Changes in Protein Expression in Spinal Neuronal Cultures Subjected to Pulsed Magnetic Field Stimulation

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INTRODUCTION: Previous studies have demonstrated that pulsed electromagnetic fields (PMF) can enhance and influence neurite outgrowth along the direction of the induced current in vitro. The purpose of this study was to determine the mechanisms by which PMF is able to influence neurite outgrowth. The hypothesis is that the expression of cellular proteins involved in neurite outgrowth is affected by PMF stimulation. Magnetic field stimulation of neural cellular components holds promise as a viable form of treatment to increase function following spinal cord injury. The mechanism of this functional enhancement is still unknown.

METHODS: Dissociated dorsal root ganglion (DRG) sensory neuron cultures from 15-day rat embryos were established and placed into an incubator equipped with copper coil energized by a waveform generator driving a power amplifier. Control cultures were placed in an incubator equipped with a sham coil. After an initial culture period of 12 hrs to allow for sensory neuron attachment to the culture dish surface, the coils were energized for 18 hrs, followed by a post-exposure period of 18 hrs. Total incubation time was 48 hrs. At termination, PMF-stimulated and unstimulated DRG cultures were harvested into a lysis buffer and total protein was extracted. After quantification, total protein was loaded into 96-well plates and ELISAs were run using commercially available antibodies to alpha-1A (calcium channel protein), alpha-1B (calcium channel protein), c-jun (DNA binding protein), calbindin (calcium binding protein), calmodulin (calcium binding protein), GAP-43 (growth cone associated protein), MAP-2 (microtubule associated protein), NF-200 (neurofilament protein), NF-68 (neurofilament protein), SMI-31 (neurofilament H protein), SMI-32 (neurofilament H protein) and tubulin (cytoskeletal protein).

RESULTS: The overall effect of PMF stimulation appears to be a decrease in expression for the proteins examined. This decrease in expression was not significant for the voltage-gated calcium channel proteins, the calcium binding proteins, tubulin, GAP43 or c-jun, indicating that PMF stimulation has little effect on intracellular signaling or growth cone formation. Significant differences were observed for NF-200 (p=0.045), NF-68 (p=0.0007) and SMI-31.
(p=0.0036) and near significant differences were observed for MAP2 (p=0.067) and SMI-32 (p=0.061). Most of these proteins are involved in neurofilament organization.

CONCLUSIONS: Neurofilament triplet proteins and microtubule-associated proteins have been shown to decrease after neuronal injury. Further down-regulation as a result of PMF stimulation may be a mechanism for the directed outgrowth of sensory neuron processes observed under our culture conditions. Understanding the mechanism of how PMFs can influence the outgrowth from neuronal cells could lead to a means by which injured and axotomized spinal neurons could be coaxed to re-grow across an injury site. This research was supported by the Richard and Barbara Raynor Medical Foundation and the Zablocki VA Medical Center.

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