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Nociceptive Polycationic Peptides Are Chemorepellents in *Tetrahymena thermophila*

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Introduction

Chemorepellents are compounds which cause a cell to move away from the source of the repellent, or down a concentration gradient of the compound. In ciliates such as *Tetrahymena thermophila* and *Paramecium tetraurelia*, this reorientation is accomplished by ciliary reversal, resulting in jerky or backward swimming which is known as an "avoidance reaction". This reaction can easily be seen under a simple dissection microscope, allowing for easy characterization of ciliate behavior in different compounds.

A number of compounds are known chemorepellents in *Tetrahymena thermophila*, including ATP and GTP which have a negative overall charge, and polycations such as lysozyme and pituitary adenylate cyclase activating polypeptide (PACAP) which have multiple positive charges. The physiological significance of these chemorepellents is uncertain. Nucleotide triphosphates, such as ATP and GTP, have been postulated to serve as a warning signal to other cells that cellular lysis has occurred, analogous to "blood in the water". Polycations such as lysozyme are thought to be similar to naturally occurring secretions of the organisms' predators, allowing some organisms to escape by sensing the polycation gradient.

Since we have previously found that polycationic peptides such as lysozyme and PACAP are chemorepellents in *Tetrahymena* which appear to signal through a G-protein linked receptor, we decided to explore whether human nociceptive peptides would also cause *Tetrahymena* to exhibit avoidance through a similar mechanism. The peptides we tested: ACTH 1-24, PTH, substance P, and bradykinin all carry a net positive charge at a pH of 7.0 and are associated with G-protein linked receptors in humans. Our hypothesis was that the efficacy of the compound in terms of causing avoidance would be linked to its charge. In addition, we hypothesized that these compounds would all work through a single receptor, the previously characterized lysozyme/PACAP receptor (Mace et al., 2000; Hassenzahl et al., 2001).

Materials and Methods

Tetrahymena thermophila, strain B2086, a generous gift from T.M. Hennessey (SUNY-Buffalo) was used for all of the experiments. Cells were grown at 25^{0}C in the axenic medium of Dentler (1988), without shaking or addition of antibiotics. Two-day old cell cultures were used for all behavioral assays described below.

Chemicals and solutions. Behavioral assays were carried out in a buffer of pH 7.0 containing 10 mM Trizma base, 0.5 mM MOPS, and 50 μM $CaCl_2$. All repellents and inhibitors used were dissolved in this buffer.

Behavioral assays. Behavioral assays were carried out as previously described (Kuruvilla et al. 1997; Mace et al. 2000). Briefly, cells were washed in buffer and 300 μl of cell suspension was transferred to the first well of a 3-well spot microtiter plate. Cells were then individually transferred by micropipette to the second well, which contained 300 μl of buffer as a control. Cells were then transferred to a third well which contained 300 μl of the nociceptive peptide of interest. Each cell was briefly observed (1-5 sec) for signs of avoidance. Avoidance behavior was characterized by jerky, backward movements, swimming in small, tight circles, sudden reversal of swimming direction, or any deviation from the normal helical swimming pattern of *Tetrahymena thermophila*. Each cell was individually scored as being positive or negative for avoidance.

Results

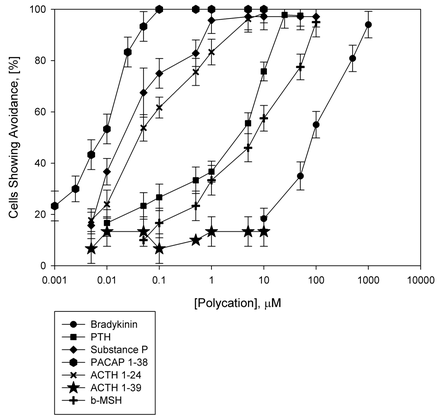

Figure 1. Nociceptive peptides are chemorepellents in *Tetrahymena thermophila*. Behavioral assays indicate that PACAP 1-38, ACTH 1-24, parathyroid hormone, substance P, b-MSH, VIP, and bradykinin all elicited an avoidance response in a concentration-dependent fashion. Each trial consisted of scoring ten cells as either positive or negative for avoidance. Each data point represents the mean ± SD of at least 6 trials.

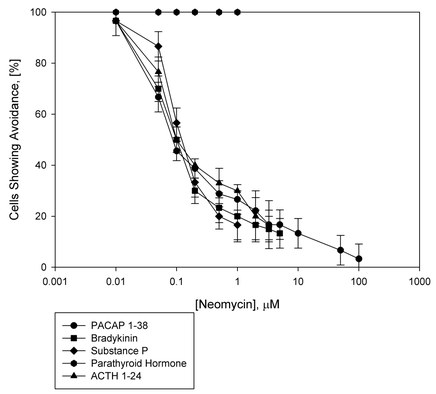

Figure 2. Neomycin sulfate inhibits avoidance to a number of polycationic peptides in *Tetrahymena thermophila*. Neomycin sulfate, a competitive inhibitor of lysozyme binding to its receptor (Kuruvilla et al., 1997) effectively eliminated avoidance to PACAP 1-38, bradykinin, substance P, and ACTH 1-24 while having no affect on avoidance of PTH. The IC_{100} of neomycin ranged from 5-50 μM, depending on the peptide tested. The IC_{50} of neomycin was approximately 0.1 μM for PACAP 1-38, bradykinin, and ACTH 1-24, and 5 μM for substance P. Each trial consisted of scoring ten cells as either positive or negative for avoidance. Each data point represents the mean ± SD of at least 6 trials. Previous studies (Mace et al., 2000) have shown that neomycin also inhibits avoidance to VIP. The effects of neomycin on b-MSH have not yet been determined.

