Electromagnetic Biology and Medicine, Early Online: 1–10, 2012

ISSN: 1536-8378 print / 1536-8386 online
DOI: 10.3109/15368378.2012.662189

informa
healthcare

Analgesic effect of the electromagnetic resonant frequencies derived from the NMR spectrum of morphine

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Exposure to various types of electromagnetic fields (EMFs) affects pain specificity (nociception) and pain inhibition (analgesia). Previous study of ours has shown that exposure to the resonant spectra derived from biologically active substances' NMR may induce to live targets the same effects as the substances themselves. The purpose of this study is to investigate the potential analgesic effect of the resonant EMFs derived from the NMR spectrum of morphine. Twenty five Wistar rats were divided into five groups: control group; intraperitoneal administration of morphine 10 mg/kg body wt; exposure of rats to resonant EMFs of morphine; exposure of rats to randomly selected non resonant EMFs; and intraperitoneal administration of naloxone and simultaneous exposure of rats to the resonant EMFs of morphine. Tail Flick and Hot Plate tests were performed for estimation of the latency time. Results showed that rats exposed to NMR spectrum of morphine induced a significant increase in latency time at time points ($p < 0.05$), while exposure to the non resonant random EMFs exerted no effects. Additionally, naloxone administration inhibited the analgesic effects of the NMR spectrum of morphine. Our results indicate that exposure of rats to the resonant EMFs derived from the NMR spectrum of morphine may exert on animals similar analgesic effects to morphine itself.

Keywords NMR, Resonance, EMFs, Analgesia, Antinociception

INTRODUCTION

Over the past three decades, the properties of electromagnetic fields (EMFs) have attracted the interest of many scientists. There are several studies of ongoing research defining that exposure to resonant frequency of EMFs can induce modifications in living systems, such as changes in protein levels, cell proliferation, alteration of cellular membrane's permeability, and Ca^{2+}, Na^{+}, K^{+} ion transfer (Islamov et al., 2002; Stronati et al., 2004; Strasák et al., 2009; Sert et al., 2011). The influence of EMFs on biological systems has been indicated by a large number of

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studies (Aaron and Ciombor, 1993; Tao and Henderson, 1999; Tofani et al., 2002; Walker et al., 2007). Exposure to electromagnetic fields (EMFs) also affects a number of other biological functions at the behavioral, cellular, and neurobiological level (Sienkiewicz et al., 2005, Spiros et al., 2006; Ayşe et al., 2010; Evangelou et al., 2011), although the processes induced by EMFs on living matter are far from being fully explained.

Effects of EMFs on nociceptive responses and pain sensitivity have been demonstrated by a number of different investigators in a variety of studies (Prato et al., 2005; Del Seppia et al., 2007; László et al., 2007). Several studies which deal with the effects of EMFs on analgesia used morphine and a variety of exogenous opiates, in order to induce analgesia, as control measurements. Analgesic effects of morphine are mainly mediated by μ-opioid receptors which are widely distributed to the central nervous system, including the spinal cord (Arvidsson et al., 1995; Hashimoto et al., 2006). Morphine, however, and other centrally acting μ-opioid analgesics produce apart from their antinociceptive effects, several side effects such as respiratory depression, inhibition of gastrointestinal motility, and tolerance that may limit their use in pain treatment (Schiller, 2005; Bonnet et al., 2010). To compare the inhibitory effects of EMFs on analgesia, the prototypic opiate antagonist, naloxone is used in a number of in vivo studies (Kavaliers et al., 1987a; Jeong et al., 2000). Most of these studies have shown that naloxone and magnetic exposure have similar inhibitory effects on analgesia (Del Seppia et al., 2000; Jeong et al., 2000). In contrast to these studies, Martin et al. (2004) presented that the analgesic effect of complex fields is eliminated by pre-injection with naloxone.

Electronic transmission of thyroxine to frogs seems also to induce the same effects as thyroxine itself on frogs (Endler et al., 1995). In previous studies, we have shown that biologically acting substances may induce the same effects on their biological targets, when substituted by their resonant electromagnetic spectrum (Evangelou et al., 2008).

