are generally ineffective (Monks et al. 1999). Furthermore, although opioids have little effect on touch-evoked pain (allodynia), tramadol has been proven effective against allodynia (Sindrup et al. 1999).

Shupak et al. (2004), also observed that sensory thresholds to non-painful warmth were not affected by the pulsed magnetic field (CNP) exposure. Thus, the analgesic effect may be quite specific, and not due to some kind of general anesthesia, which is an important consideration for potential clinical use.

Materials and Methods

Animals

Experiments were performed on 80 male Wistar rats weighing 220-250 g purchased from Center of Experimental Medicine (Medical University of Bialystok, Poland). Animals were housed in cages in a standard 12:12 h light/dark cycle. Water and food were available *ad libitum* until rats were transported to the laboratory approximately 1 h before experiments. Animals presenting any symptoms of illness were excluded from the study. All behavioral testing was performed between 9:00 am and 4:00 pm and the animals were used only once. Animal care and handling procedures were in accordance with the guidelines of the International Association for the Study of Pain

(IASP) on the use of animals in pain research and the protocol was approved by the IV Local Ethics Committee for Animal Experimentation in Warsaw, No. 02/2011, dated 14 January 2011. Two experiments were performed; the first, for evaluation of the effect of 1500 Mhz non-modulated EMF, and the second, for evaluation of the effect of pulsed 1800 Mhz on the nociceptive threshold to thermal stimulus. In each experiment 40 animals were divided into 4 main groups: 1) vehicle, 2) exposed to the EMF without medication, 3) receiving the drug without exposure of the EMF, and 4) receiving the drug and exposed to the EMF.

Exposure to electromagnetic fields and drug administration

Animals were placed in Plexiglas enclosures positioned centrally, 1 meter from the EMF source, and exposed to a far-field antenna for 15 minutes.

In the first experiment it was a non-modulated electromagnetic field of the frequency of 1500 MHz, an effective electric field with a value of 90 V/m and an effective magnetic field of 0.24 A/m.

In the second experiment rats were exposed to the far-field antenna at 1800 MHz with the additional modulation which was identical to that generated by mobile phone GSM 1800; the value of effective electric field was 20 V/m and effective magnetic field value was 0.05 A/m.

Tramadol hydrochloride (Tramal®, Grunenthal, Germany), further named TRAM, was used in the form of injectable solution in *aqua pro injectione.*

Immediately before EMF exposure rats were inraperitoneally injected with TRAM in the 20 mg/kg dose, or *aqua pro injectione* in the vehicle group, in the 1 ml/kg volume.

PWLs to thermal stimulus were measured in vehicle or tramadol (TRAM) treated animals before and after 30, 60 and 90 minutes from injections.

Thermal nociception

Assessment of thermal nociception was performed using plantar test by Hargreaves method (Hargreaves et al. 1988). To measure paw withdrawal response to noxious heat stimuli (pain inducer), each animal was placed in a Plexiglas chamber on a glass plate located above a light box. Radiant heat from a Model 336 Analgesia Meter (IITC, Inc./Life Science Instruments, Woodland Hills, CA, USA) was applied by aiming a beam of light through a hole in the light box through the glass plate to the middle of the plantar surface of the left hind paw. When the animal lifted its foot, the light beam was turned off. The length of time between the start of the light beam and the foot lift was defined in seconds as the paw withdrawal latency (PWL). Each trial was repeated 2 times at 5-min intervals. A cut-off time of 20 s was used to avoid paw tissue damage.

Statistical methods

For each of the main groups (1500 and 1800 MHz) the vehicle latency (PWL) time was identified, based on repeated measurements which were averaged (mean). Subsequently, the latency time after 30, 60 and 90 seconds were measured for all groups. The two-way analysis of variance ANOVA was applied. Distribution analysis (Shapiro-Wilk test) showed that the distribution is normal. Significance of differences between the groups was verified by Bonferroni post-test (GraphPad Prism version 5.03).

Results

The results of statistical analysis are presented graphically in Fig. 1a,b. EMF alone, of both 1500 and 1800 MHz frequencies, did not influence pain perception threshold according to thermal stimulus. There were no differences in PWLs between control group and rats exposed to EMF in three measurements. TRAM significantly increased PWLs to thermal stimulus in comparison to vehicle results at 30 ($p < 0.001$) and 60 minutes ($p < 0.05$) after drug injection. EMF exposure of both frequencies (1500 MHz, $p < 0.05$; 1800 MHz, $p < 0.001$) significantly reduced analgesic effect of TRAM at 30 min after drug injection (Fig. 1a,b). This effect disappeared at 60 min time point.

