

Electromagnetic Millimeter Wave Induced Hypoalgesia: Frequency Dependence and Involvement of Endogenous Opioids

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Millimeter wave treatment (MMWT) is based on the systemic biological effects that develop following local skin exposure to low power electromagnetic waves in the millimeter range. In the present set of experiments, the hypoalgesic effect of this treatment was analyzed in mice. The murine nose area was exposed to MMW of “therapeutic” frequencies: 42.25, 53.57, and 61.22 GHz. MMWT-induced hypoalgesia was shown to be frequency dependent in two experimental models: (1) the cold water tail-flick test (chronic non-neuropathic pain), and (2) the wire surface test (chronic neuropathic pain following unilateral constriction injury to the sciatic nerve). Maximum hypoalgesic effect was obtained when the frequency was 61.22 GHz. Other exposure parameters were: incident power density = 13.3 mW/cm², duration of each exposure = 15 min. Involvement of δ and κ endogenous opioids in the MMWT-induced hypoalgesia was demonstrated using selective blockers of δ - and κ -opioid receptors and the direct ELISA measurement of endogenous opioids in CNS tissue. Possible mechanisms of the effect and the perspectives of the clinical application of MMWT are discussed. Bioelectromagnetics 29:284–295, 2008. © 2007 Wiley-Liss, Inc.

Key words: mice; millimeter wave therapy; experimental pain; endogenous opioid subtypes

INTRODUCTION

Millimeter wave treatment (MMWT) is a therapeutic modality that originated in the former USSR in the 1960s. In this therapy, a local exposure of the skin (2–3 cm in diameter) of humans or animals to low power (<30 mW/cm²) electromagnetic waves of millimeter range results in systemic effects that include hypoalgesia, immunomodulation, anti-inflammatory, and repair-stimulating actions [Rojavin and Ziskin, 1998; Pakhomov et al., 1998a]. Since that time MMWT has been in wide use in Eastern European countries. Millions of patients with different diseases ranging from skin and bone diseases to various types of cancer and neurological disorders were reported to be treated successfully with MMWT alone or in combination with other treatments [Betskii et al., 2000; Pakhomov, 2000]. However, (a) the insufficiency of quantitative studies utilizing “blind” experimental conditions, (b) the ambiguous indications for the treatment, and (c) the absence of a reasonable theory that would explain systemic effects of the MMWT are the main reasons why this method of treatment is practically unknown to Western medicine.

In previous experiments in animals and in human volunteers, using “blind” experimental conditions, we reproduced and quantitatively evaluated one of

the systemic effects of MMWT—antinociception [Radziewsky et al., 1999, 2004a; Rojavin et al., 2000]. The 61.22 GHz MMWT-induced hypoalgesia was statistically significant ($P < 0.05$) in all the employed experimental tests. These included the cold pressor test in human volunteers; the hot-water tail-flick test (hTFT) in mice (experimental model of acute pain); the cold-water tail-flick test (cTFT; experimental model of chronic non-neuropathic pain); and the chronic constriction injury (CCI) to the sciatic nerve (experimental model of chronic neuropathic pain). Further, using the cTFT in mice, in which the effect was most pronounced and was comparable to that of 1 mg/kg of morphine, we also determined that MMWT-induced

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hypoalgesia is power dependent; pronounced after exposure of the most densely innervated skin areas; can be obliterated by denervation of the area of exposure, or by pretreatment with naloxone, the non-selective antagonist of opioid receptors (OR); and can not be reproduced by equivalent laser heating of the area of exposure [Rojavin et al., 2000; Radziewsky et al., 2004a].

We have concluded that MMWT-induced hypoalgesia is a specific and reproducible phenomenon that involves the peripheral and central nervous systems, as well as endogenous opioids. In the present set of experiments we further studied indications and mechanisms of action of MMWT. The aims of the study were: (1) to determine whether MMWT-induced hypoalgesia depends on the frequency of the MMW used in experimental models of chronic non-neuropathic and neuropathic pain, and (2) to define what types of OR are predominantly involved in the development of MMWT-induced hypoalgesia.

METHODS

Mice

All experiments were carried out on male Swiss-Webster mice (18–20 g in the beginning of experiments; obtained from Taconic, Germantown, NY). The animals were housed five per cage under standard laboratory conditions with water and food available ad libitum. The experimental procedures were approved by the Temple University Institutional Animal Care and Use Committee.

Pain Models

Cold-water tail flick test (cTFT). While gentle cooling induces a cool sensation, rapid cooling induces pain that has some characteristics of chronic non-neuropathic pain, which usually accompanies local inflammation and involves signaling from different set of nociceptors:

- Superficial myelinated fibers become functionally blocked at temperatures $<5^{\circ}\text{C}$ [Paintal, 1965]. Unlike other acute pain experimental stimuli (heat, electric shock), cold activates mostly deep unmyelinated C-fiber receptors (located along the cutaneous veins) [Fruhstorfer and Lindblom, 1983; Klement and Arndt, 1992], that are “silent” without local damage or inflammation.
- The spectrum of molecular receptors that are involved in cold-induced nociception also has some particularities: the transient receptor potential (TRP) super family was shown to play an important role in temperature sensing [Gordon, 2005]. It is important

that TRP was shown to change its functional characteristics in response to exposure to cytokines and other inflammatory mediators [Tominaga, 2004].

- Furthermore, as was shown by Rainville et al. [1992] in human volunteers, cold evokes tonic aching pain with higher estimates of unpleasantness (which is similar to that of chronic pain), and therefore was considered to be an experimental equivalent of a chronic non-neuropathic type of pain.