The aim of this study was to examine the possible analgesic effects induced by emission of the resonant EMFs derived from the NMR spectrum of morphine on Wistar rats.

MATERIAL AND METHODS

Animals Care

Twenty five Wistar rats, 2–3 months old, with average body weight (body wt) 190 ± 10 g were used in this study. Animals were kept in community cages, at room temperature (21 ± 1°C), under 12 h/12 h of light/darkness conditions, and provided *ad libitum* with rat chows Viozois S.A. (Animal Feed Company of Epirus, Greece) and water. Animals were acclimatized to laboratory conditions before the tests. To reduce variation, all experiments were performed during the light phase of the cycle (09:00–17:00). Experiments on animals were handled with human care in accordance with the National Institutes of Health guidelines and the European Union directive for the care and the use of laboratory animals (Greek presidential decree No. 160 1991 implementation of the EEC Directive 86/609/EEC).

Chemical and Reagents

Morphine hydrochloride and naloxone hydrochloride were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals were locally purchased and of analytical grade.

Electromagnetic Field Apparatus

EMFs exposure was performed using the Multi Channel Dynamic Exciter 100 V1 (MCDE), which was designed and manufactured by K. Havelas and collaborators. The MCDE system was evaluated for its safety application in animal and humans by the (Greek) National Center for Scientific Research «Demokritos» (Greek Department of the International Committee of Atomic Energy) (Karabetsos, 2000) The MCDE system was found safe for prolonged exposure of humans, animals, and other living organisms. The technical characteristics for the MCDE system are: the range intensities for the electrical field were 1.1 to 1.11 ± 0.01 V/m and for the magnetic field were 0.0027 to 0.0029 ± 0.00005 A/m. The frequencies which can be generated from the MCDE system range from 10 Hz to 1 MHz. The power density of the electromagnetic field was 100 mV/cm^3 approximately (Avdikos et al., 2007; Evangelou et al., 2011). The system was placed inside a Faraday cage, to avoid interaction with external electromagnetic fields. The apparatus provides a uniform exposure of the animal's whole body to the resonant EMFs (rEMFs).

NMR Transformation

The 45 rEMFs were selected from the transformation of chemical shift (in ppm) of morphine H^1-NMR (Table 1), representing the peaks of the morphine's NMR spectrum (Fig. 1). The fundamental frequency for the specific spectrophotometer was 200 MHz. These rEMFs were stored in the MCDE system in order to be used for emission. The equation used for this transformation was:

$$\text{rEMFs} = \text{chemical shift (ppm)} \times 200\,\text{MHz (fundamental frequency of spectrophotometer)}$$

(Keeler, 2005).

The used frequencies broad ranged from 400 Hz to 1.6 kHz (Table 1) and were emitted simultaneously. The non resonant spectrum (45 non resonant frequencies) consisted of frequencies below and above the resonant spectrum of morphine and carried the same energy as the resonant NMR spectrum of morphine (Table 1).

Analgesic Assays

The analgesic effects of morphine and rEMFs derived from H^1-NMR spectrum of morphine were evaluated using the hot-plate and the tail-flick test. The experiments were performed in a blind model study. The researchers were performing the measurements at the hot-plate and tail-flick test without knowing to which group animals belong to. In the tail flick test, animals were placed in a horizontal acrylic restraint and fixed on an analgesimeter with a portion of the tail, 5 cm from its tip, exposed to heat from a projector lamp (55 ± 1°C). A single control switch simultaneously activated the light and a timer. The timer stopped when the exposed rat tail flicks, and the interval between switching on the light and flick of the tail was recorded (latency time). A 15 s cut-off time was used to avoid thermal injury.

For the hot-plate test, animals were placed on a hot-plate enclosed by Plexiglas walls, with the temperature adjusted to 55 ± 1°C. The behavioral end point was the time measured in seconds at which the animal jumped off the plate or licked a hind paw; the cut-off time was 40 s in order to prevent tissue damage. For both tests the baseline (normal response to the noxious stimulus) was recorded before starting the experiments.

TABLE 1 The peak resonant frequencies of morphine's NMR and the non resonant frequencies spectra used in the study.

