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Expression levels of *PDYN* and *OPRM1* genes in SH-SY5Y cells exposed to 50 Hz electromagnetic field

Hamideh Mahmoudinasab, Mostafa Saadat

Department of Biology, College of Sciences, Shiraz University, Shiraz, Iran

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ABSTRACT

Introduction: Extremely low-frequency (ELF) (<300 Hz) electromagnetic fields (EMFs) may significantly affect several biological processes at the cellular and molecular level. Considering that ELF-EMF is abundant in our environment and associated with reactive oxygen species (ROS) production, exposure to EMF should be considered as a public health issue. ELF-EMF may alter the mRNA expression levels of several genes. Prodynorphin (*PDYN*, OMIM: 131340), precursor of several endogenous opioid neuropeptides, and opioid receptor mu-1 (*OPRM1*, OMIM: 600018) a member of opioid receptor family, are associated with nociception and drug-dependency.

Aim: This study was conducted to elucidate the effects of ELF-EMF on expression levels of *PDYN* and *OPRM1*.

Material and methods: Human SH-SY5Y cells were exposed first to EMF and harvested at three time points post exposure; immediately after exposure (0h), 2h and 4h after exposure. The 0.50 mT intensity of 50 Hz EMF and two exposure conditions ('15 min field-on/15 min field-off' and '30 min field-on continuously') were used. Using quantitative real-time PCR, the relative *PDYN* and *OPRM1* mRNA expression levels were calculated.

Results and discussion: After continuous exposure to ELF-EMF, analysis of variance revealed a significant reduction of *PDYN* mRNA expression levels at 0 hours and 2 hours time points ($F = 23.86$; d$f = 3, 8$; $P < 0.001$). The *OPRM1* mRNA expression levels did not show any significant alteration between the examined conditions.

Conclusions: In the present study the continuous exposure condition of ELF-EMF was associated with the lower expression levels of the *PDYN*.

Corresponding author: Mostafa Saadat, Department of Biology, College of Sciences, Shiraz University, Shiraz 71467-13565, Iran. Tel: +9871 36137432; Fax: +9871 32280916.
E-mail address: saadat@shirazu.ac.ir, msaadat41@yahoo.com.

1. INTRODUCTION

Prodynorphin (*PDYN*, OMIM: 131340) is a precursor of several endogenous opioid neuropeptides including dynorphin related peptides which play an important role in several complex traits such as nociception and drug-dependency.[1,2] The *Pdyn* knockout mice showed increased explorative behavior in anxiety tests suggesting an anxiogenic role of the peptides derived from PDYN.[3] Although in SH-SY5Y cells treated with morphine the *PDYN* mRNA expression level is increased after a short exposure, a longer exposure time resulted in a decreased level of *PDYN* mRNA expression.[4] It has been shown previously that a 68-bp sequence within the promoter region of *PDYN* occurs as a polymorphic element, either singular or as tandemly repeated element up to 5 times.[5,6] This polymorphism plays an important role in the *PDYN* expression level. Alleles with 3–5 repeats have an approximately 50% greater level of transcriptional activity compared to alleles with 1–2 repeats.[5,7] Previous studies have been indicating that the high repeat alleles decrease the risk of heroin dependency.[2,6,8]

The opioid receptor mu-1 gene (*OPRM1*, OMIM: 600018) encodes the mu opioid receptor which is the primary site of action for morphine and methadone and is involved in drug dependency.[9,10] In SH-SY5Y cells treated with morphine the mRNA expression levels of *OPRM1* decreased significantly in a dose dependent manner after a short exposure time; however, a longer exposure time led to an increased *OPRM1* expression.[4] In the A118G variant of *OPRM1* (rs1799971, Asn40Asp), which is a functional polymorphism, the G allele showed a modest protective effect on substance dependence.[11,12]

