GLUTAMATE REGULATES NEURITE OUTGROWTH OF DESCENDING BRAIN NEURONS IN CULTURE FROM LARVAL LAMPREY

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ABSTRACT

In spinal cord-transected larval lamprey, descending brain neurons, most of which are reticulospinal (RS) neurons, regenerate their axons across a transection site and contribute to 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 be due, in part, to calcium influx via voltage-gated calcium channels, since application of high potassium media inhibited neurite outgrowth, an effect that was blocked by Co^{2+} or Cd^{2+}. Glutamate application in the presence of ω-conotoxin MVIIC still inhibited neurite outgrowth, suggesting calcium influx via chemically-gated channels may also contribute. Particularly, N-methyl D-aspartate (NMDA) application elicited neurite retraction. Glutamate application in the presence of tetrodotoxin (TTX) inhibited neurite outgrowth. Interestingly, TTX inhibited neurite growth, suggesting the neurons may be spontaneously active.

Glutamate-induced neurite retraction may be due, in part, to 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 (IBMX) inhibited neurite outgrowth, suggesting that cAMP is normally produced in cultured RS 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