Thus, the cTFT that was originally designed to quantitatively assess the antinociceptive actions of drugs with opioid-mediated effects [Pizziketti et al., 1985] was used to evaluate chronic non-neuropathic pain in mice.

Previously we described the cTFT in detail [Radziewsky et al., 2004a]. The key elements of the method are: (1) “Blind” experimental setting: two individuals do the testing. One investigator applies the treatment, while the other one makes the measurements, not knowing which treatment the animal received. (2) Test is conducted four times: once before the MMWT (Baseline), and three times (with 15-min intervals) after the treatment. During the entire time of the experiment mice were restrained in a specially designed plastic tube with an opening in the front of the restrainer for breathing and exposure purposes. Results of the post-treatment tests for each mouse are averaged and converted to percent of each mouse baseline response. (3) Animals were assigned to MMWT or Sham groups (paired) based on the latency of their response in the test before MMWT (baseline). As a result, the initial average time of the resistance to the cold-induced pain was approximately equal in the Sham and MMWT groups.

Wire surface test following unilateral chronic constriction injury to the sciatic nerve

Surgery. For our experiments we slightly modified the surgical method described by Bennett and Xie [1988] for rats. In brief, mice were anesthetized and the right common sciatic nerve was exposed at the level of the mid-thigh, 2–3 mm proximal to its trifurcation. The nerve was freed of surrounding tissue and two ligatures (10–0; Alcon, Sinking Spring, PA), separated by 1 mm, were tied loosely around the nerve. The sciatic nerve was constricted to 1/3–1/2 of its diameter. The wound was then closed in layers. For sham operations, all of the above was performed, except for the nerve ligation.

Wire surface test (WST). It has been reported that after a CCI to the sciatic nerve, mechanical allodynia develops on the side of the surgery [Bennett and Xie,

1988; Malmberg and Basbaum, 1998]. On a flat floor, when the pressure on the entire footpad is even, the rats were often seen to raise the affected hind paw from the surface and hold it in a protected position. Bennett and Xie [1988] emphasized that they did not use cages with wire mesh floors (traditional at that time) to avoid additional discomfort arising from the injured hind paw. Using this observation, we developed a wire surface test. A cage (14 cm × 12 cm × 14 cm) with a wire mesh floor was used to conduct the test (Fig. 1). The mice were placed into the cage (on the treatment days, 20 min following MMWT) for a period of 9 min (first 4 min—adjustment period; 5 min—observation period). In this test, the following end points were recorded: (1) number of paw-protective movements (lifting, licking) in horizontal position (PPMh) (Fig. 1A); (2) number of paw-protective movements in vertical position (PPMv) (Fig. 1B); (3) the total cumulative time the injured paw was held up from the wire-mesh floor during the 5 min observation period (TT); and (4) the number of times the mouse was “standing” (“vertical activity;” VA).

To avoid possible distraction to the mouse by the presence of the researcher, the test was digitally recorded then analyzed later.

The time-schedule of the tests is shown in Figure 2. Each mouse was tested 14 times: training and baseline tests before the surgery; training and two baseline tests in 9–11 days following the CCI; four times during the course of MMWT; and five times in the post-treatment period. As in the experiments using cTFT, a WST was conducted using “blind” experimental settings: The treatment and recording were done by one researcher, while the further analysis was completed by the other individual.

Nine experimental groups were created:

- Cage control (no CCI and no MMWT were done; $N = 10$).

- CCI + Sham MMWT (animals following CCI were restrained, but not exposed to MMW; $N = 10$).
- CCI + MMWT 61.22 (MMW with frequency 61.22 GHz; $N = 11$).
- CCI + MMWT 53.57 (MMW with frequency 53.57 GHz; $N = 10$).
- CCI + MMWT 42.25 (MMW with frequency 42.25 GHz; $N = 10$).
- Sham CCI + Sham MMWT (Sham surgery was followed by the series of restrainings; $N = 10$).
- Sham CCI + MMWT 61.22 ($N = 10$).
- Sham CCI + MMWT 53.57 ($N = 10$).
- Sham CCI + MMWT 42.25 ($N = 10$).

In our preliminary experiments we noticed that mice had individual behavioral particularities after CCI: some held the injured paw for a long time over the surface without a dramatic increase of PPMh, while others demonstrated a much higher increase in the number of PPMh, with less effect on TT. To provide an overall measure of mechanical allodynia that would accommodate different behavioral particularities, we created an integral index of pain and discomfort (IIPD). The index incorporates all four of the above end points, and was calculated using a system of four linear equations that we obtained from the empirical evaluation of the level of pain and discomfort in 50 test mice (on a scale from 0 to 10, where 0 indicates no pain or discomfort, and 10 indicates an animal is in most severe pain and discomfort).

