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Elevated Levels of Stress Proteins (Hsp32 and Hsp70i) in H9c2 Cells Exposed to 60Hz, 120μT Magnetic Field

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1 Introduction

The purpose of this study was to determine the effect of electromagnetic fields on cellular heat-shock protein expression.

2 Materials and Methods

The embryonic heart derived cell line H9c2 used for the study was grown in 75cm^2 flasks in DMEM with 10% FBS and 5% CO_2 . Cells were exposed to a 120 μT magnetic field for 2 hours by placing them within paired Helmholz coils in a 37^{o}C cell culture incubator. Control cells grown under identical conditions were not exposed to the magnetic field. Positive control samples were exposed to heat for 30minutes or 1hour. Following EMF or heat exposure cells were incubated at 37^{o}C for different time periods until the cells were harvested for RNA or protein extraction. Cytosolic RNA extraction was performed following the protocol specified by Qiagen RNeasy Mini Kit. RT-PCR was performed using custom designed hsp32 and hsp70i primers and transcripts quantified. Proteins were extracted using a modified RIPA lysis buffer followed by separation on a 10% SDS- polyacrylamide gel and blotted and probed with specific Mouse monoclonal anti-Hsp32 and 70i.

3 Results

Exposure to Extremely Low Frequency Electromagnetic Fields has been shown to produce a variety of biological responses in different cell types, tissues and animal models. Experimental evidence has demonstrated that such responses exhibit well-defined characteristics, including dose and time dependence ("temporal sensing"), which suggest a possible receptor-mediated response via signal transduction pathways although the exact mechanism of interaction between EMF and the cell is not yet well understood. Chicken embryos exposed to a weak, 60 Hz, magnetic field (MF) have shown nearly double the survival to hypoxia-reoxygenation than did embryos with no MF exposure prior to hypoxia. Similar results were seen with rat-heart derived H9c2 cells exposed to hypoxia-reoxygenation. These results suggested that MF exposure might precondition myocardium against the damaging effects of ischemia and reperfusion that occur with myocardial infarction and restoration of coronary blood flow, respectively. Our current hypothesis is that this is brought about by up-regulation of stress proteins. Consistent with our hypothesis we found transcripts levels of stress proteins-hsp32 and hsp72 increase a few hours after exposure to MF. While both transcripts showed elevated levels immediately after 2 hours of MF exposure, their levels declined after 2, 4 and 6 hours of incubation post exposure. The protein levels were less consistent and Hsp 32 showed a two-fold increase 12 hours after EMF exposure. Surprisingly Hsp70i levels did not show any appreciable increase after exposure.

4 Conclusion

These preliminary studies suggest the role of stress proteins in magnetic field induced cellular responses.

Future work using real time (quantitative) RT-PCR (already underway) will help to determine the kinetics and specificity of the MF-induced response. Post hypoxia exposures to Magnetic field have also been designed to look into possible protective

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effects of magnetic filed subsequent to an ischemic episode.

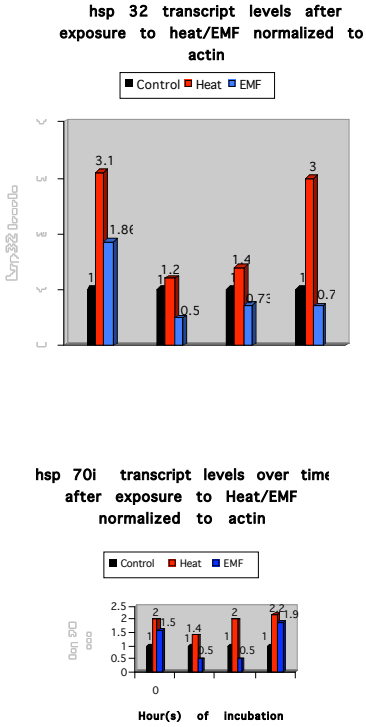

Figure 1 : Transcript levels of hsp32 and hsp70i after varying time period post EMF exposure.

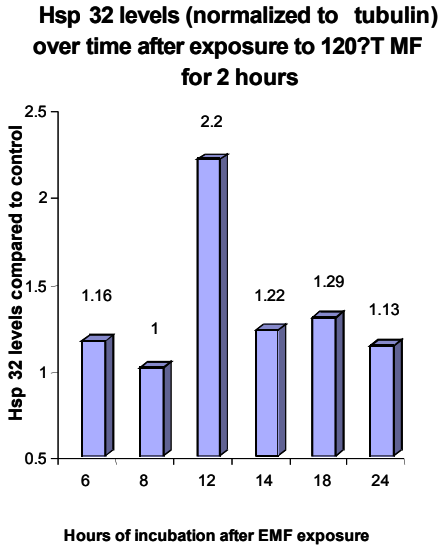

Figure 2 : Protein levels of hsp32 after varying time period post EMF exposure.