

## **Effects of Hypothermia on RBM3 and CIRP Cold-Shock Proteins Expression in Murine Organotypic Hippocampal Slice Cultures**

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Therapeutic hypothermia has emerged as a highly effective neuroprotective therapy for cardiac arrest survivors. There are a number of purported mechanisms, but the exact mechanism still remains to be elucidated.

Although hypothermia generally down-regulates protein synthesis and metabolism in mammalian cells, a small subset of homologous (>70%) cold-shock mRNAs and proteins (RBM3, RNA-binding motif protein 3 and CIRP, cold-inducible RNA-binding protein) are induced under these conditions. In this study, we compared the effects of moderate (33.5°C) and deep (17°C) hypothermia with standard normothermia (37°C) on the regulation of RBM3 and CIRP mRNA and protein expressions in organotypic slice cultures from mouse hippocampus (OHSC).

### Methods

Organotypic hippocampal slice cultures (OHSC) were prepared from C57BL/6 mice postnatal 5 days old. Brain slices were incubated under moderate (33.5°C) and deep (17°C) hypothermia in a specially developed incubator in an atmosphere of 5% CO<sub>2</sub> at 100% humidity for a maximum of 48 h. Normothermic control cells were incubated at 37°C throughout the experiments. Real-time RT-PCR, Western Blotting, and MTT Assay were also performed.

### Results

Exposure to moderate hypothermia resulted in a significant up-regulation of both RBM3 and CIRP mRNAs in the murine OHSC, as compared to normothermia control. Up-regulation in mRNAs started after 4 hours cooling and continued to increase till experimental end (48 hours). Interestingly, exposure to deep hypothermia did not result in a significant increase in RBM3 or CIRP transcripts. RBM3 protein expression was also significantly up-regulated by moderate hypothermia, but starting after 24 hours exposure and remained elevated till experimental end. No significant up-regulation of CIRP protein expression was observed in the slice cultures at any time points.

### Conclusion

We observed that RBM3 gene and protein expressions in brain slices were significantly up-regulated upon exposure to moderate hypothermia. These findings further support the implication of RBM3 as a potential effector for hypothermia-induced neuroprotection.