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ACTH: The Uninhibitable (or is it)?

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ACTH—the Uninhibitable (or is it?)

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Abstract

Adrenal corticotropic hormone, or ACTH, is a peptide hormone secreted by the anterior pituitary gland. The full-length peptide is 39 amino acids long. ACTH signals through a G-protein linked receptor in humans, using the adenylyl cyclase pathway. Potassium and chloride channels have also been implicated in human ACTH signaling.

Tetrahymena thermophila are free-living, ciliated protozoans. These organisms exhibit avoidance behavior toward many polycationic peptides, which serve as chemorepellents. The reason for this is unknown; however, it is hypothesized that natural predators of *T. thermophila* secrete polycationic peptides, and that polycation avoidance allows *T. thermophila* to escape predation. We obtained a number of peptides derived from ACTH, including ACTH 1-39, ACTH 1-24, ACTH 11-24, ACTH 6-24, and ACTH 1-14. We hypothesized that the more highly charged peptide derivatives would be the most effective chemorepellents. This hypothesis was proven correct, with the most highly charged ACTH derivative, ACTH 6-24, demonstrated as the most effective chemorepellent. The least charged form of ACTH, ACTH 1-39, was least effective at causing avoidance.

We hypothesized that ACTH signaling in *T. thermophila* would use similar signaling pathways to those previously identified in humans. This, however, has not proven to be the case. We have tested G-protein inhibitors, adenylyl cyclase inhibitors, potassium channel blockers, and chloride channel blockers in *T. thermophila*. None of these drugs had any measurable effect on ACTH signaling. In addition, we have chelated extracellular calcium (using EGTA) and depleted ER calcium stores (using thapsigargin). Neither of these interventions inhibited ACTH signaling in this organism. Calcium channel blockers also failed to affect avoidance. This is highly unexpected, since all known chemorepellent pathways discovered in *Tetrahymena* to date are calcium-dependent. It is possible that ACTH is using a novel signaling pathway in *T. thermophila*. We hope that further testing will enable us to discover more about this signaling mechanism.

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.
- Inhibition was carried out by individually transferring cells into the inhibitor for 10-15 minutes before transferring cells to a mixture of the inhibitor and the ACTH solution. Cells were scored as either avoiding or not avoiding. Cells were counted in groups of 10 for each trial. A minimum of 6 trials was done for each assay.

Results

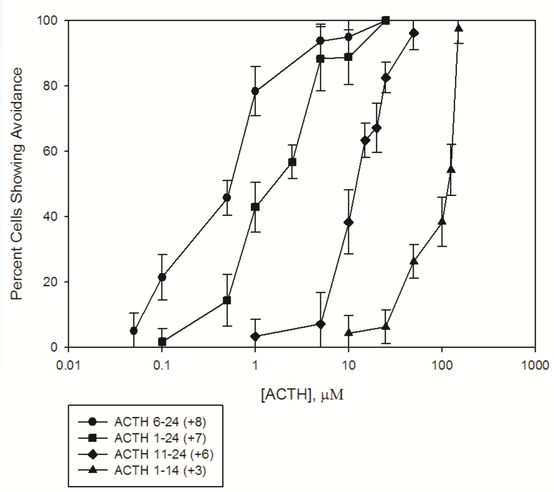

Figure 1. Charged peptides derived from ACTH are chemorepellents in *Tetrahymena thermophila*. Each point represents the mean ± SD of at least 6 trials.

Table 1. Calcium is not involved in ACTH signaling in *Tetrahymena thermophila*.

Inhibitor	Mechanism of Action	Effect on Avoidance
EGTA	Extracellular Calcium Chelator	None
Thapsigargin	Depletes ER Calcium Stores	None
ω-conotoxin	Broad Spectrum Calcium Channel Blocker	None
Amlodipine	L-type Calcium Channel Blocker	None

Table 2. Potassium and chloride are not involved in ACTH signaling in *Tetrahymena thermophila*.

Inhibitor	Mechanism of Action	Effect on Avoidance
TEA	Broad Spectrum Potassium Channel Blocker	None
NPPB	Broad Spectrum Chloride Channel blocker	None

Table 3. G-protein signaling and tyrosine kinase signaling are not involved in ACTH signaling in *Tetrahymena thermophila*.

Inhibitor	Mechanism of Action	Effect on Avoidance
GDP-β-S	G-protein antagonist	None
Rp-cAMPs	cAMP antagonist	None
Genistein	Broad Spectrum Tyrosine Kinase Inhibitor	None
J-11397	Inhibitor of Human Nociceptin Inhibitor	None

Conclusions

- ACTH 6-24 is a potent chemorepellent in *Tetrahymena thermophila,* with an EC_{100} of just 5 μM.
- Charge is important in signaling. More highly charged fragments of ACTH were more effective repellents.
- Neither intracellular nor extracellular calcium appear to contribute to ACTH signaling. This is unlike other known chemorepellent pathways found in *Tetrahymena* to date, all of which have involved a calcium-based depolarization.
- GPCR antagonists and tyrosine kinase inhibitors do not affect ACTH avoidance in *Tetrahymena thermophila*. This contrasts with humans, where GPCRs are involved in ACTH signaling.
- Further experiments may help us determine a signaling mechanism.

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