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Cell Biology International 33 (2009) 419–423

Cell Biology International

www.elsevier.com/locate/cellbi

Short communication

Effect of 60 Hz electromagnetic fields on the activity of hsp70 promoter: An *in vitro* study

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Received 20 March 2008; revised 10 June 2008; accepted 29 September 2008

Abstract

We have evaluated the effect of 60 Hz sinusoidal magnetic fields (MF) at 8 and 8 μT on expression of the luciferase gene contained in a gene construct labelled as Electromagnetic Field-plasmid (pEMF). The vector included the hsp70 promotor containing the 3 nCTCTn sequences previously described for the induction of hsp70 expression by magnetic fields, as well as the reporter of the luciferase gene. We also replicated the study of Lin et al. [Lin H, Blank M, Rossol-Haseroth K, Goodman R. Regulating genes with electromagnetic response elements. J Cell Biochem 2001;81(1):143–48]. The pEMF plasmid was transfected into HeLa and BMK16 cell lines that were later exposed to either MF or thermal shock (TS). An increased luciferase expression was found in both the cells exposed to MF and TS compared with their control groups ($P < 0.05$). Furthermore, the combined effect of MF and TS was also analyzed. A synergistic effect between two factors was observed for this co-exposure condition in terms of luciferase gene expression.

Keywords: 60 Hz electromagnetic fields; Hsp70 promoter; Gene expression; Thermal shock

1. Introduction

Extremely low frequency electromagnetic fields (ELF-EMF) can interact with biological systems and several reports emphasize that these fields are able to affect the synthesis of DNA and RNA as well as the cellular proliferation (Marron et al., 1988; Tenforde, 1992; Goodman and Blank, 2002).

Since 1983, however, it has been demonstrated that weak, pulsing electromagnetic fields increase the transcription process in dipteran salivary gland cells, suggesting an activation of DNA (Goodman et al., 1983).

On the other hand, the therapeutic effect of the ELF-EMF has been widely studied over the last few years, focusing on knowing the effect of these fields on genetic expression (Dicarlo et al., 1999; Ronchi et al., 2004). Also, Tokalov and Gutzeit (2004) evaluated the effect of 50 Hz MF on stress genes in the cellular line HL-60 on the expression of hsp27, hsp60, hsp70(A, B and C), hsc70, hsp75, hsp78 and hsp90 genes using RT-PCR. Their results showed an increased hsp70 expression in cells exposed to MF at 60–80 μT. However, they observed that the response to these fields was similar to the one observed when cells received a thermal shock (TS). On the other hand, opposed results were obtained by Coulton et al.

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doi:10.1016/j.cellbi.2008.09.014

(2004) when leucocytes from human blood were exposed to 50 Hz MF in the range of 0 to 100 μT for 4 h, evaluating the expression of the thermal shock genes (hsp27, hsp70 and hsp70B) compared to cells exposed to 42 °C for 2 h. Their results did not show that 50 Hz MF were able to induce the expression of thermal shock proteins. Due to the conflicting results, and the inability to replicate a previous report from Lin et al. (2001) who earlier described that hsp70 promoter contains specific sequences for the induction of expression by magnetic fields, we undertook to evaluate the effect of 60 Hz EMF on the hsp70 activity. The combined effect between ELF-EMF and TS was also analyzed for this co-exposure condition in terms of luciferase gene expression.

2. Material and methods

2.1. Gene construct

The vector pNFKB-luc (cat # 631904, Clontech) was used after removal of the original promoter by using the enzymes HindIII and Nhel. The promoter hsp70 obtained from human whole blood using PCR was inserted. The resultant vector was called pEMF (Electromagnetic field-plasmid). This vector also has the luciferase gene with a length of 1689 bp, and a signal for polyadenilation of SV40 and the ampicilin-resistance gene.