Number of frequencies	Peak from NMR Spectrum (ppm)	Resonant-Frequencies (Hz)	Non resonant-Frequencies (Hz)
1	2.005	401	200
2	2.042	408	203
3	2.048	410	210
4	2.086	417	215
5	2.269	454	221
6	2.279	456	228
7	2.32	464	235
8	2.33	466	240
9	2.682	536	252
10	2.725	545	265
11	3.336	667	272
12	3.341	668	285
13	3.347	669	296
14	3.352	670	300
15	3.358	672	305
16	3.476	695	312
17	3.757	751	318
18	3.777	755	323
19	3.789	758	335
20	4.267	853	342
21	4.278	856	350
22	4.906	981	1535
23	5.043	1009	1542
24	5.468	1094	1555
25	5.478	1096	1568
26	6.307	1261	1570
27	6.341	1268	1572
28	6.36	1272	1575
29	6.395	1279	1582
30	6.797	1359	1590
31	6.805	1361	1595
32	6.825	1365	1601
33	6.832	1366	1605
34	6.944	1389	1615
35	6.95	1390	1619
36	6.957	1391	1622
37	6.963	1393	1630
38	6.971	1394	1635
39	6.978	1396	1639
40	6.984	1397	1642
41	6.991	1398	1650
42	7.093	1419	1658
43	7.097	1420	1662
44	7.581	1516	1668
45	7.634	1527	1675

There were no significant statistical differences among the baseline latencies in the rats of the experiment, which later were randomly allocated in the various experimental groups. The latency times measured at each time point were normalized by an index of analgesic activity (IAA):

Index of analgesic activity (IAA) = (test latency time)-(baseline latency time) / (cut off time)-(baseline latency time).

The results are expressed as mean ± SD of the latencies times and the IAA values and plotted against time of the recordings. Area under the curve (AUC) in the IAA-time curves was also calculated in computer units.

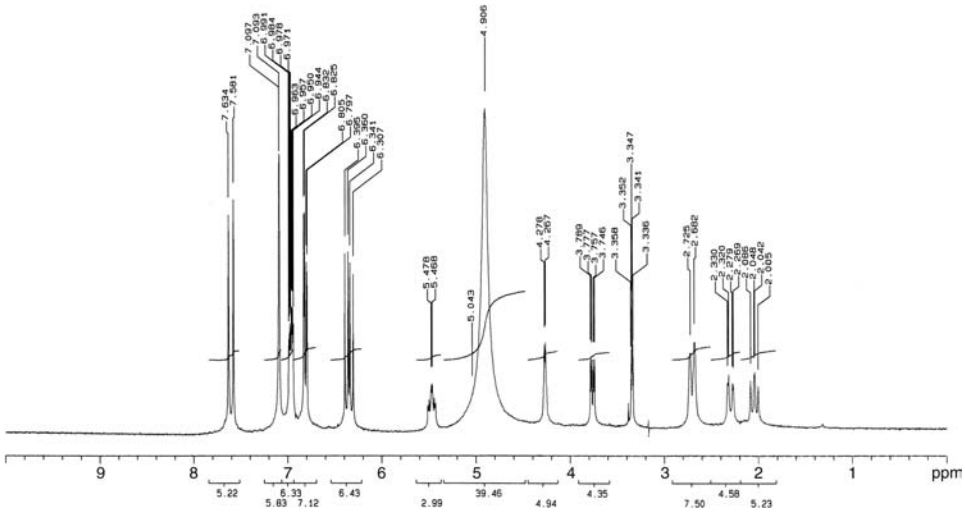

FIGURE 1 [1]H-NMR spectrum of morphine. The number above each peak represents the chemical shift in ppm.

Mode of Exposure to EMFs

Animals during treatment were kept in community cages with free access to food and water, placed inside the Faraday cage, at stable room temperature and humidity, and at a given distance from the dipole antenna of the device so that the rEMFs emitted were equally absorbed by the tissues of each animal. There was no significant variation (± 0.3°C) in temperature at all time points of exposure.

