

Suppressive effect of the pulsed electromagnetic field 1800 MHz on analgesic action of tramadol in animal model of persistent inflammatory state

PAWEŁ BODERA¹, WANDA STANKIEWICZ¹, BOŻENA ANTKOWIAK², MAŁGORZATA PALUCH², JAROSŁAW KIELISZEK¹, JAROMIR SOBIECH¹, ROBERT ZDANOWSKI^{2,3}, ANDRZEJ WOJDAS⁴, EWA SKOPIŃSKA-RÓŻEWSKA^{1,5}

¹Department of Microwave Safety, Military Institute of Hygiene and Epidemiology, Warsaw, Poland

²Department of Pharmacology and Toxicology, Military Institute of Hygiene and Epidemiology, Warsaw, Poland

³Department of Regenerative Medicine, Military Institute of Medicine, Warsaw, Poland

⁴Department of Otolaryngology, Military Institute of Medicine, Warsaw, Poland

⁵Pathology Department, Center for Biostructure Research, Warsaw Medical University, Warsaw, Poland

Abstract

The aim of this study was the evaluation of the influence of a physical factor (electromagnetic field – EMF) on the analgesic efficacy of tramadol in rats with complete Freund's adjuvant (CFA) induced paw inflammation. Complete Freund's adjuvant significantly increased sensitivity of inflamed paws to thermal stimulus. Tramadol (TRAM) administered to rats with paw inflammation significantly increased paw withdrawal latency at 30, 60 and 90 minute after drug injection in comparison with non-treated group. Electromagnetic field exposure did not markedly influence nociceptive threshold to thermal stimulus, but significantly decreased paw withdrawal latency in rats treated with tramadol at 30 and 60 minutes from the drug injection.

Conclusion: Electromagnetic field exposure of 1800 MHz frequency and 20 V/m intensity (similar to that of cell phone), did not influence nociceptive threshold to thermal stimuli, however it transiently decreased analgesic efficacy of tramadol in rats with CFA-induced paw inflammation.

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

(Centr Eur J Immunol 2012; 37 (1): 25-29)

Introduction

We began a series of studies on the effects of electromagnetic fields (EMF) on the analgesic activity of opioid drugs. Currently, the issue is not moving a question of the potential impact of the high-frequency effect of the drugs in the central nervous system, which in the absence of appropriate legislation is the threat for the health of the population of patients treated with analgesic agents.

In our study, we investigated the influence of EMF on parallel use of the opioid drug tramadol with endogenous regulation of complete Freund's adjuvant (CFA)-induced

inflammatory pain, which is connected with adaptive T cell immune response.

Inflammatory pain is characterized by an increased response to mechanical or heat stimuli which are normally perceived as only mildly painful, occurring during mechanical or thermal hyperalgesia. After tissue injury, inflammatory mediators are produced in the circulation (e.g. bradykinin) and by local resident cells (e.g. tissue macrophages and dendritic cells). The inflammatory response is amplified by migration of leucocytes into the inflamed tissue, by production of cytokines, chemokines, growth factors (e.g. nerve growth factor), and tissue acidification.

Correspondence: Wanda Stankiewicz, Department of Microwave Safety, Military Institute of Hygiene and Epidemiology, Warsaw, Poland, e-mail: wanda.stankiewicz@gmail.com

Intra-plantar injection of CFA was used to study inflammatory pain in rodents.

Analgesia, regulation of the autonomic nervous system and neuroendocrine activities, respiration and gastrointestinal motility, which are well known critical physiological functions, depend on endogenous opioid peptides and their receptors, prevalent in the organism [1, 2].

The seven transmembrane G-protein coupled receptor (GPCR) superfamily which contains three classes of opioid receptors named μ MOR, δ DOR and κ KOR, mediate the endogenous biological activities of the opioid neuropeptides, endorphins, enkephalins and dynorphins [2, 3].

Receptors of selected opioids, which are involved in the modulation of the pain networks, also influence certain parameters of both the innate and adaptive immune system [4, 5].

Immune cells, particularly under stressful conditions, produce endogenous opioids such as enkephalins locally at the site of inflammation, completely apart from cells of the central and peripheral nervous system [6].

Analgesia is elicited by opioids, acting on the peripheral sensory nerve terminals. It also exerts a range of immunomodulatory effects on T cell responses [7].

The peripheral blood lymphocytes have opioid receptors on their membranes, which was demonstrated during numerous studies [8].