2.2. Cell culture and transfection

HeLa (human adenocarcinoma cell line was obtained from American Type Culture Collection, Manassas, VA) and BMK16 (Baby Mouse Kidney cells) were plated at 2×10^5 cells/ml on Falcon dishes and grown in RPMI 1640 medium (Life Technologies, Gaithersburg, MD) with 10% fetal calf serum (Gibco/BRL, Gaithersburg, MD) at 37 °C. The cells were transfected using a transfection mixture (Invitrogen # Cat. 18324-111). In the first test tube 1.0 μg of DNA were added to 100 μl of DMEM-serum free medium. In the second test tube, 10 μl of lipophectamine were added to 100 μl of DMEM-serum free medium. The pre-acomplejed DNA mixture was diluted with the lipid mixture and was then incubated for 15 min at room temperature. After the complexes were formed, the medium where the cells were grown was changed with 100 μl of serum free medium. After this, the complex of the DNA mixture with lipids and fresh medium was added to the cells and incubated at 37 °C under 5% of CO_2 for 3 h. After this time, 1.0 ml of culture medium with 10% fetal calf serum was added, and then after 48 h the cells were exposed to 60 Hz MF, TS, or combined exposure.

2.3. Magnetic field exposure facilities and measurements

Transfected cell cultures were incubated at 37 °C and exposed to 8 and 8 μT for 20 min. A coil was built by winding 340 turns of 1.3 mm diameter enamel insulated copper wire to form a cylindrical solenoid with a radius of 5.27 cm and a length of 25 cm. This solenoid was connected to a step-down transformer and to a variable transformer that was plugged in to a 110 V AC source. This exposure system was placed inside a dry incubator set at 37 ± 0.1 °C. Cultures were allocated in the middle of the solenoid where the MF was homogeneous, an equal number of sham treated cultures were used as a negative control and placed into an MF device of identical design as the one mentioned above, but it was turned off.

Magnetic flux density (rms) was measured using an axial Hall effect probe (Bell FW 6010 gaussmeter, Orlando, Fl, USA). An oscilloscope (BK-Precision model 2120) was coupled to the system to monitor the resulting field, and a 60 Hz alternating sinusoidal MF was generated. The magnetic flux density was monitored during the experiments by using an appropriate unshielded probe coil calibrated with the gaussmeter. The local geomagnetic field was also measured, setting the gaussmeter in DC mode and using an axial high sensitivity Hall probe (Integrity Design IDR-321 geomagnetometer, Essex Jct., VT). The average value was 20 μT within the exposure room.

2.4. Heat shock conditions

Cells in Falcon flasks were immersed in water bath at 43 °C for 20 min, and then removed and maintained at 37 °C for an additional 30 min period before lysate was prepared.

2.5. Luminescence assays

Cells that received the MF or TS treatments and their controls were processed according to the luciferase assay system (Promega, Madison, WI, USA) as described by Martin et al. (1996). Briefly, cellular lysates were assayed for luciferase activity by the addition of 20 μl lysate to 100 μl luciferin substrate. Luminescence was measured using a MGM Instruments model Optocomp I Luminometer (Serial no. 202379). Total protein was precipitated from 50 μl lysate with 10% trichloroacetic acid and dissolved in 0.1 N NaOH. Protein concentrations were determined using the principle of dye binding (Bradford, 1976). Luciferase activity was adjusted for protein content by dividing the relative light units by the protein concentration (RLU/mg of protein).

2.6. Experimental schedules

To evaluate MF effects on luciferase gene expression in Hela cell line at 8.0 and 80.0 μT, 2 independent experiments were performed. Each assay included the following treatment regimens: (a) non-transfected cells as a control and without magnetic or thermal treatments, (b) pEMF-transfected cells but not exposed to MF or TS, (c) transfected cells treated with a TS, (d) transfected cells exposed for 20 min to MF.

To evaluate MF effects on BMK16, one experiment was carried out on 5 groups: (a) non-transfected cells as a negative control and without MF or heat shock treatments, (b) pEMF-transfected cells without MF exposure or TS treatment, (c) transfected cells treated with a TS, (d) transfected cells treated with MF at 8.0 μT, and (e) transfected cells exposed to MF at 8 μT.