

Major: Biochemistry
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Funded by: Life Sciences Undergraduate Research Opportunity Program

The mechanism by which potassium causes neurite retraction in lamprey descending neurons in cell culture

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Severe spinal cord injury (SCI) disrupts descending axons from reticulospinal (RS) neurons that project to the spinal cord. In most "higher" vertebrates, including humans, recovery is very minimal due to limited regeneration in the central nervous system, and paralysis is usually permanent below the injury site. In several lower vertebrates, including the lamprey, behavioral recovery is almost complete following SCI due to robust axonal regeneration. To study the cellular and molecular mechanisms that regulate axonal regeneration, neurons are often isolated in cell culture so that the factors that influence neurite outgrowth can be studied under controlled conditions. In our laboratory, we have developed a cell culture system in which neurite outgrowth of RS neurons can be studied (Hong et al., 2002; Ryan et al., 2004). Application of glutamate, an excitatory neurotransmitter, to the growth cones of RS neurons results in neurite retraction, presumably because of depolarization, calcium influx, and an increase in intracellular calcium. Intracellular calcium is thought to be one of the important regulators of the rate and direction of neurite outgrowth. Calcium influx could result from at least two different channels: chemically-gated channels (e.g. NMDA channels); or voltage-gated calcium channels. The purpose of the present study was to determine if calcium influx via voltage-gated calcium channels is sufficient to elicit neurite retraction. First, focal application of a 31 μM potassium to growth cones of DiI-labeled RS neurons in culture to open voltage-gated calcium channels significantly reduced neurite growth rates, including neurite retraction, compared to pre-control periods. Second, 2 μM of Co^{++} or 300 μM Cd^{++} , which block calcium channels, abolished potassium-induced neurite retraction. In conclusion, the results suggest that calcium influx via voltage-gated calcium channels is sufficient to cause neurite retraction. Other experiments will determine if influx through voltage-gated channels is necessary for glutamate to elicit neurite outgrowth. Determination of the factors that regulate neurite outgrowth may provide information about the mechanism by which RS neurons regenerate their axons following spinal cord injury and restore locomotor function.