From Table 1, which is in fact a system of four linear equations with four unknowns:

$$\begin{aligned} 0.9a + 2.1b + 1.7c + 42.6d &= 0 \\ 12.7a + 6.5b + 22.2c + 22.6d &= 4 \\ 22.2a + 6.5b + 30.7c + 22.0d &= 6 \\ 26.7a + 1.3b + 50.5c + 5.7d &= 8 \end{aligned}$$

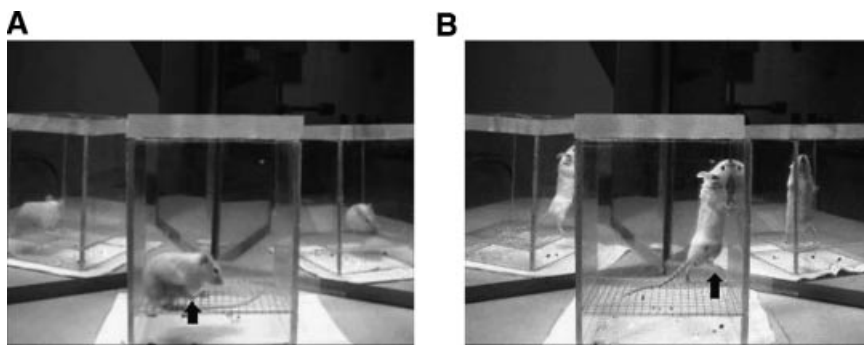


Fig. 1. Wire surface test. Mice are placed in a cage (14 cm × 12 cm × 14 cm) with a wire mesh floor. Two mirrors are positioned behind the cage to observe an animal from all angles. **A:** (↑) Example of a paw-protective movement in horizontal position (hPPM). **B:** (↑) Example of a paw-protective movement in vertical position.

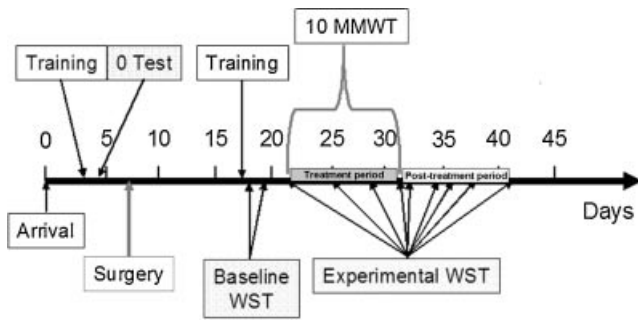


Fig. 2. Time-schedule of experiments in mice with CCI to the sciatic nerve. WST, wire surface test; MMWT, millimeter wave treatment.

we have calculated coefficients for all of the above end points. In our experiments they were:

PPMh: $a = 0.141$; PPMv: $b = 0.095$; TT: $c = 0.083$; and VA: $d = -0.011$. The subsequent comparison of the empirical and calculated IIPD demonstrated a very close concurrence. An average difference was ~ 0.5 . Thus, to calculate an individual IIPD in our further experiments we have used the formula:

$$\text{IIPD} = 0.141 \text{ PPMh} + 0.095 \text{ PPMv} + 0.083 \text{ TT} - 0.011 \text{ VA}$$

Such an approach allowed comparing the level of pain in all animals, regardless of their behavioral particularities.

Millimeter Wave Treatment

Previously we described the exposure procedure and dosimetric calculations in detail [Radzievsky et al., 2004b]. Key elements of the experimental MMW treatment were:

(1) To prevent the influence of electromagnetic “noise,” exposure of mice to MMW radiation was conducted in a shielded chamber made of 0.5-in. thick low-carbon steel. The generators of the MMW (Models G4–142 and G4–141; made in the former USSR), the

power meter (ML 4803A; Anritsu, Tokyo, Japan), and the spectrum analyzer (Hewlett Packard 8565B, Houston, TX) were located outside the shielded area. (2) Based on our previous results, the nose was chosen as the area of exposure in all the experiments. Each mouse’s nose during the exposure was located at the center of the antenna aperture at a distance of 0–1 mm from its front edge. (3) In the cTFT, mice were exposed to the frequencies 42.25, 53.57, 56.22, 61.22, and 66.22 GHz. In the WST, animals were treated with only the “therapeutic” frequencies 42.25, 53.57, and 61.22 GHz. (4) The peak power density at an output power of 30 mW and frequency 61.22 GHz was 56 mW/cm^2 . The average incident power density within the antenna aperture was 13.3 mW/cm^2 . Based on our previous dosimetric calculations using the Amber 4256 IR camera (Amber Engineering, Inc., Goleta, GA; spatial resolution of 256×256 pixels per frame and a temperature sensitivity of 0.02 K) [Radzievsky et al., 2004a], for the frequency dependence experiments we equalized the average power density for each of the other MMW frequencies used by varying the output power of the generators. (5) During the exposure to MMW animals were restrained for 15 min (duration of exposure). (6) Animals were taken into the WST in 20 min following the exposure; in the cTFT mice were tested immediately after the exposure and then two more times as described above.

Selective Opioid Receptor Blockers

The general logic of the present experiments is as follows: if the selective pharmacological blockage of a certain type of OR is followed by the disappearance (or significant reduction) of the MWT-induced hypoalgesia in the cTFT, then this particular type of OR is involved in the development of the effect [Shinoda et al., 2007]. The following selective blockers of OR were used: (1) μ_1/μ_2 —OR antagonist β -funaltrexamine (β -FNA). The blocker was injected s.c., 20 mg/kg, 24 h prior to testing. (2) δ -OR antagonist naltrindole methanesulfonate (NTI). The blocker was injected s.c., 1 mg/kg, 24 h prior to testing. (3) κ -OR blocker nor-binaltorphine

TABLE 1. Results of Empirical Evaluation of the IIPD in 50 Mice

Number of mice per group	Average number of Paw protective movements in horizontal position (a)	Average number of paw protective movements in vertical position (b)	Average total time (s) the injured paw was held up from the floor (c)	Average number of vertical movements (vertical activity) (d)	Subjectively evaluated integral index of pain and discomfort (IIPD)
18	0.9	2.1	1.7	42.6	0
14	12.7	6.5	22.2	22.6	4
11	22.2	6.5	30.7	22.0	6
7	26.7	1.3	50.5	5.7	8

Animals were grouped in accordance with the level of their individual subjective IIPD values and their recorded end points were averaged.

hydrochloride (nor-BNI). The blocker was injected s.c., 20 mg/kg, 18 h prior to testing.

