The Chemorepellent, Netrin-1, Appears to Signal Through a Tyrosine Kinase in Tetrahymena thermophila

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Presenters
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Abstract

Netrin-1 is a pleiotropic peptide signaling molecule. Its most well-known role in vertebrate development is neuronal guidance. Depending upon the cell type and signal concentration gradient, netrin-1 may serve either as a chemoa ctactant, causing formation of axonal growth cones, or as a chemorepellent, causing growth cone collapse within the axon. Netrin-1 can bind to at least two types of receptors, and uses a variety of signaling proteins to convey its message. In some vertebrate cell types, the netrin-1 signal is G-protein mediated, while in other cell types, netrin signaling requires a tyrosine kinase or some other combination of kinases in order to signal. *Tetrahymena thermophila* are free-living, eukaryotic cells that can respond to chemotacticants and chemorepellents by moving toward attractants and away from repellents. By studying the behavior of these organisms, we have found that netrin-1 acts as a chemorepellent in *T. thermophila*. Response to netrin-1 is concentration dependent, with an EC_{50} of approximately 1 µM, and an EC_{90} of approximately 10 µM. Netrin-1 avoidance may be effectively eliminated by the addition of the broad-spectrum tyrosine kinase inhibitor, genistein, to the behavioral assay. The IC_{100} of genistein was near 50 µM. G-protein inhibitors, calcium chelators, and a number of other pharmacological inhibitors had no effect on netrin-1 signaling in this organism. These data show that netrin-1 is a chemorepellent in *Tetrahymena thermophila* and that netrin signaling appears to implicate a tyrosine kinase in this organism. Further studies will help us to determine whether genistein is specifically acting upon a tyrosine kinase or whether the inhibition is occurring via some other genistein-mediated effect.

Materials and Methods

Behavioral assays were conducted using a dissection microscope, a 3-well microtiter plate, and a modified Pasteur pipette as described in Mace et al., 2000, and as pictured below.

Netrin-1 Peptide

**KFOQREKKGKCKKA**

Net charge = +6 at pH 7.0

Results

Figure 1. Amino Acid Sequences of Netrin-1 Peptide used in this study show that it is polycationic at our assay pH of 7.0. Positively charged amino acids are shown in red, while negatively charged amino acids are shown in blue.

Table 1—Many other pharmacological inhibitors had no effect on avoidance to netrin-1. This implies that these peptides are not signaling through a G-protein coupled receptor or the previously described polycation receptor (Keedy et al., 2003). Intracellular and extracellular calcium do not appear to be involved in this response.

![Figure 2. Netrin-1 is a chemorepellent effective at micromolar concentrations in Tetrahymena thermophila. The EC_{50} of the peptide was approximately 1 µM.](image)

![Figure 3. Genistein, a broad-spectrum tyrosine kinase inhibitor, blocks behavioral avoidance to netrin-1 in *Tetrahymena thermophila*.](image)

![Figure 4. Cross-adaptation of Netrin-1 Peptide vs. ACTH 6-24 indicates that the two chemorepellents could share some aspect of their signaling pathway.](image)

Table 2. Cross-adaptation of Netrin-1 Peptide vs. ACTH 6-24 indicates that the two chemorepellents could share some aspect of their signaling pathway.

<table>
<thead>
<tr>
<th>Inhibitor Used</th>
<th>Mechanism of Action</th>
<th>Effect on Netrin-1 Avoidance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neomycin sulfate</td>
<td>Competitive inhibitor of polycation receptor</td>
<td>None</td>
</tr>
<tr>
<td>Thapsigargin</td>
<td>Depletes calcium from ER stores</td>
<td>None</td>
</tr>
<tr>
<td>Rp-8</td>
<td>Broad-spectrum G protein inhibitor</td>
<td>None</td>
</tr>
<tr>
<td>Rp-1080</td>
<td>Analogue of UTP, inhibits PKA</td>
<td>None</td>
</tr>
<tr>
<td>U-73122</td>
<td>Phospholipase C inhibitor</td>
<td>None</td>
</tr>
<tr>
<td>OD-0881</td>
<td>Broad-spectrum kinase inhibitor</td>
<td>None</td>
</tr>
<tr>
<td>OD-2025</td>
<td>Guanylyl cyclase inhibitor</td>
<td>None</td>
</tr>
</tbody>
</table>

Conclusions

- Netrin-1 peptide is a chemorepellent in *Tetrahymena thermophila* in the micromolar range.
- Avoidance to netrin-1 peptide is blocked by the addition of genistein, suggesting tyrosine kinase involvement.
- Cross-adaptation of Netrin-1 Peptide vs. ACTH 6-24 indicates that the two chemorepellents could share some aspect of their signaling pathway.
- Using diazolin as a negative control, we hope to ascertain whether the genistein-mediated inhibition is specific.

References


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