Extremely low-frequency (<300 Hz) electromagnetic fields (ELF-EMFs) are commonly present in daily life all over the world. They may significantly affect several biological processes at cellular and molecular level. On the other hand, the therapeutic effects of ELF-EMF have been proven. EMF at low frequency of 60 Hz (in USA and Canada) and 50 Hz (in Europe and Asia) is one of the EMF modalities were used for therapeutic purposes.[13] It is recommended that the field intensity of EMF producing medical devices not exceeded 0.50 mT. Because it interferes with cardiac pacemakers, ferromagnetic implants and other implanted medical and surgical devices.[14] Therefore, EMFs in these ranges have proven to be a safe, easy-to-use, non-invasive and low cost method for therapeutic purposes such as reducing pain.[15] It has been reported that ELF-EMF enhanced production of reactive oxygen species (ROS).[16–18] Cells maintain redox balance through production of ROS and cellular antioxidant capacity. The altered balance between generation and elimination of ROS plays a critical role in a variety of multifactorial complex traits including neurodegenerative diseases. Considering the ELF-EMF abundance in our environment and its association with ROS production, exposure to EMF should be considered as a public health issue.[19] It has been reported that ELF-EMF may alter the mRNA expression levels of several genes[20,21] including the brain,[22,23] neuroblastoma cells,[24] and embryonic neural stem cells.[25,26] As there is no study published yet about investigation on the alteration of the *PDYN* and *OPRM1* mRNA expression levels in human SH-SY5Y cells due to ELF-EMF, we designed and carried out the following experiment.

2. AIM

This study was conducted to elucidate the effects of ELF-EMF on expression levels of *PDYN* and *OPRM1*.

3. MATERIAL AND METHODS

3.1. Cell culture

Human SH-SY5Y neuroblastoma cells were obtained from National Cell Bank of Iran (Pasteur Institute, Iran). The cells were seeded at 3×10^5 cells/mL in 10 cm tissue culture Petri dishes approximately 24 hours prior to each treatment and kept in a cell culture incubator, using a 1 : 1 mixture of Dulbecco's Modified Eagle's Medium (DMEM) and Ham's F12 medium enriched with glutamax, supplemented with 10% FBS (Gibco), 100 U/mL penicillin and 100 μg/mL streptomycin (Sigma).

3.2. Electromagnetic field exposure system and exposure conditions

A solenoid with 44 cm length and 14 cm diameter was used for EMF exposure. It consisted of 2000 turns of 1 mm diameter copper wire, performing with 50 Hz alternating current (AC) as described previously.[20] The solenoid was placed in a box wrapped with 2 layers of aluminum sheets and 1 layer of copper sheet. The field intensity is calculated by

$$B = m_0 NI / L \text{ formula,}$$

where B represents the field intensity (T), m_0 represents the vacuum permeability and equals to $4\varpi \times 10^{-7}$ (N/A^2), N represents the number of turns, I represents the current in the wire (A), and L represents the solenoid length (m). Field accuracy was measured by a digital teslameter (Lutron Electronic Enterprise). The uniformity of the magnetic field in the central region of the solenoid was confirmed by a magnetic field simulation program (Vizimag 3.185, SoftNewsNet s.r.l.). No significant changes in the temperature of solenoid were observed during exposure. In each experiment, one 10-cm culture petri dish was placed horizontally in the center of the solenoid from 15 cm to 29 cm, where the uniform magnetic field equals to 0.50 ± 0.01 mT.

We designed two exposure conditions with the total exposure time of 30 minutes: (1) continuous exposure condition: cells were exposed to EMF for 30 minutes continuously; (2) intermittent exposure condition: cells were exposed to two 15 minutes EMF with a 15 minutes interval without EMF exposure. In our previous studies, we observed that total EMF exposure time of 30 minutes could induce significant changes in the mRNA levels of DNA repair and antioxidant genes.[20,24,27,28]

Therefore, we chose the time of 30 minutes for EMF exposure in this study. Control cells were kept under the same conditions without EMF exposure. Cells were harvested at three time points post exposure; immediately after completing exposure (0 hours), 2 hours and 4 hours after exposure.