Discussion

Our study showed that single, 15-minute exposure to EMF of microwave high-frequencies did not influence pain threshold but induced transient suppression of tramadol antinociceptive effect in rats. The presented study of the mutual effects of 1500 MHz and 1800 MHz electromagnetic field and synthetic opioid drug – tramadol, on pain perception in rats was performed for the first time.

In other studies performed on rat models authors obtained conflicting results on the effects of EMFs on analgesia induced by morphine and other compounds.

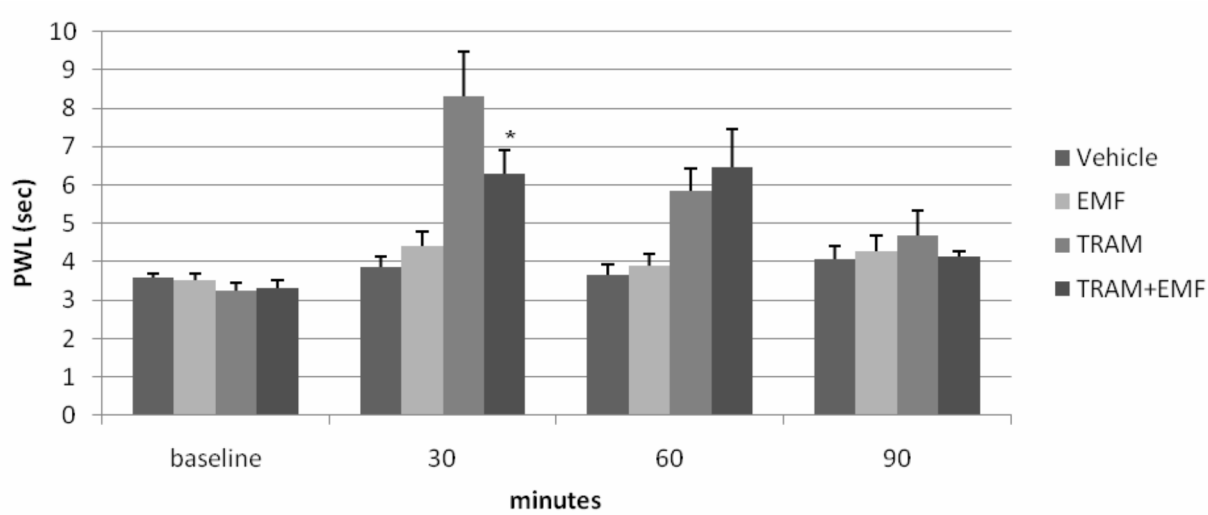

Fig. 1a. 1500 MHz.

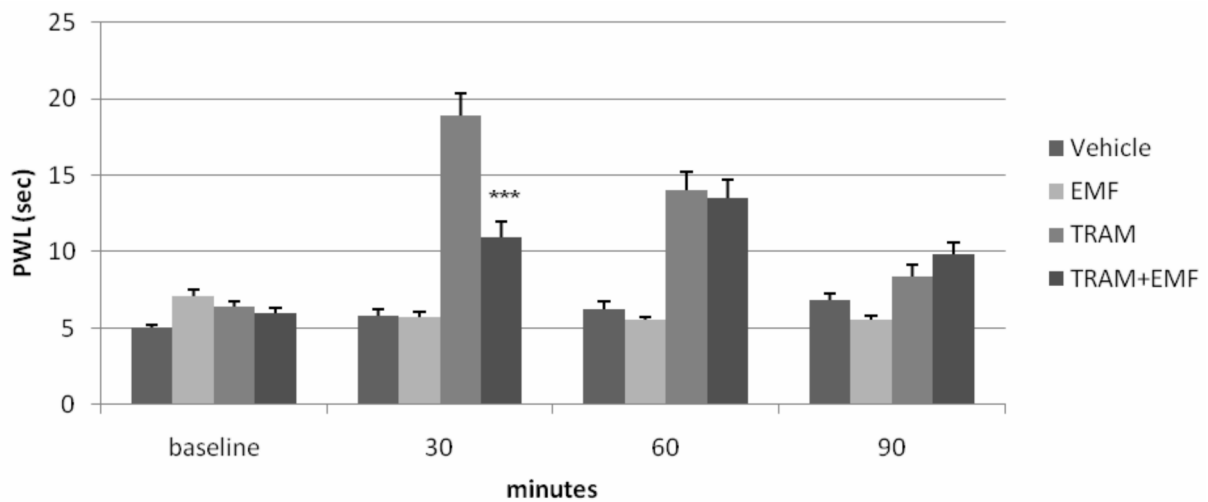

Fig. 1b 1800 MHz

Fig. 1 a,b. Paw withdrawal latency to radiant heat (pain inducer) in irradiated (EMF, EMF+ TRAM) and non-irradiated (vehicle, TRAM) groups of rats before, and after 30, 60 and 90 minutes from tramadol (TRAM, 20 mg/kg ip.) or *aqua pro injectione* injection (vehicle). Results are shown as mean ± SEM. *** – $p < 0.001$; * – $p < 0.05$ TRAM vs TRAM+EMF