Five experimental groups of mice were created:

- Sham MMWT. Twenty-four hours prior to the experiment mice were injected with 0.9% NaCl. The next day animals were restrained, but not exposed to MMW ($N = 15$).
- MMWT. Animals were sham injected and 24 h later were exposed to MMW ($N = 18$).
- Three experimental groups ($N = 15$ in each), where (18–24 h prior to the MMWT and subsequent cTFT) mice were injected with one of the above selective blockers of ORs.

Measurement of Endogenous Opioid Concentration in CNS Tissue Samples by Enzyme Immunoassay

Using blockers we indirectly determined involvement of certain types of OR. The measurement of endogenous opioids (EO) content in the CNS provides direct evidence of EO changes following MMWT. Three experimental groups (10 animals in each) were created:

- Cage control mice (mice were not restrained or exposed to MMW).
- Sham MMWT group, in which mice were restrained for 15 min, but not exposed to MMW.
- MMWT group, where each animal's nasal area was exposed to MMW for 15 min.

The CNS tissue samples were collected from decapitated mice 20–25 min following a single exposure to MMW or the restraining. According to our previous results, this is a time when MMWT-induced hypoalgesia is most pronounced in the cTFT. The CNS tissue was quickly removed, placed on ice, and dissected. Three tissue samples (average weight ~15–20 mg) were taken from each mouse: the Midlumbar spinal cord; the Brain Stem (periaqueductal gray area), and the Hypothalamic region of the CNS. These regions were shown to be among the key pain regulatory areas [Willis and Westlund, 1997]. The tissue samples were frozen in liquid nitrogen, and stored at -70°C .

For analysis, the frozen tissue samples, placed in 200 μl of lyses buffer containing protease inhibitors (Active Motif, Carlsbad, CA), were packed in ice and then insonated until complete homogeneity was obtained. The samples were centrifuged at 10,000g for 10 min at 4°C . The clarified supernatants were collected and used for the enzyme immunoassay. The

endogenous opioid concentration was determined using a competitive Enzyme Immunoassay kit (Peninsula Laboratories, San Carlos, CA) according to the manufacturer's instructions. Briefly: 50 μl of tissue extract, 25 μl biotinylated opioid peptide, and 25 μl primary antibody were mixed in a 96-well plate in which the wells were specially treated to bind the primary antibody. The biotinylated peptide competes for the antibody binding sites with the sample peptide. After 2 h of incubation, unbound biotinylated peptides were removed by vigorous washing and 100 μl streptavidin-conjugated Horseradish Peroxidase (SA-HRP) was added and allowed to bind to the immobilized primary antibody/biotinylated peptide complex for 1 h. After washing away excess SA-HRP, 100 μl TMB (3,3',5,5'-tetramethyl benzidine dihydrochloride) was added to react with the bound HRP. The developing color reaction was terminated after 1 h by adding 100 μl of 2N HCl and the color intensity was measured photometrically at 450 nm. The optical densities are directly proportional to the amount of biotinylated peptide-SA-HRP complex, but inversely proportional to the amount of the free peptide in the samples. The opioid concentration of samples was calculated by extrapolation of optical density measurements data on the standard curve and was expressed in ng/ml/g units. The concentrations of Endomorphin 1 ($\mu\text{-EO}$; the Brain Stem and the Hypothalamic region); Endomorphin 2 ($\mu\text{-EO}$; the Spinal Cord); Enkephalin ($\delta\text{-EO}$); and Dynorphine ($\kappa\text{-EO}$) were determined.

Statistical Analysis

Because all the behavioral tests were characterized by a rather high individual variability of results, and the distribution of variants within the experimental groups was not normal, raw data from the cTFT were converted to percent of each mouse's baseline response. The average percent change was calculated for each mouse. Post-treatment group means were compared to those of the control groups means using the Kruskal–Wallis ANOVA test. A level of $P < 0.05$ was taken to indicate statistical significance.

To analyze the results of the WST the IIPD was used. The results were analyzed at the time periods indicated in Fig. 2: (1) the levels of IIPD in the 0 test; (2) the average of two Baseline tests; (3) the average of IIPD in the first half of the MMWT (tests after first and fourth exposures); (4) the second half of the treatment (tests following the 7th and 10th MMWT); (5) the average of IIPD in the first half of the post-treatment period (on days 1 and 3 or 5 after the last MMWT); and (6) the second half of the post-treatment period (on days 7 and 9 or 10 after MMWT) were compared using the Kruskal–Wallis ANOVA test.

The internal variability of the ELISA method is also rather high. Thus, for quantitative analysis it is important that the tissue samples of the Cage control, Sham-control, and MWT groups of animals are equally represented in any ELISA run. In our study, for the quantitative comparative analysis, all the results are presented as a percentage of EO concentration against the level of the given peptide in the Cage Control mice, which was taken as 100%. The Kruskal–Wallis ANOVA test ($P < 0.05$) was used for further statistical analysis.