3.3. RNA extraction, cDNA synthesis and Real--time RT-PCR

Total RNA was extracted using RNX-plus kit (Cinnagen) according to the manufacturer's protocol. RNA was then reverse transcribed to cDNA pool by primerscript RT reagent kit (TaKaRa Bio) in accordance with the provider's instructions. Primers for the investigated genes and TATA box-binding protein gene, as a housekeeping gene (*TBP*; OMIM: 600075), were reported previously.[4] Designed primers were specific to mRNAs and did not amplify genomic DNA. Quantitative real-time PCR analysis was performed using sybr premix Ex Taq II (TaKaRa Bio) in Rotor-Gene 6000 HRM (Corbett Research). The two-step real-time PCR program was: pre-amplification denaturation at 95°C for 30 s, 40 cycles of denaturation at 95°C for 5 s, followed by annealing and extension at 60°C for 45 s. The relative gene expression level was measured according to the $2^{-\Delta\Delta Ct}$ method based on the threshold cycle (Ct) values.[29] All data were normalized to the value of control cells placed in switched off solenoid which assumed 1.

3.4. Statistical analysis

All experiments were done in triplicates. Data are shown as the mean ± SE of three independent experiments. The differences between treatments were evaluated using one-way Analysis of Variance (ANOVA) followed by Duncan post hoc test. Statistical analysis was conducted using SPSS statistical software package (SPSS Inc., Chicago, IL, USA) (version 11.5). A probability of $P < 0.05$ was considered statistically significant.

4. RESULTS

The relative *PDYN* mRNA expression levels in SH-SY5Y cells exposed to ELF-EMF is shown in Figure 1 for both exposure conditions. After continuous exposure to ELF-EMF, analysis of variance revealed a significant reduction of *PDYN* mRNA expression levels at 0 hours and 2 hours time points ($F = 23.86$; $df = 3, 8$; $P < 0.001$; Figure 1A). In contrast to the continuous exposure condition, in the intermittent ex-

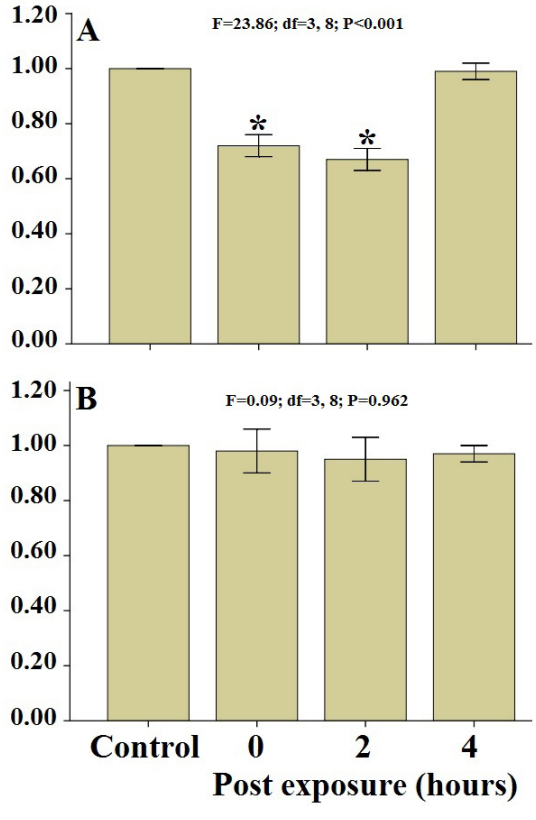

Figure 1. Relative *PDYN* mRNA expression levels in SH--SY5Y cells after exposure to '30 min field on continuously' (A) and '15 min field-on/15 min field-off' (B) electromagnetic fields (EMF, 50 Hz, 0.50 mT) at 0, 2, and 4 hours post exposure. Comments: $n = 3$, mean ± *SE*. *$P < 0.05$ all values were compared with unexposed cells (=1) using Duncan post hoc test.