Activation of the T cell through the T cell receptor (TCR) significantly upregulates both the percentage of T cells that express DOR as well as the number of DORs expressed by each T cell [9]. The range of immunomodulatory effects on T cell responses, which are exerted by DOR agonists include, but are not limited to, T cell proliferation, cytokine production, chemotaxis, thymic T cell selection, opioid mediated modulation of the release of chemokines and expression and/or functionality of chemokine receptors on leukocytes [10-15].

The opioid drugs have affinity to μ -opioid receptors, by the indirect influence on some ion receptors e.g. calcium channels. There is no assertion in the available literature that the EMF can directly influence the molecular equilibrium among ionotropic transmembrane receptors.

Bearing in mind that there is an increasing appreciation for the ability of cytokines to directly impact excitatory neuronal function, resulting in spontaneous activity and pain facilitation, we take into allowance further assessment of such molecules in the received blood samples.

Tramadol (TRAM) is an analgesic drug that is used broadly worldwide, but its mechanisms of action have not been fully elucidated [11]. This drug was initially thought to have acted primarily through the activation of μ -opioid receptors and the inhibition of monoamine reuptake.

Nevertheless, the major advances have recently been made in our understanding of the physiology and pharmacology of GPCR signaling.

Tramadol has been shown to affect GPCRs, including muscarinic acetylcholine receptors and 5-hydroxytryptamine receptors. The effects of TRAM on monoamine transporters, GPCRs, and ion channels are presented in several studies which have also shown that GPCRs and ion channels are targets for analgesics and anesthetics.

The attention of many research groups has focused on the influence of weak EMF exposure on the Ca^{2+} transmission through the transmembrane ion channel [12].

The interaction site in the cell is still unknown but the cell membrane area and the DNA have been suggested [13, 14].

Other observable bioeffects which have been reported concerning EMF exposure include changes in cell membrane function, metabolism, cellular signal communication, cell stress, and cell death.

The contribution of animal studies is worth touching upon in order to understand the cellular and molecular components of neuropathic pain generation in the context of increased T-type calcium channel activity. Changes in the expression of transmitters and receptors in the sensory ganglia are triggered by nociceptor sensitization, including increased prevalence of cells with ongoing activity [15].

The aim of the present study was the evaluation of the influence of a physical factor (EMF) on the analgesic efficacy of TRAM in rats with CFA induced paw inflammation.

Material and methods

Animals

Experiments were performed on 50 male Wistar rats (five groups of 10 animals each) weighing 220-250 g purchased from Center of Experimental Medicine (Medical University of Białystok, Poland). Animals were housed in cages on 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 a.m. and 4.00 p.m. 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.

Thermal nociception

Assessment of thermal nociception was performed using plantar test by Hargreaves method [16]. To measure paw withdrawal response to noxious heat stimuli, 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 as the paw withdrawal latency (PWL). Each trial was repeated 2 times at 5-min intervals for each paw. A cut-off time of 20 s was used to avoid paw tissue damage.

Drugs

Complete Freund's adjuvant (heat killed *Mycobacterium tuberculosis* suspended in paraffin oil, 1 mg/ml) was purchased from Sigma-Aldrich. Persistent inflammation was elicited with CFA injected into the plantar surface of the left hind paw in 0.1 ml volume 24 hours before EMF exposure and drug application.

Tramadol hydrochloride (Tramal®, Grünenthal, Germany) was used in the form of injectable solution in aqua pro injection, 20 mg/kg body mass, by intraperitoneal route.

Experimental procedures

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

The far-field region is the most important, as this determines the antenna's radiation pattern. Also, antennae are used to communicate wirelessly over long distances, so this is the region of operation for most antennas.

Rats were exposed in pairs. The propagation vector of the incident wave was parallel to the long axis of the animal's body. Each pair was given 15 min exposure. The same num-

ber of rats was sham-exposed with no voltage applied to the field generator. In order to assess the influence of EMF exposure on pain threshold to thermal stimulus and thermal hyperalgesia, PWLs were measured in control saline-treated animals and after inflammatory state induction. During particular EMF exposure two rats were placed in plexiglas enclosures positioned centrally, 1 meter from the EMF source. Immediately before EMF exposure rats were injected with TRAM in the 20 mg/kg dose or vehicle in the 1 ml/kg volume. Paw withdrawal latency to thermal stimulus was measured 30, 60 and 90 min after TRAM injection.