RESULTS

Frequency Dependence of the MMWT-Induced Hypoalgesia

Chronic non-neuropathic pain. Previously we demonstrated that a single 15 min exposure to MMW with frequency of 61.22 GHz and average incident power of 13.3 mW/cm² can significantly suppress cold-induced pain in mice in the cTFT. In the present set of experiments, using the same cTFT, we have compared this effect with the hypoalgesia induced by the other “therapeutic” [Rojavin and Ziskin, 1998] frequencies: 42.25 and 53.57 GHz (Fig. 3). The MMWT using these frequencies (duration of exposure and average incident power density were the same) also statistically significantly increased the resistance of mice to cold-induced pain. However, the effect was significantly lower than that of treatment with 61.22 GHz MMW. Furthermore, if the frequency was decreased or increased by 5 GHz from the “optimal” 61.22 GHz (frequencies 56.22 and 66.22 GHz), the

MMWT-induced hypoalgesia decreased. We concluded that MMWT-induced hypoalgesia in the model of chronic non-neuropathic pain is a frequency-dependent phenomenon.

Chronic neuropathic pain Our experiments have demonstrated that CCI to the sciatic nerve in mice is a reliable and reproducible experimental model for neuropathic pain. Using the WST, the first signs of pain and discomfort can be detected 3–4 days after the surgery. Starting from day 10 after the trauma, the symptoms of neuropathic pain became relatively stable, and (if untreated) stay at approximately the same level for at least a month. Sham surgery, as well as sham exposure, does not change murine behavior significantly in the WST. Levels of the recorded WST end points were statistically indistinguishable amongst the Cage Control, the Sham CCI + Sham MWT, the Sham CCI + MWT 61, the Sham CCI + MWT 53, and the Sham CCI + MWT 42 experimental groups (data not shown). In contrast, all the end points changed significantly following CCI. The total number of Paw Protective Movements increased more than four times: from 5 in the “0 test” recording to 20.9 in the baseline tests. The Total Time increased even more: from 2.93 to 29.17 s on average. Significantly reduced was the VA: from 35.2 in the “0 test” to 17.0 in the Baseline tests. And finally, the IIPD rose more than 10 times: from 0.48 in the “0 test” to 4.97 on average in the Baseline tests.

Ten 15-min exposures to MMW changed the level of neuropathic pain in mice after CCI (Fig. 4). The effect was frequency dependent, and most pronounced

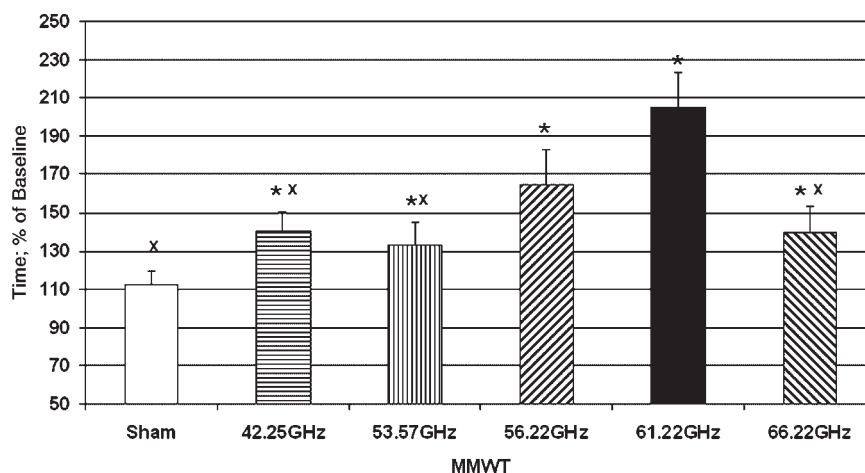


Fig. 3. Cold water tail-flick test in mice. Comparison of the hypoalgesic effects of a single exposure to MMW with different frequencies. Other MMW characteristics: average incident power density = 13.3 mW/cm²; duration of exposure = 15 min; area of exposure—nose. $N \geq 15$ for all groups. Each column represents the group mean + SE. Values with statistically significant difference from Sham control group are marked with “*”; from 61.22 GHz group—with “x”

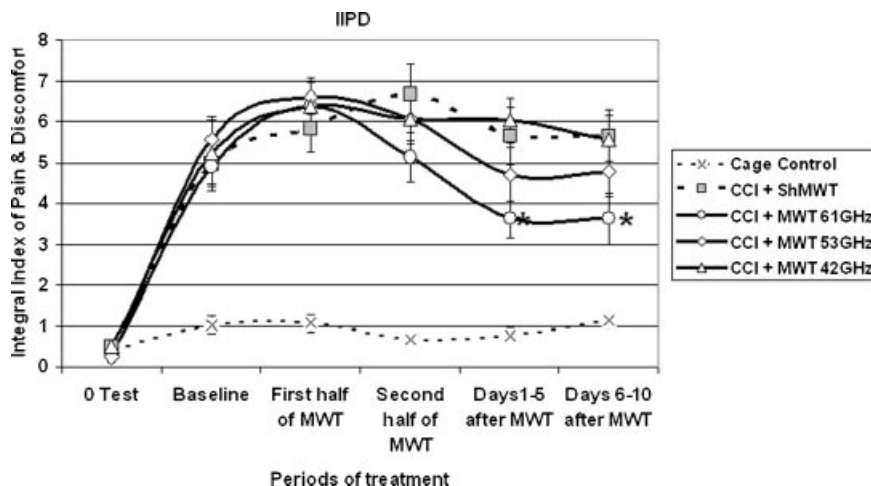


Fig. 4. Dynamic of MMWT influence on the integral index of pain and discomfort (IIPD) in mice following CCI to the sciatic nerve. Comparison of the hypoalgesic effects of exposures to MMW with different frequencies. Other MMW characteristics: average incident power density—13.3 mW/cm²; duration of a single exposure—15 min; area of exposure—nose. *N* ≥ 10 for all groups. Each point represents the group mean ± SE. Values with statistically significant difference from CCI + Sham MMW control group marked with “*”.

when the frequency of 61.22 GHz was used. IIPD suppression appeared starting from the second half of the treatment and was statistically significant up to the end of the experiment (10 days after the MMWT was over). In the post-treatment period, the beneficial effect of the 61.22 GHz MMWT reaches a statistically significant level not only for IIPD, but for all the recorded end points except the VA, where the increase was registered but was not statistically significant.