Animals used for the hot plate and tail flick tests were divided in five different groups ($n = 5$, per group): (a) control group; (b) intra-peritoneal (i.p.) administration of 10 mg/kg body wt of morphine; (c) exposure to 45 rEMFs of morphine; (d) administration of 1 mg/kg body wt of naloxone and exposure to the 45 rEMFs of morphine; and (e) exposure of rats to randomly selected non resonant EMFs.

Animals of groups c, d, and e were exposed to electromagnetic fields for 5 h and measurement of latency times was performed after 1 and 5 h of exposure for both tail flick and hot plate tests.

Statistical Analysis

The statistical significance between data means was determined by Student's t-test and two-way analysis of variance (ANOVA) was used for the statistical evaluation of differences between groups (SPSS version 16.0, Statistical Package for the Social Sciences software, SPSS, Chicago, USA). P-values $p < 0.05$ were considered as significant.

TABLE 2 Tail flick test latency times (in seconds) for all groups at different time points.

Groups	Start point (0 h)	1 h	End point (5 h)
Control	5.2 ± 0.5	5.4 ± 0.6	5.8 ± 0.7
MRF 10 mg/kg body wt	5.3 ± 1.2	$>15.00^{a,b}$	$>15.00^{a,b}$
rEMFs	5.1 ± 1.2	$7.8 \pm 1.2^{a,b}$	$10.0 \pm 1.7^{a,b}$
NLX + NMR-rEMFs	5.0 ± 0.5	4.8 ± 0.6	4.9 ± 0.8
Non rEMFs	5.0 ± 0.8	5.2 ± 1.2	5.6 ± 1.0

[a] Significantly different from starting point (0 h) ($p < 0.05$); [b] Significantly different from Control ($p < 0.05$). Data are presented as mean ± S.D; Abbreviations: MRF, Morphine; rEMFs, resonant Electromagnetic Frequencies; NLX, Naloxone.

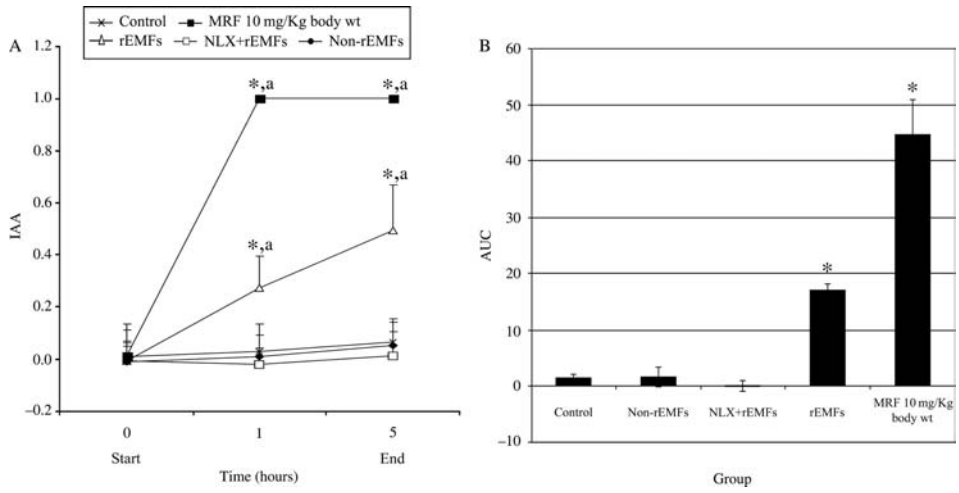

FIGURE 2 Antinociceptive effect for the 5 groups (A) Time course of the latencies times in the tail flick test induced by morphine and EMFs. (B) AUC of the groups shown in A. The results are expressed as mean ± SD. * significantly different from Control ($p < 0.05$), [a] significantly different from starting point (0 h) ($p < 0.05$). Abbreviations: IAA, index of antinociceptive activity; AUC, area under curve of antinociception activity; MRF, morphine; rEMFs, resonant Electromagnetic Frequencies; NLX, naloxone.