Statistical methods

For statistical evaluation of the results two-way analysis of variance ANOVA was applied. Significance of differences between the groups was verified with a Bonferroni test (GraphPad Prism software).

Results

Performed analysis of variance revealed, that variation among columns means is significantly greater than expected by chance. The p -value is < 0.0001 , considered as very significant.

The results are presented graphically on Fig. 2. Complete Freund's adjuvant injection alone elicited thermal hyperalgesia and highly significant PWL reduction ($p < 0.01$ at 30 and 60 min, $p < 0.001$ at 90 min) in comparison to rats non-treated with CFA.

Electromagnetic field exposure did not markedly influence nociceptive threshold to thermal stimulus, both in baseline and after CFA measurements. In animals with paw inflammation TRAM significantly increased PWLs to thermal stimulus in all three measurements ($p < 0.001$). Electromagnetic field exposure in TRAM treated rats signifi-

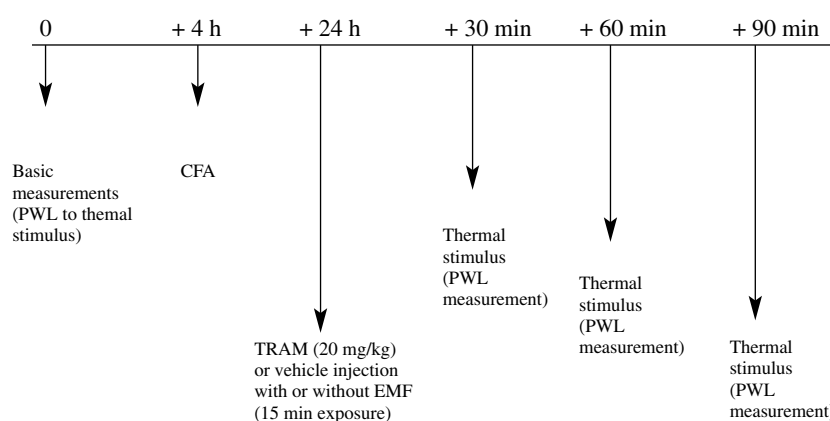


Fig. 1. Scheme of experiment in rats with paw inflammation induced with complete Freund's adjuvant (CFA)

PWL – paw withdrawal latency, EMF – electromagnetic field, TRAM – tramadol

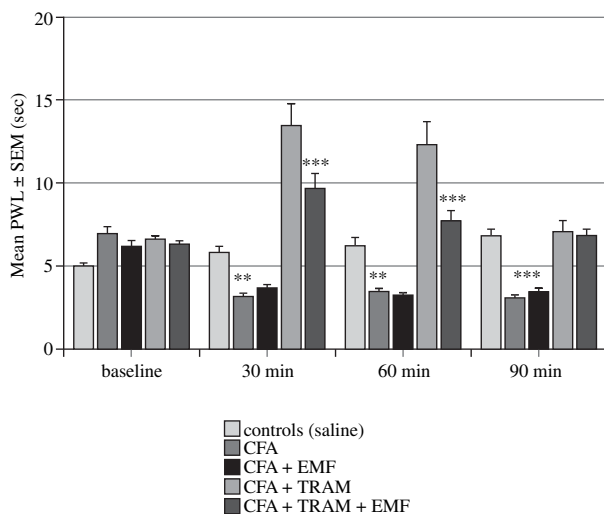


Fig. 2. Paw withdrawal latency (PWL) to radiant heat (pain inducer) in irradiated (CFA + EMF, CFA + TRAM + EMF) and non-irradiated (saline, CFA, CFA + TRAM) groups of rats before and after 30, 60, and 90 min from tramadol (TRAM, 20 mg/kg, *i.p.*) or vehicle injection

** $p < 0.01$, *** $p < 0.001$

cantly reduced PWL at 30 and 60 min from the drug injection ($p < 0.001$).

Discussion

Opioid peptides play an important role in mediating the expression of analgesia and a variety of behavioral, physiological, endocrinological and immunological functions. They have also been identified in invertebrates, including land snails, where they have also been shown to subserve a variety of basic functions including that of antinociception.

This analgesia presents the day–night variations and simultaneously the analgesic effects of EMFs have presented maximum analgesia and inhibitory effects at night [17].

Studies employing specific agonists for the various opioid receptor classes demonstrated in mice that EMFs differentially suppressed the analgesia mediated by various opioid receptors, but not that mediated by others, such as sigma receptor-targeted ligands [18].