Types of Opioid Receptor in MMWT-Induced Hypoalgesia

The cTFT, where the MMWT-induced hypoalgesia develops after a single exposure, was used for this part of our experiments. As is evident from the data presented in Figure 5, the biggest suppression of the MMWT-induced hypoalgesia in the cTFT was registered after pretreatment of mice with the selective

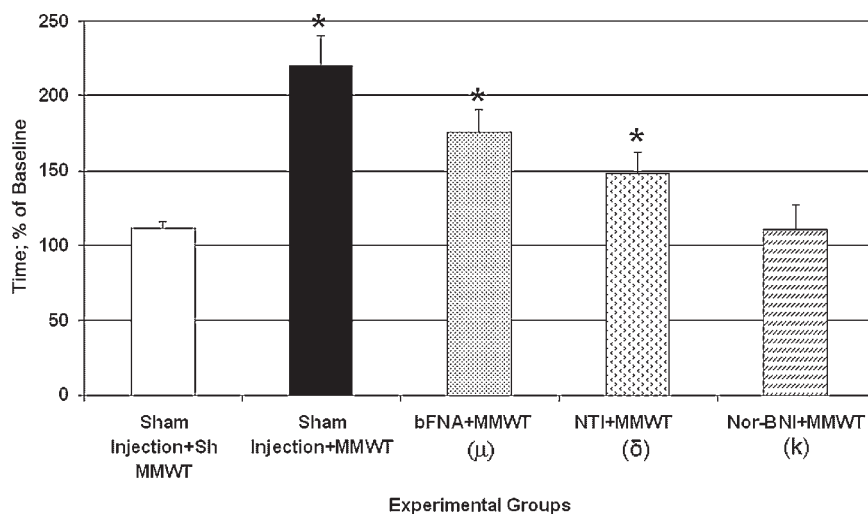


Fig. 5. Cold water tail-flick test in mice. Influence of pretreatment with selective blockers of opioid receptors (μ — β FNA; δ —NTI; and κ —Nor-BNI) on MMWT-induced hypoalgesia. MMWT characteristics: Frequency = 61.22 GHz; average incident power density = 13.3 mW/cm²; duration of exposure = 15 min; area of exposure is nose. *N* ≥ 15 for all groups. Each column represents the group mean + SE. Values with statistically significant difference from Sham control group marked with “*”.

blocker of κ -OR Nor-BNI, when MMWT-induced increase of pain resistance completely disappeared. A rather significant suppression was determined after pretreatment with the blocker of δ -OR (NTI), while the selective blocker of μ -OR (β -FNA) did not change the response of animals in the cTFT considerably.

Endogenous Opioid Content in CNS Tissue Following MMWT

The involvement of δ -opioids in the systemic reaction to MMW was confirmed with further ELISA analysis of EO contents in various parts of the CNS in 20 min following the exposure to MMW (frequency 61.22 GHz). The concentration of the selective δ -EO Enkephaline significantly increased in the midbrain and hypothalamic areas (Fig. 6). No significant changes in the concentration of the selective κ - or μ -EO were determined using the present experimental approach.

DISCUSSION

To be accepted by Western physicians, a pharmacological agent or therapeutic modality has to pass through intensive testing procedures. These procedures include careful preclinical testing and four phases of clinical trails [Spilker, 1996]. An obligatory requirement for this testing is that the evaluation has to be quantitative and has to be conducted in a “double-blind” manner. Unfortunately, in the former USSR during 1960–1970 when the beneficial biological effects of low power MMW were first discovered, the

circumstances were different. The legal base for the introduction of new treatments into clinical practice was undeveloped, and the availability of effective pharmacological agents was limited. Thus, a new treatment, namely MMWT, which demonstrated a high initial success and almost complete absence of side effects, was immediately accepted for wide clinical usage. At the same time, mechanisms of action of MMWT, specific indications (and possibly contra-indications) for the treatment were, and still are, unclear. For instance, certain MMW frequencies (42.25, 53.57, and 61.22 GHz) were declared “therapeutic” by the Soviet Ministry of Health [Rojavin and Ziskin, 1998], but no reports in the available literature explain its reasoning. The closest is the publication by Samosiuk et al. [2000]. In this experimental study in mice the frequencies 60 and 118 GHz (modulated by 4 Hz), and also MMW “noise” in the range of 42–95 GHz (modulated by 1–60 Hz) caused more hypoalgesia than “noise” 90–140 GHz, or combination of fixed frequency 60 GHz + noise 42–95 GHz; and 118 GHz + noise 90–140 GHz. However, “blind” experimental conditions were not utilized. Furthermore, unmodulated “therapeutic” frequencies in this study were not reviewed at all. The same is true for the other variables of MMWT: average incident power density; site of exposure; duration and number of exposures. The parameters used in MMWT were chosen by physicians mainly arbitrarily. There are still no publications about comparative studies that would quantitatively assess the effects of different regimes of

