Four weeks of close proximity to the 4.7T magnet does not stimulate heat shock protein expression in the rat brain

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Introduction. Directive 2004/40/EC of the European Parliament and of the Council on the minimum health and safety requirements regarding the exposure of workers to the risks arising from physical agents (electromagnetic fields) evoked discussion concerning possible impact of magnetic resonance equipment on health of NMR personnel. We are not aware of any data in this respect, although a single report by Guisasola et al. [1] suggested that exposition to magnetic field during MRI procedure does not induce cellular stress *in vitro* as measured by heat shock proteins, their messenger ribonucleic acids, and adenosine-3',5'-cyclic monophosphate. Heat shock proteins (Hsp) are 'molecular chaperones' that serve multiple functions such as preventing protein misfolding and inhibiting apoptosis. The level of these proteins inside cells may be considered as a marker of cellular stress. In unstressed tissues some of them (e.g. Hsp70) are abundant while the other (e.g. Hsp25 in rats and its human homologue Hsp27) are barely detectable. Generally Hsp are induced by various cellular stressors through activation of a specific transcription factor called Heat Shock Factor (HSF) which binds to the Heat Shock Element (HSE) of the heat shock-inducible genes. Their pattern of expression depends on the kind of stressor. In the present study we attempted to reveal a possible effect of close proximity of an animal MRI/MRS system on the expression of heat shock proteins Hsp70 and Hsp25 in the rat brain.

Methods and Materials. The 4.7 T superconducting Bruker magnet of 310 mm bore which has the gradient of the order of 1 T/m to 0.5 T/m in front of the bore was used to expose animals to the EM fields. 18 male Wistar rats (young adult, 175 g body weight at the beginning of the experiment) were randomly divided two groups and placed in animal cages, 3 animals per cage. The animals belonging to the experimental group were kept at the front of the aforementioned magnet for 17 h per day on the average, for 4 weeks. The cages were positioned directly in front of the bore of the magnet. The animals belonging to the control group were housed in the same room, but outside the Faraday cage of the magnet. At the end of the 4-week long exposure the animals were anesthetized with ketamine-xylazine mixture, and decapitated. The brains were dissected into 4 regions: cortex, cerebellum, medulla oblongata and thalamus and snap-frozen. Tissue specimens were homogenized with a ultrasonic processor (Cole-Parmer) in 10 volumes of 20 mM Tris-HCl buffer pH 7.4 containing 150 mM NaCl, 1 mM EDTA, 1mM EGTA, 1% Triton X-100, 2.5 mM sodium orthovanadate, 1 mg/mL leupeptin, 1 mM Pefablock (Fluka) and centrifuged at 3 000 x g. Homogenates were frozen and stored at -70C until assay. The content of heat shock proteins Hsp70 and Hsp25 in homogenates was measured by ELISA kits (R&D Systems). Hsp concentration was standardized to total protein concentration in the sample as determined by Bradford method. The significance of the interregional differences and the effects of exposure to EM field on Hsp70 and Hsp25 level was assessed by two-way ANOVA and *post hoc* Scheffe test.

Results. Hsp70: Two-way ANOVA showed no significant effect ($P>0.6$) of magnetic field exposure, but protein levels in different brain regions differed significantly ($P<0.05$; Fig. 1). **Hsp25:** Two-way ANOVA showed a significant effect of both exposure to the magnetic field ($P<0.01$) and brain region($P<0.001$). However, *post hoc* Scheffe test did show no significant difference between control and experimental group for any of the brain regions (Fig. 2).

Conclusion. Although Hsp70 appears to be a sensitive marker of cellular stress, since over-expression of Hsp70 was noted after application of many stressful stimuli including stressful social situations [2], we were unable to show any significant effect of one-month exposure of rats to the 4.7T magnet at close proximity on this protein expression in the brain. However, one-month exposure to magnetic field resulted in slight increase of Hsp25 level in the brain tissue. Hsp25 and Hsp70 reveal different patterns of expression. It seems that magnetic field stimulates Hsp25 more efficiently than Hsp70 expression, but the mechanisms involved are unknown. On the basis of our results we are unable to conclude that 1-month exposure of rats to the 4.7T magnet produces cellular stress in the rat brain. This issue needs further evaluation. Our results clearly show that, when attempting to evaluate potential stressful condition at the cellular level, examination of multiple biological markers may be essential.

References

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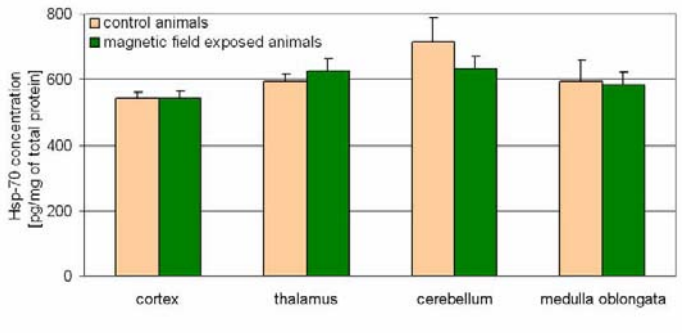

Fig. 1. Hsp70 expression in in brain regions of control and magnetic field exposed animals. Results are expressed as means ± SEM.

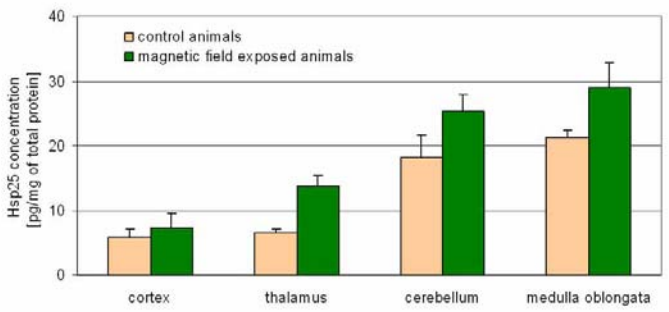

Fig. 2. Hsp25 expression in brain regions of control and magnetic field-exposed animals. Results are expressed as means ± SEM.