12-2006

Characterizing the Polycation Receptor of Paramecium

Eric D. Robinette

Heather G. Kuruvilla
Cedarville University, heatherkuruvilla@cedarville.edu

Recommended Citation

Robinette, E.D., Kuruvilla, H. G. Characterizing the polycation receptor of *Paramecium*. Mol. Biol. Cell 17 (supplement), Presented at the 46th Annual ASCB Conference, December, 2006.

Characterizing the Polycation Receptor of *Paramecium*

Eric D. Robinette, Heather G. Kuruvilla

Department of Science and Mathematics, Cedarville University, Cedarville, OH 45314 USA

Introduction

Unicellular eukaryotes are complex systems, performing all the tasks needed for survival within the context of a single cell. Protozoans, such as *Tetrahymena* and *Paramecium*, use chemosensory systems to detect food and to avoid predation.

Both *Tetrahymena* and *Paramecium* have been used as models for studying chemorepellents. Lysozyme, ATP, and GTP have been found to have chemorepellent activity in both ciliates. In *Tetrahymena*, several PACAP isoforms have been shown to bind to the same receptor as lysozyme, indicating that this receptor may be a more general "polycation receptor" (Keedy et al., 2003). The polycation receptor in *Tetrahymena* appears to be a G-protein linked receptor which activates adenylyl cyclase and phospholipase C (Keedy et al., 2003).

The lysozyme receptors have been affinity purified from both *Paramecium* and *Tetrahymena*. The molecular weight of the *Tetrahymena* protein is approximately 42 kD, while the molecular weight of the *Paramecium* protein is approximately 58 kD (Kuruvilla and Hennessey, 1998).

In our current study, we examined the hypothesis that *Paramecium* have a polycation receptor similar to that of *Tetrahymena*. We found that although both organisms bind similar ligands, there are multiple differences between the two pathways (see Conclusions).

Materials and Methods

Tetrahymena thermophila, strain B2086, and *Paramecium tetraurelia,* strain nd6, both generous gifts of T.M. Hennessey (SUNY Buffalo) were used in this study.

Behavioral studies were conducted as described by Hennessey *et al.,* 1995. Briefly, individual cells were transferred from buffer into the polycation of interest, under a dissection microscope. Cells were then observed to determine whether backward swimming occurred. Ten cells were observed for each trial. At least three trials were done for each concentration point.

When inhibitors were used, cells were exposed to the inhibitor for one to two hours prior to exposure to the polycation of interest in order to allow for uptake of the inhibitor.

Polycation Avoidance in *Paramecium* differs widely from that of *Tetrahymena*

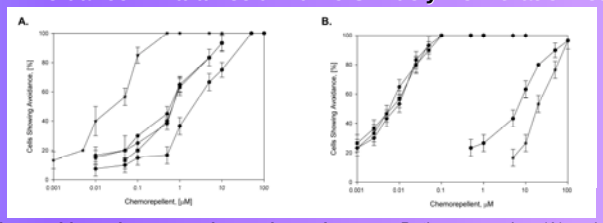

Figure 1. Polycation avoidance in *Paramecium* and *Tetrahymena*. Both *Paramecium* (A) and *Tetrahymena* (B) avoid VIP (circles), PACAP 1-27 (squares), PACAP 1-38 (diamonds), PACAP 6-27 (hexagons) and lysozyme (stars). Each point represents the mean ± SD of three trials. **A.** The concentration of ligand required for 100% avoidance in *Paramecium* is 50 μM VIP, 10 μM for PACAP 1-27 and 1-38, and 0.5 μM for lysozyme. We could not obtain 100% avoidance with PACAP 6-27 because of its toxicity. The EC50 of these polycations in *Paramecium* was approximately 3 μM for VIP, 0.75 μM for all PACAP isoforms, and 0.1 μM for lysozyme. **B.** The concentration of ligand required for 100% avoidance in *Tetrahymena* is 100 μM VIP, 0.1 μM for all PACAP isoforms, and 100 μM for lysozyme. The EC_{50} of these polycations in *Paramecium* was approximately 7.5 μM for VIP, 0.05 μM for all PACAP isoforms, and 50 μM for lysozyme (see also Keedy *et al.,* 2003).

The Pharmacological Profiles of the Receptor Also Differ Between the Two Organisms

Table 2. Summary of Pharmacological Data in *Tetrahymena* and *Paramecium*.