Dixon and Persinger (2001) showed that weak magnetic field reduces analgesia induced by morphine (4 mg/kg) or by L-NAME (agmatine) which is inhibitor of nitric oxide synthase (50 mg/kg). On the other hand, Martin et al. (2004) reported that weak complex magnetic fields potentiate analgesia induced by morphine (4 mg/kg) and agmatine (10 mg/kg). For our study we chose tramadol, widely used weak opioid drug effective in the treatment of moderate to severe pain states. Our results correspond to Dixon and Persinger (2001) findings.

Tramadol was found to noncompetitively inhibit N-methyl-d-aspartate (NMDA) receptors at clinical concentrations and to inhibit γ-aminobutyric acidA receptors at large concentrations. NMDA receptor inhibition may contribute to the antinociceptive property of tramadol.

To date, the only neurotransmitter or neuromodulator known to exhibit antagonist activity at NMDA receptors at a non-glycineB site is agmatine. In rat hippocampal neurons agmatine has been shown to block NMDA channels because of the interaction between agmatine's guanidine group and the channel pores (Yang and Reis 1999). Furthermore, in neurons and PC12 cells agmatine blocks the induction of excitotoxicity by glutamate (Zhu et al. 2003). Agmatine also acts as an agonist at imidazoline receptors, inhibits nitric oxide synthase and interacts with alpha-2-adrenoceptors (Berkels et al. 2004). Feng et al. (2002) have suggested that agmatine reduces brain

injury in neonatal rats exposed to hypoxia and ischemia as a result of its inhibitory effect on nitric oxide synthase. Because of its multiple interactions with receptors and enzymes agmatine represents a neurotransmitter that could increase in concentration during conditions such as cardiac arrest to prevent a variety of injurious brain activities.

It is also possible that agmatine may participate in the potential interaction of tramadol with electromagnetic radiation.

In the available literature we have found a few papers presenting some positive and some negative biological effects of cellular phones electromagnetic fields. Gerner et al. (2010) have observed increased protein synthesis by various human cell lines in *vitro* exposed to 1800 MHz radiofrequency mobile phone EMF. It may reflect an increased rate of protein turnover stemming from protein folding problems caused by the interference of radio-frequency EMFs with hydrogen bonds. It was also observed by Franzelitti (2010) that high-frequency EMF (GSM 1800 MHz) induced transient DNA damage in the human trophoblast cell line.

According to Nylund and Leszczynski (2004) 900 MHz radiation exposure of human endothelial cell line resulted in altered expression of some cytoskeletal proteins. Study of Stankiewicz et al. (2006, 2011) revealed immunostimulatory influence of 900 MHz exposure on human lymphocytes and monocytes *in vitro* in cultures activated with mitogens and on murine endothelial cells proliferation *in vitro*.

Based on the above observations, such changes caused by EMF influence on the electrochemical environment of the cell, resulting in binding ions or dipoles, may be accompanied by alterations in the conformation of molecular entities (such as lipids, proteins and enzymes) in the cellular structures.

Bearing in mind that most of the cellular structures are electrically charged and ion transfer is broadly involved in pain perception, it may be assumed that MF/EMF possesses the potential to influence opioid drugs' effects via altering the 3-D structure of water dipoles surrounding receptors and their physical-chemical properties, such as hydration and salvation ability, surface tension, pH, and electro-conductivity. The role of ions in the regulation of cell structure and function is determined by potential changes in the water dipoles' structure and behavior.

Bearing in mind all facts presented above, we formulated a hypothesis that water molecules surrounding particular structures such as trans-membrane receptors (especially voltage-gated ion channels) play an extremely important role in the detection of magnetic/electromagnetic field signals, as well as in signal transduction cascades.

Conclusion

High frequency electromagnetic fields of 1500 and 1800 MHz when applied alone, did not influence pain perception threshold to thermal stimulus, however it presented an unwanted effect diminishing analgesic action of tramadol.

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