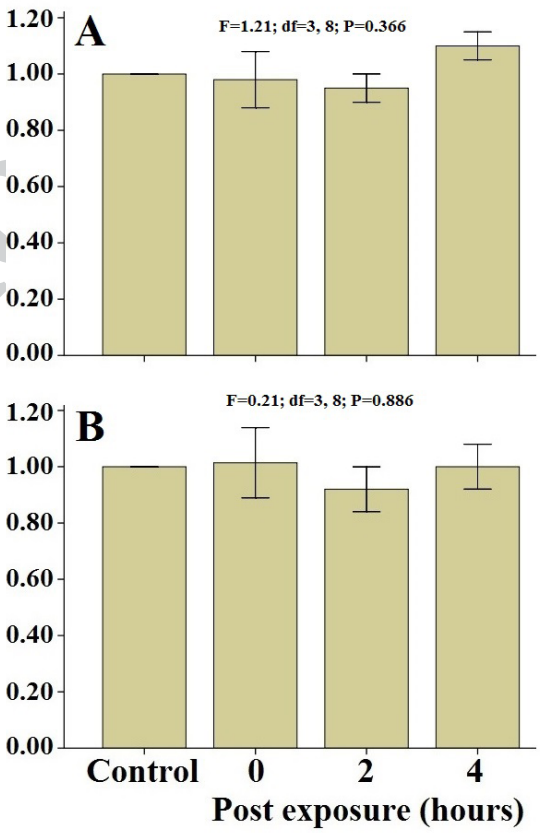

Figure 2. Relative *OPRM1* mRNA expression levels in SH-SY5Y cells after exposure to '30 min field on continuously' (A) and '15 min field-on/15 min field-off' (B) electromagnetic fields (EMF, 50 Hz, 0.50 mT) at 0, 2, and 4 hours post exposure. Comments: $n = 3$, mean ± *SE*.

posure, the *PDYN* mRNA expression levels showed no significant difference in cells exposed to ELF-EMF compared to control cells ($F = 0.09$; d$f = 3, 8$; $P = 0.962$; Figure 1B).

The relative expression levels of *OPRM1* in the continuous exposure (Figure 2A) and in the intermittent exposure conditions (Figure 2B) showed no significant alteration after any of the examined treatments (continuous exposure condition: $F = 1.21$; d$f = 3, 8$; $P = 0.366$; intermittent exposure condition: $F = 0.21$; d$f = 3, 8$; $P = 0.886$).

5. DISCUSSION

PDYN plays an important role in neuropsychiatric diseases such as drug abuse.[1-3] The low repeated alleles of variable number of tandem repeats of *PDYN* showed approximately 50% lower transcriptional activity compared to high repeated ones[5] and were associated with increased risk of dependency to heroin.[6]

It has been shown that heroin increases the production of ROS.[30,31] Furthermore, drug abusers are at oxidative stress.[32,33] Interestingly, the mRNA expression levels of several anti-oxidant genes significantly decreased in SH-SY5Y cells treated with morphine.[34] It has been reported that oxidative stress plays an important role in drug dependency. Previous studies have demonstrated that ELF-EMF is associated with production of ROS.[16–18] Moreover, reduced *PDYN* expression in the human brain is associated with opiate dependency.[35,36] Interestingly, in the present study the continuous exposure condition of ELF-EMF was associated with the lower expression levels of the *PDYN*. It may indicate that in cells exposed to ELF-EMF the *PDYN* expression level significantly decreased via production of ROS. Consequently, it might be concluded that exposure to ELF-EMF may appear as a risk factor for using illegal drugs.

However, the present study has some limitations; there is no data available on experimental animal models and human subjects exposed to ELF-EMF. In the present study, only one intensity and two exposure conditions of the ELF-EMF were investigated. Moreover, there is no data dealing with the protein level of the selected genes. It will be interesting to further investigate the effects of other intensities and conditions of ELF-EMF exposure on opioid system related genes. Moreover, epidemiological studies should help to elucidate the association between exposure to ELF-EMF (environmentally and/or occupationally) and the risk of drug dependency.

6. CONCLUSIONS

In the present study we demonstrate that the continuous exposure condition of ELF-EMF is associated with a lower expression level of the *PDYN*. Our data suggests that in cells exposed to ELF-EMF the *PDYN* expression levels significantly decreased via production of ROS. Epidemiological studies should help to elucidate the association between exposure to ELF-EMF and the risk of drug dependency.

Conflict of interest

No competing interests are declared by any of the authors.

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