Bao *et al.* (2006) raised the possibility of a direct effect of EMFs on opioid receptor numbers/binding activity and/or their functional activity [19]. It was showed that 4-day magnetic exposure increased the levels of β -endorphin and substance P in the hypothalamus of rats.

The large superfamily of seven transmembrane-spanning G protein-coupled receptors gathers the opioid receptors [17].

Inhibition of neurotransmitter release, as a result of activation of opioid receptors, is considered as the mechanism underlying analgesia. G-protein subunits interact with mul-

tipule cellular effector systems, inhibiting adenylyl cyclase and voltage-gated Ca^{2+} channels and stimulating G protein-activated inward rectifying K^{+} channels and phospholipase C_{β} (PLC_{β}) and inhibiting neuronal activity [20].

Opioids in low concentrations can also have stimulatory effects on neurotransmission, increasing the rate of neuronal firing, which have been associated with changes in m-opioid receptor-G protein coupling and G signaling to adenylyl cyclase. These effects of opioids have been linked to an increase in Ca^{2+} conductance and a decrease in K^{+} conductance [21].

The binding sites for opioid drugs are divided into ‘classical’ opioid sites, which are blocked by naloxone or the other opioid antagonists, and ‘non-classical’ opioid receptors, which can not be inhibited by classical opioid antagonists. In spite of the many properties of G protein coupled receptors (GPCR) sharing the ‘non-classical’ binding sites, such as saturability and ion sensitivity, their anomalous pharmacology is still unexplained.

Being aware that cytokines regulate the acute and long-term changes in voltage-gated and ligand-gated ion channels, we would like to posit a new idea that there is a feedback between cytokines and the subunits of ion channels. The understanding of this issue is that the external influence on ion channels (EMF) would be reflected by an elevated cytokine level, which could be easily investigated in an irradiated rats model, where the inflammation is artificially generated (CFA).

Besides crossing the cell membrane through voltage-gated channels from the outside, Ca^{2+} can enter the cytosol from internal stores in response to many environmental signals. Several G protein-linked receptors activate a pertussis toxin (PTX)-insensitive G protein to stimulate the activity of membrane-bound phospholipase C_{β} (PLC_{β}). This enzyme specifically cleaves phosphatidylinositol-4,5-bisphosphate (PIP_2) in the membrane to generate two important second messengers, diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3) [22].

Inositol 1,4,5-trisphosphate triggers Ca^{2+} release from internal stores by binding to and opening specific channels found along the surface of the endoplasmic reticulum (ER).

Activity of various enzymes such as calcium-sensitive or calcium/calmodulin-dependent kinases and phosphatases, is dependent on signals which are transduced throughout the cell [23]. Activity of protein kinase C (PKC) is partially dependent on the binding of Ca^{2+} to its regulatory domain.

Another Ca^{2+} -sensitive kinase, calcium/calmodulin-dependent kinase II (CaMKII), has been linked to the phosphorylation of various channels and receptors [24].

We would like to formulate a hypothesis that water molecules surrounding particular structures such as transmembrane receptors (especially voltage-gated as ion channels) play an extremely important role in the detection of magnetic/electromagnetic field signals, as well as in signal trans-

duction cascades. Our efforts addressed the evaluation of the contribution of EMF in a change of an opioid drug's effect on pain which is simultaneously associated with the inflammation. Electromagnetic field is perceived as capable of influencing some changes in the microenvironment around and within the cell, as well as in the cell membranes.

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 magnetic field/electromagnetic field (MF/EMF) possess 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 solvation ability, surface tension, pH, and electroconductivity.

The changes, which are causing by EMF 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.

The role of ions in the regulation of cell structure and function is determined by potential changes in the water dipoles' structure and behavior.

Conclusion

The presented study of the effects of EMF on pain perception in rats with persistent paw inflammation was performed for the first time.

Electromagnetic field exposure of 1800 MHz frequency and 20 V/m intensity (similar to cell phone), did not markedly influence nociceptive threshold to thermal stimuli, however it transiently decreased analgesic efficacy of TRAM in rats with CFA-induced paw inflammation.