RESULTS

Tail Flick Test

The average latency time for animals at 0 hours was the same in all five procedures. Addition of 10 mg/kg body wt of morphine increased latency time in all time points (1, 5, and 6 h) beyond the cut off point (15 s) (Table 2, Fig. 2). Animals exposed to rEMFs showed a 56% and 99% increase in latency time at 1 and 5 h, respectively ($p < 0.05$). Animals were also exposed to random non resonant EMFs, which showed no significant difference in latency time at any time point. Additionally, when animals were treated with naloxone at a dose of 1 mg/kg body wt before the exposure to rEMFs of morphine, there was no increase in average latency time. The analgesic effect to animals exposed to NMR spectrum of morphine was significantly increased when compared to control ($p < 0.05$) but not as strong as animals treated with 10 mg/kg body wt of morphine (Fig. 2B). Analysis of the tail flick test latencies showed significant effects of treatment with the rEMFs spectrum of morphine (Figs 2A, 2B). The results showed that rEMF spectrum of morphine produces less than half of the antinociceptive effect of morphine (Fig. 2B). The tail flick test also confirmed that neither random exposure to non resonant EMFs nor pretreatment with naloxone before the exposure to the rEMF spectrum of morphine result in any significant antinociceptive effect (Table 2, Figs 2A, 2B).

TABLE 3 Hot plate test latency times (in seconds) for all groups at different time points.

Groups	Start point (0 h)	1 h	End point (5 h)
Control	18.3 ± 1.9	18.9 ± 2.1	19.9 ± 2.5
MRF 10 mg/kg body wt	18.5 ± 2.1	$>40^{a,b}$	$>40^{a,b}$
rEMFs	17.8 ± 1.9	$29.3 \pm 4.0^{a,b}$	$29.5 \pm 4.4^{a,b}$
NLX + NMR-rEMFs	17.4 ± 1.1	17.1 ± 1.2	16.9 ± 2.2
Non rEMFs	17.7 ± 2.7	18.7 ± 4.4	20.2 ± 3.7

[a] Significantly different from starting point (0 h) ($p < 0.05$); [b] Significantly different from Control ($p < 0.05$). Data are presented as mean ± S.D; Abbreviations: MRF, Morphine; rEMFs, resonant Electromagnetic Frequencies; NLX, Naloxone

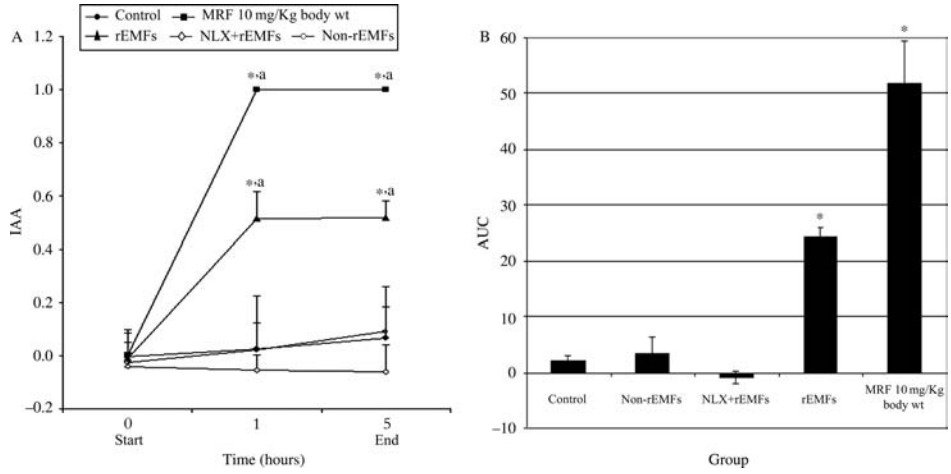

FIGURE 3 Antinociceptive effect for the 5 groups (A) Time course of the latencies times in the hot plate test induced by morphine and EMFs. (B) AUC of the groups shown in A. The results are expressed as mean ± SD. * significantly different from Control ($p < 0.05$), [a] significantly different from starting point (0 h) ($p < 0.05$) Abbreviations: IAA, index of antinociceptive activity; AUC, area under curve of antinociception activity; MRF, morphine; rEMFs, resonant Electromagnetic Frequencies; NLX, naloxone.