Table 1: Characteristics of Polycationic Peptides. Positively charged amino acids are indicated in **bold** font; negatively charged amino acids are indicated in *italic* font. Net charge was calculated by subtracting the negatively charged amino acids from the positively charged amino acids. EC_{100} values were obtained from Figure 1.

Peptide	Amino Acid Sequence	Total Number of Positive Charges	Net Charge at pH 7.0	EC_{100}, μM
b-MSH	Ala-*Glu*-**Lys**-**Lys**-*Asp*-*Glu*-Gly-Pro-Tyr-**Arg**-Met-*Glu*-**His**-Phe-**Arg**-Trp-Gly-Ser-Pro-Pro-**Lys**-*Asp*	6	+1	100
ACTH 1-39	Ser-Tyr-Ser-Met-*Glu*-**His**-Phe-**Arg**-Trp-Gly-**Lys**-Pro-Val-Gly-**Lys**-**Lys**-**Arg**-**Arg**-Pro-Val-**Lys**-Val-Tyr-Pro-Asn-Gly-Ala-*Glu*-*Asp*-*Glu*-Ser-Ala-*Glu*-Ala-Phe-Pro-Leu-*Glu*-Phe	8	+2	Not determined
Bradykinin	**Arg**-Pro-Pro-Gly-Phe-Ser-Pro-Phe-**Arg**	2	+2	1000
Substance P	**Arg**-Pro-**Lys**-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-**NH2**	3	+3	1
PTH	Ser-Val-Ser-*Glu*-Ile-Gln-Leu-Met-**His**-Asn-Leu-Gly-**Lys**-**His**-Leu-Asn-Ser-Met-*Glu*-**Arg**-Val-*Glu*-Trp-Leu-**Arg**-**Lys**-**Lys**-Leu-Gln-*Asp*-Val-**His**-Asn-Phe	8	+4	10
VIP	**His**-Ser-*Asp*-Ala-Val-Phe-Thr-*Asp*-Asn-Tyr-Thr-**Arg**-Leu-**Arg**-**Lys**-Gln-Met-Ala-Val-**Lys**-**Lys**-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-**NH2**	7	+5	50
ACTH 1-24	Ser-Tyr-Ser-Met-*Glu*-**His**-Phe-**Arg**-Trp-Gly-**Lys**-Pro-Val-Gly-**Lys**-**Lys**-**Arg**-**Arg**-Pro-Val-**Lys**-Val-Tyr-Pro	8	+7	5
PACAP 1-38	**His**-Ser-*Asp*-Gly-Ile-Phe-Thr-*Asp*-Ser-Tyr-Ser-**Arg**-Tyr-**Arg**-**Lys**-Gln-Met-Ala-Val-**Lys**-**Lys**-Tyr-Leu-Ala-Ala-Val-Leu-Gly-**Lys**-**Arg**-Tyr-**Lys**-Gln-**Arg**-Val-**Lys**-Asn-**Lys**-**NH2**	13	+11	0.1
lysozyme	**Lys**-Val-Phe-Gly-**Arg**-Cys-*Glu*-Leu-Ala-Ala-Ala-Met-**Lys**-**Arg**-**His**-Gly-Leu-*Asp*-Asn-Tyr-**Arg**-Gly-Tyr-Ser-Leu-Gly-Asn-Trp-Val-Cys-Ala-Ala-**Lys**-Phe-Gly-Ser-Asn-Phe-Asn-Thr-Gln-Ala-Thr-Asn-**Arg**-Asn-Thr-*Asp*-Gly-Ser-Thr-*Asp*-Tyr-Gly-Ile-Leu-Gln-Ile-Asn-Ser-**Arg**-Trp-Trp-Cys-Asn-*Asp*-Gly-**Arg**-Thr-Pro-Gly-Ser-**Arg**-Asn-Leu-Cys-Asn-*Asp*-Gly-**Arg**-Thr-Pro-Gly-Ser-**Arg**-Asn-Leu-Cys-Asn-Ile-Pro-Cys-Ser-Ala-Leu-Leu-Ser-Ser-*Asp*-Ile-Thr-Ala-Ser-Val-Asn-Cys-Ala-**Lys**-**Lys**-Ile-Val-Ser-*Asp*-Gly-Asn-Gly-Met-Asn-Ala-Trp-Ala-Trp-**Arg**-Asn-**Arg**-Cys-**Lys**-Gly-Thr-*Asp*-Val-Gln-Ala-Trp-Ile-**Arg**-Gly-Cys-**Arg**-Leu	20	+11	100

Conclusions:

- **Many polycationic nociceptive peptides are chemorepellents in *Tetrahymena thermophila*.**
- **In general, higher positive charge correlates with lower EC_{100} values. However, peptide folding and size also appear to play a role (compare lysozyme and PACAP).**
- **Neomycin sulfate blocks signaling of a number of these peptides, suggesting a possible shared receptor or second messenger pathway. However, PTH may use an alternative pathway according to these data.**

References:

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