Introduction

Spinal cord injuries (SCI) are suffered by approximately 250,000 and 500,000 people each year according to the World Health Organization. Even with modern medicine, spinal injuries are impossible to cure. Spinal injuries sever the neurons in the spine, disrupting the pathway the brain uses to send commands to various parts of the body. As a result, communication with neurons below the site of injury is permanently altered. One method used to attempt to restore function to the affected parts of the body is to inject a virus that expresses a protein that promotes axon growth into the motor cortex. This is done so that new connections can form around the permanent damage created by the injury. However, the newly sprouted axon-to-cell connections can be mistargeted. To solve this issue, proteins, such as the calcium-binding protein calbindin, are used to identify and study where the connection became misdirected.

Structure of Calbindin: A calcium-binding protein found in interneurons.

Calbindin is composed of 3 EF-hand domains, each connected by hydrophobic contacts to amino acids on each arm. Ile19, trp20, and phe23 from the first arm make contact with leu36, leu39, and ala46 on the second hand while an EF-hand loop is made by contacts between asp24 and ala46. The two cystine residues, cys100 and cys219 play an important role in calcium binding in the protein. Each EF-hand domain contains two α-helices. The α-helix of each of each pair is differentiated as a light or dark shade of green, orange, or purple.

Spinal cord function as it relates to spinal cord injury.

- Spinal cord injuries affect motor function beneath the site of injury
- Injuries higher on the spinal cord are associated with greater motor damage
- Newly sprouted axon-to-cell connections, synaptogenesis can be mistargeted following SCI
- Study of the expression of postsynaptic cell markers allows visualization of post-synaptic cell type (calbindin is one of these markers)

Immunohistochemistry is used to identify interneurons in the spinal cord.

Immunofluorescence methods. Spinal cord slices are cut to 30um and mounted on a slide. This tissue is first incubated with the calbindin-specific primary antibody, followed by an fluorescently-tagged secondary antibody; The secondary antibody specifically binds to the primary. A confocal microscope is used to visualize the fluorescently labeled cells. This schematic highlights C (calbindin) and P (parvalbumin) which are markers for different types of interneurons. Immunofluorescence methods are used to specifically identify calbindin-positive cells.

Calbindin identifies a subset of neurons in the ventral horn of spinal cord.

Summary

- Antibodies locate calbindin to make a map of the spinal cord which will help to identify axonal targeting in injured and uninjured tissue
- Further research may lead to the identification of therapeutic targets for appropriate synaptic integration
- Research of this nature is necessary to create a foundation for treatment of spinal cord injuries

References:

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