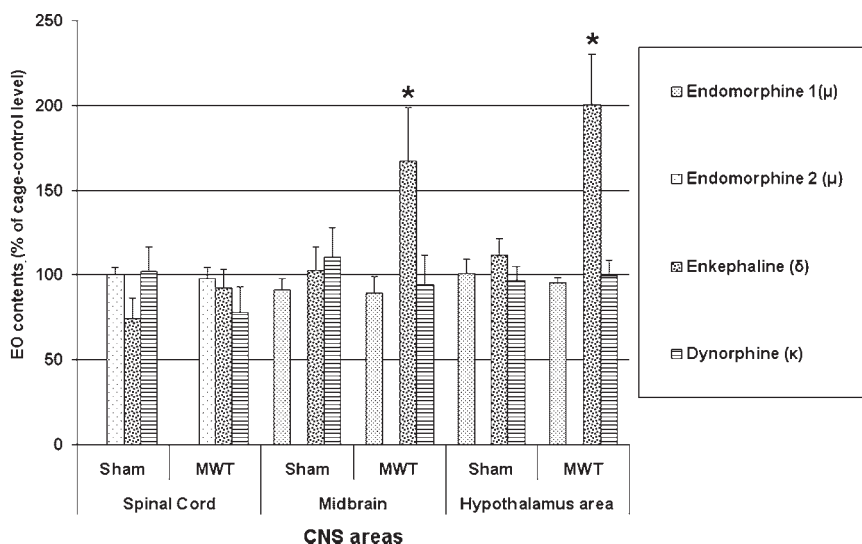


Fig. 6. Influence of a single MMWT on the endogenous opioids content in different parts of murine CNS. MMWT characteristics: frequency = 61.22 GHz; average incident power density = 13.3 mW/cm²; duration of exposure = 15 min; area of exposure—nose. N = 10 for all experimental groups. Each column represents the group mean + SE. Values with statistically significant difference from Sham control group marked with “*.”

MMWT under “blind” experimental conditions. So, despite the reported high success of the treatment in “millions” of patients with very different diseases, its acceptance by Western physicians has been very guarded. Is this panacea-like treatment real? Is it possible to reproduce and quantitatively evaluate the systemic biological effects of MMWT under “blind” experimental conditions using sham-treatment control groups?

In our work we have concentrated on one of the reported systemic effects of MMWT—hypoalgesia. Using strict “blind” experimental conditions we have quantitatively evaluated the ability of MMWT to suppress different types of pain in mice. The hypoalgesia was most pronounced in the model of chronic non-neuropathic pain, in which a single exposure to MMW results in more than a twofold increase in cold-induced pain resistance. Further experiments demonstrated that: MMWT-induced hypoalgesia is specific, and cannot be reproduced by equivalent heating of the same area of exposure; is power dependent; and its effectiveness is highest when the exposed skin area possesses the greatest neural innervation density [Rojavin et al., 2000; Radziewsky et al., 2004a]. In the present set of experiments, we have determined that MMWT-induced hypoalgesia is also frequency-dependent. The highest level of chronic pain suppression can be obtained using a frequency of 61.22 GHz. Furthermore, as is apparent from our previous and present results, different types of pain have different “sensitivities” to MMWT: while a single exposure to MMW (frequency = 61.22 GHz; average power density = 13.3 mW/cm²; and duration = 15 min) can significantly reduce pain sensitivity in the models of acute and chronic non-neuropathic pain, it was ineffective in the model of neuropathic pain. Only after a course of 10 exposures can MMWT-induced hypoalgesia be registered.

Previously, using pretreatment with Naloxone (a non-specific blocker of OR), we indirectly demonstrated involvement of endogenous opioids in MMWT-induced hypoalgesia [Rojavin et al., 2000]. Present work has determined that δ - and κ -OR are predominantly involved in this effect. Furthermore, a significant increase of the selective agonist of δ -OR content (enkephalin) was detected in the brain stem and hypothalamic regions of the murine CNS in 20 min following a single MMWT. Thus, we can conclude that the system of endogenous opioids plays an important role in the systemic effects of MMWT. At the same time, we may only hypothesize why the ELISA test used in our experiments did not detect changes in dynorphine concentration in the CNS tissue. Can it be insufficient “sensitivity” of the method? Or is it a very

local effect, which was “swamped” by the size of the sample? Or maybe a different “time window” should be used to “catch” changes in the dynorphine content within the CNS tissue? Or have all of the above contributed to the results of our study? Additional experiments are required to answer all these questions.

According to the International Association for the Study of Pain, neuropathic pain can be defined as pain initiated or caused by a primary lesion or dysfunction in the nervous system [Ossipov et al., 2000]. Neurogenic pain can result from peripheral nerve trauma (deafferentation events, entrapment, amputation), infection (post-herpetic neuralgia, human immunodeficiency syndrome-associated neuralgia), pressure due to growth (neoplasia), infarct, metabolic disturbance (diabetic neuralgia), or it may be idiopathic [MacFarlane et al., 1997]. Following CCI (that was used in the present experiments) neuropathic pain development is a result of complex functional and morphological changes that involve not only peripheral neural structures, but various parts of the central nervous system as well [Attal and Bouhassira, 1999]. Moreover, the endogenous opioid system plays a key role in the cascade of events in response to peripheral neural trauma. Both the cell surface OR spectrum and the levels of endogenous opioids are changed by chronic neuropathic pain [Przewlocki and Przewlocka, 2001]. It is clinically important that while the effectiveness of exogenously injected opiates is often reduced in neuropathic pain conditions [Mayer et al., 1999], and may cause many intolerable or even unmanageable adverse effects [McQuay, 1999], endogenous opioids can effectively suppress the level of neuropathic pain [Zadina et al., 1999; Przewlocki and Przewlocka, 2001]. Thus, the ability of MMWT to modulate the functional activity of the system of endogenous opioids has a definite clinical advantage. Furthermore, the ability of selective κ - and δ -OR agonists to alleviate neuropathic pain was reported [Catheline et al., 1998; Walker et al., 1999; Dondio et al., 2001; Mika et al., 2001]. Therefore, the capacity of MMWT to selectively influence κ - and δ -ORs (discovered in the present set of experiments) can be very promising for the treatment of the most recalcitrant type of chronic pain—neuropathic.