Hot Plate Test

The average latency time at 0 h was the same for all 5 procedures. The addition of 10 mg/kg body wt of morphine increased latency time in all time points (1 h, 5 h) beyond the cut off point (40 s) (Table 3, Fig. 3). Animal exposure to rEMFs of morphine resulted in a significant increase in latency time at any time point ($p < 0.05$). Latency time was increased 64% and 65% ($p < 0.05$) after 1 and 5 h of exposure, respectively (Fig. 3A). When animals were treated with naloxone at a dose of 1mg/kg body wt before the exposure to rEMFs of morphine, no increase was observed in average latency time. In hot plate test the greatest antinociceptive effect for the rEMFs group was observed after exposure for 1 and 5 h ($p < 0.05$) (Fig. 3A). Exposure of animals to the rEMF spectrum of morphine produced similar but weaker antinociceptive effects as i.p. administration of morphine (Figs 3A, 3B). The AUCs from the hot plate test revealed that the spectrum produces half the effect of i.p. administration of morphine (Fig. 3B). Furthermore, when animals were treated with naloxone before exposure to the rEMFs there was no significant change to the latency times (Table 3) and consequently to the antinociceptive indexes (Figs 3A, 3B). Finally, random exposure of rats to non resonant EMFs had no effect on latency times as shown in Table 3 and no antinociceptive effect as shown in Figs 3A, 3B.

DISCUSSION

Exposure of rats to the resonant electromagnetic frequencies (rEMFs) of morphine obtained from its NMR spectrum resulted into a similar to the substance analgesic effect estimated by both analgesic tests. In contrast, exposure of rats to a randomly selected set of 45 non resonant frequencies had not any antinociceptive effect on animals. The latter is indicative of some type of specificity in analgesic effect of the NMR spectrum of morphine. This type of spectrum specificity was previously shown by us, exposing cells and animals to the NMR spectra of other biologically active substances, such as an organometallic complex of Tin (SnMNA) exerting potent anticancer effects on malignant cells and tumor- bearing animals (Evangelou et al., 2008). Moreover, previous studies of ours on malignant cells and tumor-bearing

animals showed that only resonant coherent electromagnetic fields may exert anticancer effects (Karkabounas et al., 2006; Avdikos et al., 2007). Electromagnetic fields as above exert their effects at a non thermal way due to the low intensities and frequencies of the fields.

There have been several attempts by researchers to address and explain the possible cellular modes of EMFs action on biological systems although the mechanisms of EMF induced nociception and analgesia remain to be fully identified. The most proposed mechanisms are the calcium ion involvement (Kavaliers et al., 1987b; Prado, 2001; Pessina et al., 2001; Ikehara et al., 2002), nitric oxide and protein kinase C involvement (Kaczmarek, 1987; Miura et al., 1993; Bawin et al., 1996), and free radical production (Wolf et al., 2005; Simko, 2007). In our study, none of the recommended mechanisms could explain the analgesic effects of the rEMFs of the NMR spectrum of morphine on Wistar rats.

The analgesic effect of NMR spectrum of morphine observed in our experiment cannot be due to stress-induced analgesic effects of electromagnetic fields since no analgesia was induced by exposing the rats to a similar energy non resonant spectrum (non rEMFs). No side effects, such as dizziness, nausea, vomiting, or constipation, were registered either by morphine administration or by emission of morphine's NMR spectrum to rats.

In a study of Martin et al. (2004), the analgesic effect of magnetic fields was limited by pre-injection with naloxone. In our study, injection of naloxone inhibited both the analgesic effects either of morphine itself or of its NMR spectrum. The latter indicates that the morphine's-NMR analgesic effect is probably exerted through direct or indirect activation of the μ-opioid receptors.

In conclusion, our results indicate that the analgesic effects of morphine may possibly be exerted by emitting the resonant EMFs of the substance's NMR spectra at low, harmless intensities. Further studies are, however, needed in order to confirm whether emission of the resonant NMR spectra of analgesics could be used as an adjuvant treatment in pain management.

ACKNOWLEDGEMENTS

All authors gratefully appreciate the technical assistance provided by Mr. Konstantinos Havelas.

Declaration of interest

All authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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