References

1. Tegeder I, Geisslinger G (2004): Opioids as modulators of cell death and survival-unraveling mechanisms and revealing new indications. *Pharmacol Rev* 56: 351-369.
2. McCarthy L, Wetzel M, Sliker JK, et al. (2001): Opioids, opioid receptors, and the immune response. *Drug Alcohol Depend* 62: 111-123.
3. Williams JT, Christie MJ, Manzoni O (2001): Cellular and synaptic adaptations mediating opioid dependence. *Physiol Rev* 81: 299-343.
4. Vallejo R, de Leon-Casasola O, Benyamin R (2004): Opioid therapy and immunosuppression: a review. *Am J Ther* 11: 354-365.
5. Roy S, Wang J, Kelschenbach J, et al. (2006): Modulation of immune function by morphine: implications for susceptibility to infection. *J Neuroimmune Pharmacol* 1: 77-89.
6. Shahabi NA, McAllen K, Sharp BM (2003): Phosphorylation of activating transcription factor in murine splenocytes through delta opioid receptors. *Cell Immunol* 221: 122-127.
7. Shahabi NA, McAllen K, Sharp BM (2006): Delta opioid receptors stimulate Akt-dependent phosphorylation of c-jun in T cells. *J Pharmacol Exp Ther* 316: 933-939.
8. Shen H, Aeschlimann A, Reisch N, et al. (2005): Kappa and delta opioid receptors are expressed but down-regulated in fibroblast-like synoviocytes of patients with rheumatoid arthritis and osteoarthritis. *Arthritis Rheum* 52: 1402-1410.
9. Nguyen K, Miller BC (2002): CD28 costimulation induces delta opioid receptor expression during anti-CD3 activation of T cells. *J Immunol* 168: 4440-4445.
10. Bénard A, Boué J, Chapey E, et al. (2008): Delta opioid receptors mediate chemotaxis in bone marrow-derived dendritic cells. *J Neuroimmunol* 197: 21-28.
11. Minami K, Uezono Y, Ueta Y (2007): Pharmacological aspects of the effects of tramadol on G-protein coupled receptors. *J Pharmacol Sci* 103: 253-260.
12. Bauréus Koch CL, Sommarin M, Persson BR, et al. (2003): Interaction between weak low frequency magnetic fields and cell membranes. *Bioelectromagnetics* 24: 395-402.
13. Lednev VV (1993): Possible mechanisms for the effect of weak magnetic fields on biological systems: correction of the basic expression and its consequences. In: *Electricity and Magnetism in Biology and Medicine*. Blank M (ed.). San Francisco Press, San Francisco, CA; 550-552.
14. Blanchard JP, Blackman CF (1994): Clarification and application of an ion parametric resonance model for magnetic field interaction with biological systems. *Bioelectromagnetics* 15: 217-238.
15. Koltzenburg M, Bennett DL, Shelton DL, McMahon SB (1999): Neutralization of endogenous NGF prevents the sensitization of nociceptors supplying inflamed skin. *Eur J Neurosci* 11: 1698-1704.
16. Hargreaves K, Dubner R, Brown F, et al. (1988): A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain* 32: 77-88.
17. Waldhoer M, Bartlett SE, Whistler JL (2004): Opioid receptors. *Ann Rev Biochem* 73: 953-990.
18. Bauréus Koch CL, Sommarin M, Persson BR, et al. (2003): Interaction between weak low frequency magnetic fields and cell membranes. *Bioelectromagnetics* 24: 395-402.
19. Bao X, Shi Y, Huo X, Song T (2006): A possible involvement of beta-endorphin, substance P, and serotonin in rat analgesia induced by extremely low frequency magnetic field. *Bioelectromagnetics* 27: 467-472.
20. Prinster SC, Hague C, Hall RA (2005): Heterodimerization of G protein-coupled receptors: specificity and functional significance. *Pharmacol Rev* 57: 289-298.
21. Crain SM, Shen KF (2000): Antagonists of excitatory opioid receptor functions enhance morphine's analgesic potency and attenuate opioid tolerance/dependence liability. *Pain* 84: 121-131.
22. Suh BC, Hille B (2007): Regulation of KCNQ channels by manipulation of phosphoinositides. *J Physiol* 582: 911-916.
23. Fan RS, Jácomo RO, Jiang X, et al. (2005): G protein-coupled receptor activation rapidly stimulates focal adhesion kinase phosphorylation at Ser-843. Mediation by Ca²⁺, calmodulin, and Ca²⁺/calmodulin-dependent kinase II. *J Biol Chem* 280: 24212-24220.
24. Dupont G, Houart G, De Koninck P (2003): Sensitivity of CaM kinase II to the frequency of Ca²⁺ oscillations: a simple model. *Cell Calcium* 34: 485-497.