Public Abstract
Sarah Kathleen Ryan
M.S.
Interdisciplinary Neuroscience Program
Glutamate Regulates Neurite Outgrowth of Descending Neurons in Culture from Larval Lamprey
Advisor: Dr. Andrew McClellan

_____
Advisor's signature

Graduation Term: Fall 2005

In spinal cord-transected larval lamprey, descending brain neurons, most of which are reticulospinal neurons, regenerate their axons across a transection site and mediate behavioral recovery. In the present study, DiI-labeled descending brain neurons in larval lamprey were dissociated and cultured. Glutamate application to neurons elicited neurite inhibition and often retraction, which was abolished by kynurenic acid. Glutamate-induced neurite retraction appeared to result, partially, from calcium influx via voltage-gated calcium channels, since high potassium media inhibited neurite outgrowth, an effect that was blocked by cobalt or cadmium. Glutamate application in the presence of ω-conotoxin MVIIC still inhibited neurite outgrowth, suggesting calcium influx via chemically-gated channels may also contribute. Particularly, NMDA application elicited neurite retraction.

Glutamate application with tetrodotoxin inhibited neurite outgrowth. Interestingly tetrodotoxin alone also inhibited neurite growth, suggesting the neurons may be spontaneously active.

Glutamate-induced neurite retraction may result, partially, from increases in intracellular calcium levels, which possibly involve second messengers (e.g. cAMP). Application of dibutyryl cyclic AMP or Forskolin induced neurite retraction, while 3-isobutyl-1-methylxanthine inhibited neurite outgrowth, suggesting that cAMP is produced in cultured reticulospinal neurons. The agent H89 abolished neurite retraction mediated by glutamate, suggesting that cAMP and protein kinase A are involved in the signaling pathway for glutamate-induced neurite retraction.

Results from the present study suggest that glutamate inhibits neurite outgrowth by acting on glutamate receptors, mediating calcium influx via voltage-gated and chemically-gated channels, increasing intracellular calcium, and activating cAMP. Similar intracellular signaling mechanisms may be important for axonal regeneration following spinal cord injury in the lamprey.

Keywords: growth cone, reticulospinal neurons, regeneration, spinal cord injury