Based on the known facts about biological effects of MMW, the chain of events initiated by skin exposure to MMW can be subdivided into four major steps: (1) the “Initiation” phase, (2) the “Transmission of the signal” to the CNS, (3) the “Modulation of the CNS function” phase, and (4) the “Systemic response” phase. Among them the “Initiation” and “Transmission of the signal” phases are probably the most puzzling. The penetration depth of the MMW into mammalian skin is less than 0.5 mm. This limits the number of possible targets of

MMW to structures within the epidermis. However, free nerve endings that can reach into superficial layers of the skin [Cauna, 1980; Reilly et al., 1997], are within range of the MMW. According to our previous results, direct or indirect (through keratinocytes, mast cells, or local immunocompetent cells) interaction of MMW with free nerve endings is an obligatory step in the development of the MMWT-induced hypoalgesia: denervation of the area of exposure cancels the effect. Importantly, several independent in vitro studies showed that neurons and isolated nerves changed their functional characteristics in response to MMW exposure [Khramov et al., 1991; Pakhomov et al., 1997, 1998b; Alekseev and Ziskin, 1999]. Furthermore, this interaction may not be purely thermal: an equivalent local heating of the area of exposure with the Holmium YAG laser did not produce any systemic hypoalgesia in the cTFT [Radzievsky et al., 2004a]. However, considering the inability of MMW (at therapeutic power levels) to cause the direct excitation of neurons and generate action potentials, we may speculate that MMW are influencing ectopic spike activity of sensory neurons by modulating their subthreshold membrane potential oscillation. One possible mechanism for this process could be our recent discovery of the reversible externalization of phosphatidylserine molecules as the result of the cells' exposure to MMW [Szabo et al., 2006]. Two other known facts may also contribute to the "uniqueness" of the MMW-skin interaction:

- Molecular oxygen has two resonance absorption lines at 62 and 120 GHz [Betsky et al., 2004]. It is remarkable that our most pronounced biological effects of MMWT were found following exposures to MMW with frequency 61.22 GHz.
- The water content of the outer skin layers is lower than 50–60% [Warner et al., 1988]. In the striatum corneum it can be even less than 30%. At the same time, the water content of the free nerve endings (that extend to the outer most skin layers [Cauna, 1980; Munger and Ide, 1988]) is 70–80%. Taking into consideration that MMW are selectively absorbed by water and water-containing tissues, one may hypothesize that free nerve endings may "selectively absorb" MMW energy and experience large temperature gradients.

There are several studies that confirm presence of the third phase of the systemic reaction to MMWT and support the responsiveness of the brain to the peripheral MM electromagnetic signal:

- The presence of electroencephalographic changes was shown in healthy human volunteers [Lebedeva,

- 1997] and in children with cerebral palsy [Antonova et al., 1995] as a result of their exposure to MMW.
- Exposure of the rat's hip to MW caused changes in concentration of dopamine and noradrenalin in rats' brains [Bazian et al., 1997].
- Changes in the c-fos content in the hypothalamic region of rat's CNS were found following paw exposure to MMW [Novikova et al., 2002].

Our discovery of changes in enkephalin content in the brain stem and in the hypothalamic regions of murine CNS after MMWT also confirms the CNS function modulatory effect of MMW.

The "systemic response" phase of the biological effects of MMWT is most probably preconditioned by the functional effects of endogenous opioids. It is known that they are involved not only in regulation of ascending and descending pain pathways, but also in regulation of many other systems [Carr and Serou, 1995; Kowalski, 1998; Terenius, 2000; Bodnar et al., 2005; Molina and Molina, 2006]. Thus, the surprisingly broad spectrum of MMWT effects can be explained by the broad spectrum of endogenous opioid biological effects. As an illustration: the pretreatment with Naloxone not only cancels the hypoalgesic effect of MMWT, but also abolishes MMWT-induced prolongation of ketamine and chloral hydrate anesthesia [Rojavin and Ziskin, 1997], cancels the antipruritic [Rojavin et al., 1998] and antitumor [Radzievsky et al., 2004b] effects of MMWT in mice.

MMWT has big clinical potential. A number of experimental studies, including our present experiments, have clarified the possible biological mechanisms of the treatment [Pakhomov et al., 1998b; Logani et al., 2002; Makar et al., 2003; Radzievsky et al., 2004a; Alekseev et al., 2005; Szabo et al., 2006]. Some clinical studies have demonstrated the effectiveness of MMWT under "double-blind" conditions [Korpan and Saradeth, 1995; Megdiatov et al., 1995; Radzievsky et al., 1999; Usichenko et al., 2003]. However, strict indications for the treatment still need to be developed. As our results have established, even for a single symptom such as pain different regimes of MMW exposure have different effectiveness. More preclinical and clinical studies are needed before introducing MMWT into broad clinical practice.

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