Netrin-1-like Peptides Are Secreted by Tetrahymena thermophila

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Netrin-1-like Peptides are Secreted by Tetrahymena thermophila

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Introduction

Netrin-1 is a neurite outgrowth attractant involved in vertebrate axon guidance processes. In invertebrates, Tetrahymena thermophila, netrin-1 acts as a chemoattractant, causing cells to exhibit a characteristic avoidance behavior. In the ciliated protozoan, Tetrahymena thermophila, netrin-1 is implicated in netrin-1 signaling in some vertebrate cell types. However, none of these inhibitors affected Tetrahymena avoidance to netrin-1. Finally, we wished to answer the question, “is netrin-1 actually serving an autocrine signaling role in Tetrahymena, or is the peptide merely serving as an agonist for another receptor?” In order to answer this question, we prepared a whole cell extract of Tetrahymena using 0.1% SDS. We also washed Tetrahymena in our behavioral buffer and allowed them to sit in that buffer for 24 hours. The Tetrahymena were centrifuged out of the buffer, and the supernatant, containing the proteins which the Tetrahymena had secreted, was kept for ELISA assay. An ELISA, using a polyclonal anti-netrin-1 antibody, was run on the whole cell extract and the secreted proteins against a netrin-1 standard curve. Both the secreted proteins and the whole cell extract tested positive for netrin-1 in the ELISA. Further experimentation will allow us to determine the nature of these netrin-like peptides.

Materials and Methods

For ELISA, a polyclonal goat anti-netrin-1 peptide antibody was used as the primary antibody at a 1:100,000 dilution. HRP-conjugated rabbit anti-goat antibody was used as a secondary antibody at a 1:2,000,000 dilution. Pierce’s Quanta Red was used as substrate. Nitrocellulose was used as a positive control.

Conclusion

- Daidzein studies indicate that blocking of netrin-1 signaling is specific, and is likely mediated by a tyrosine kinase.
- Cross-adaptation studies indicate that netrin-1 does not signal through the same pathways as either GTP or PACAP.
- Netrin-1 signaling in Tetrahymena is unaffected by src, focal adhesion kinase, or Rho kinase.
- ELISA indicates that a netrin-1 like peptide is present both in Tetrahymena whole cell extract, as well as in proteins secreted from Tetrahymena. This indicates that Tetrahymena may be using netrin-1 like peptides to communicate with each other or with other organisms.

References


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