Fast rearrangement of the neuronal growth cone's actincytoskeleton following VEGF stimulation

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Background

Development of nervous system involves neuronal migration and axonal navigation. Growth cone which is a highly motile structure at the distal tip of growing axon leads to its final destination. Various guidance cues plays crucial role in cytoskeletal rearrangement by forming finger like structure filopodia and flat veil like lamellipodia. Out of these cues vascular endothelial growth factor (VEGF) is the putative factor for angiogenesis or vascular sprouting, also has trophic activity similar to the nerve growth factor (NGF) on neuronal cells. It also stimulates axonal growth and also enhances survival of dorsal root ganglion or cervical neurons.¹ VEGF is known to inhibit hypoxic death of cortical neurons in-vitro² and in cerebral ischemia.³ The signaling by VEGF through its receptor VEGFR2 and NRP-1 (neuropilin-1) have been speculated within the neuronal growth cone.

The author's objective of this study was to investigate the effect of VEGF and NGF on neuronal growth cone and its motility. Further they identified the receptors and downstream signaling pathway by VEGFR2 and NRP-1 by using corresponding antibody and inhibitor axitinib respectively.

Study Design

Dorsal root ganglion (DRG) explant culture has done from 10-day old chicken embryo for 3 days with incubation of VEGF, NGF, VEGF + NGF, axitinib and anti-NRP-1. Immunostaining of NF-H has done and confocal laser scanning microscopy (CLSM) has used for analyzing the neurite outgrowth and growth cone morphology. DRG exposed to the NGF showed difference in axonal radial outgrowth morphology with many neurites. VEGF incubation resulted in enhanced outgrowth whereas by adding VEGF + NGF together lead to the extreme outspread of extensions of neuronal cells to

several micrometers. In contrast, after blocking by antibody and axitinib this effect has blocked.

Dissociated DRG cell culture was performed and cytoskeletal vector was inserted into single neuronal cell by microinjection technique. Growth cone motility has been studied by time-lapse imaging performed after 24 hrs with CLSM. By adding the stimulating factors growth cone circumference motility has increased after 60 minutes and this effect has drastically enhanced when incubated with VEGF + NGF. Blocking by antibody treatment and axitinib were completely reversed these effect.

Implications

The present study has demonstrated that VEGF and NGF have their direct effect on cytoskeletal dynamics by enhancing motility within few minutes, visualized by using transfected neuronal cells by microinjection. This effect enhanced when adding both factors together. VEGFR2 and NRP-1 has identified to be involved in signaling pathway by blocking these by antibody and axitinib treatment.

doi : 10.5214/ans.0972.7531.200307

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