Inhibitor	*Tetrahymena*	*Paramecium*
GDP-β-S	Inhibits at 1 mM[1]	No effect on avoidance at 1 mM[3]
GP-Antagonist-2 (BIOMOL™)	Toxic to cells	No effect on avoidance at 100 μM[3]
Rp-cAMPs	Inhibits at 50 μM[1]	No effect on avoidance at 100 μM[3]
Neomycin Sulfate	Inhibits at 5 μM[1]	Inhibits electrophysiological response to lysozyme at 10 μM[4]
U-73122	Inhibits at 1 μM[2]	Toxic to cells[3]
Genistein	No effect on avoidance at 100 μg/ml[1]	Inhibits at 0.1 μg/ml[3]
Daidzein	No effect on avoidance at 100 μg/ml[1]	No effect on avoidance at 100 μg/ml[3]

[1]Keedy et al., 2003. [2]Bartholomew et al., unpublished data [3]Robinette and Kuruvilla, unpublished data [4]Hennessey et al., 1995

The Tyrosine Kinase Inhibitor, Genistein, Eliminates Lysozyme Avoidance in *Paramecium*

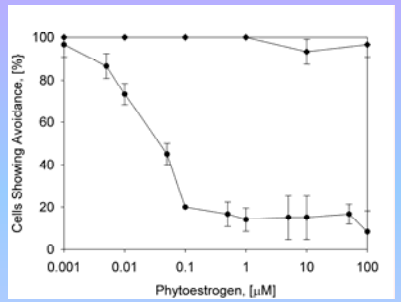

Figure 2. Genistein (closed circles) eliminates behavioral avoidance to 0.5 μM lysozymein *Paramecium*, while daidzein (closed diamonds), a control, has no effect on avoidance. Each point represents the mean ± SD of ≥three trials. Each trial consisted of ten cells which were individually scored for avoidance.

Cross-Adaptation between lysozyme and members of the PACAP family suggest that these ligands use the same receptor and/or second messenger pathway.

Table 1. Cells were adapted to either 0.5 μM lysozyme or 50 μM VIP for 15 minutes prior to exposure to 0.5 μM lysozyme, 50 μM VIP, 10 μM PACAP 1-38, or 10 μM PACAP 1-27. Cells were not adapted to any of the PACAP isoforms because prolonged exposure to high concentrations of PACAP is lethal to *Paramecium*. Each trial represents 10 cells. A minimum of three trials was conducted for each concentration. These data are similar to results previously obtained for *Tetrahymena* (Keedy *et al.,* 2003)

	Lysozyme	PACAP 1-38	PACAP 1-27	VIP
Lysozyme	15 ± 5%, N = 4	20 ± 10%, N = 3	20 ± 10%, N = 3	23.3 ± 5.8%, N = 3
VIP	15 ± 5%, N = 6	6.6 ± 5.8%, N = 3	16.6 ± 5.8%, N= 3	10 ± 0%, N = 3

Conclusions

- The polycation receptor of *Paramecium* appears to have a higher affinity for lysozyme than for PACAP, while the polycation receptor of *Tetrahymena* has a higher affinity for PACAP than for lysozyme, as indicated by behavioral studies.
- Both receptors have a relatively low affinity for VIP, as indicated by behavioral studies.
- Both organisms show cross-adaptation between the various polycations tested (lysozyme, PACAP, VIP), indicating that a single receptor is probably interacting with all of these polycations.
- In *Tetrahymena*, pharmacological studies indicate that the polycation receptor is a G-protein linked receptor. However, in this study, G-protein inhibitors failed to have any effect on lysozyme avoidance in *Paramecium*.
- *Paramecium* avoidance to lysozyme is inhibited by the tyrosine kinase inhibitor, genistein. This inhibitor has no effect on lysozyme/PACAP avoidance in *Tetrahymena.*
- The receptors in the two organisms exhibit very different characteristics, including molecular weight, interaction with ligands, and interaction with second messenger pathways.

References

1. Hennessey, T.M., Kim, M.Y., Satir, B.H. 1995. Lysozyme acts as a chemorepellent and secretagogue in *Paramecium* by activating a novel receptor-operated Ca^{2+} conductance. J. Membrane Biol. 148: 13-25.
2. Keedy, M., Yorgey, N., Hilty, J., Price, A., Hassenzahl, D., Kuruvilla H. 2003. Pharmacological evidence suggests that the lysozyme/PACAP receptor of *Tetrahymena thermophila* is a polycation receptor. Acta Protozool. 42: 11-17.
3. Kuruvilla, H.G., Hennessey, T.M. 1998. Purification and characterization of a novel chemorepellent receptor from *Tetrahymena thermophila. J. Membrane Biol.* 162: 51-57.
4. Kim, M.Y., Kuruvilla, H.G., Hennessey, T.M. 1997. Chemosensory adaptation in *Paramecium* involves changes in both repellent binding and the consequent receptor potentials. Comp. Biochem. Physiol. 118A(3): 589-597.