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Nociceptin Is a Chemorepellent in *Tetrahymena thermophila*

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Introduction

Tetrahymena thermophila are free-living, ciliated, unicellular eukaryotes that respond to stimuli by moving toward chemoattractants and away from chemorepellents. It is hypothesized that this behavior guides them toward food sources, and away from potential predators.

A number of polycationic peptides are chemorepellents in *T. thermophila*, including lysozyme, PACAP, VIP, and charged fragments of ACTH. A number of human nociceptive peptides are chemorepellents as well, including Substance P and bradykinin.

In this study, we show that the nociceptive peptide, nociceptin, is a chemorepellent in *T. thermophila,* and that a calcium-based depolarization is involved in signaling.

Methods

- Behavioral assays were conducted by individually transferring cells using a modified Pasteur pipette. Cells were transferred from buffer into a solution containing the chemorepellent in question.
- Whole-cell electrophysiology was carried out using a 2-electrode setup. An inverted drop method was used for capturing and recording the cells.

Results

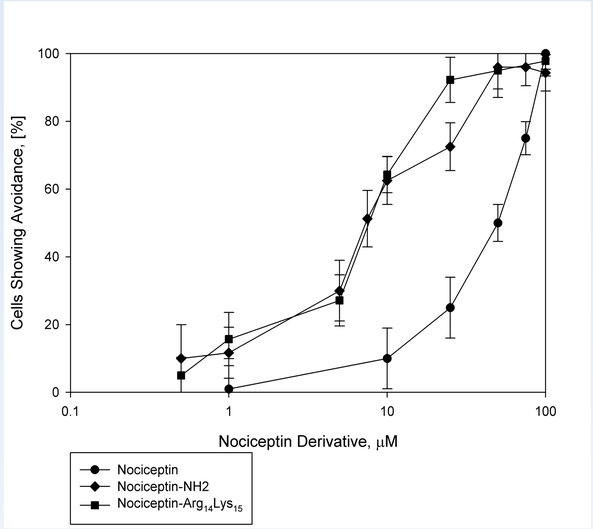

Figure 1. Nociceptin is a chemorepellent in *Tetrahymena thermophila*. Percentage avoidance was determined by scoring groups of ten cells as either positive or negative for avoidance. Number of trials was ≥ 6 (sixty cells total) for each data point. The most positively charged derivative of nociceptin, nociceptin-Arg-Lys (overall charge +6), was a more effective repellent than nociceptin-NH2 (overall charge +5), which was more effective than nociceptin alone (overall charge +4).

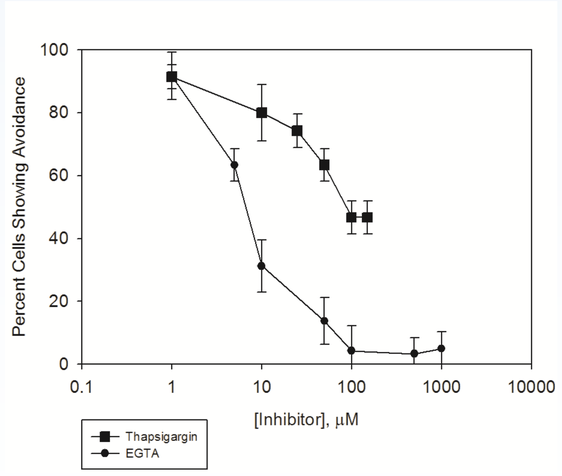

Figure 2. Avoidance to Nociceptin-NH2 is dependent on calcium. Addition of external EGTA to the buffer completely eliminated avoidance, implicating extracellular calcium in signaling. Addition of thapsigargin to the buffer reduced avoidance, implicating intracellular calcium stores in signaling.

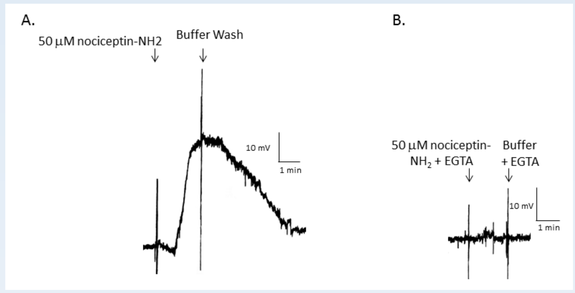

Figure 3. Nociceptin causes a depolarization in *Tetrahymena thermophila* which is nearly eliminated by the addition of EGTA. Representative traces of whole-cell electrophysiology experiments are shown. **A.** The depolarization caused by the addition of 50 μM nociceptin-NH2 was approximately 30 mV. **B.** Addition of 100 μM EGTA to the buffer nearly eliminated this depolarization.

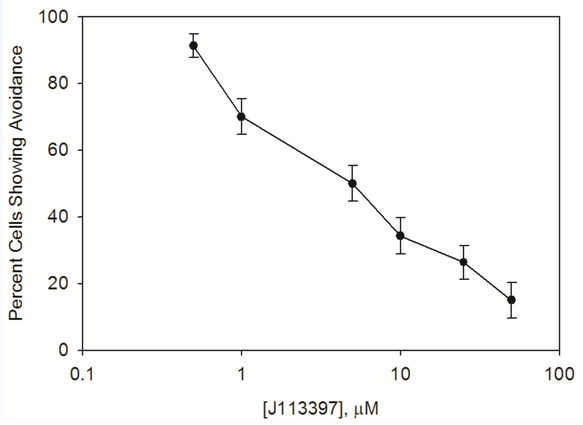

Figure 4. J113397, a competitive inhibitor of the human nociceptin receptor, blocks nociceptin avoidance in *Tetrahymena thermophila*. This suggests that the polycation receptor of *Tetrahymena* may share a similar ligand binding region with the nociceptin receptor of humans.

Conclusions

- Nociceptin is a chemorepellent in *Tetrahymena thermophila*.
- Charge is important in signaling. More highly charged species of nociceptive were more effective repellents.
- Intracellular and extracellular calcium both appear to contribute to nociceptin signaling.
- A competitive inhibitor of the human nociceptin receptor, J113397, inhibits nociceptin avoidance in *Tetrahymena thermophila,* suggesting some shared features between the nociceptin binding proteins in these two species.
- GPCR antagonists and tyrosine kinase inhibitors do not affect nociceptin avoidance in *Tetrahymena thermophila* (data not shown). This contrasts with humans, where GPCRs are involved in nociceptin signaling.

Contact Information

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