Investigation of the Functions of Insect Arginine Vasopressin-like Peptides

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Abstract

The neuropeptide arginine vasopressin (AVP) is a multifunctional signaling peptide that is highly conserved across eukaryotic animals. In mammals, it primarily acts as an anti-diuretic and a regulator of blood pressure. These traits among others merit the study of the arginine vasopressin-like (AVPL) hormone found in insects (Aikins et al., 2008). We investigated the bioactivity of AVPL in an assay measuring the recovery time after knockout in water, indicating hormonal activity for alert states in the red flour beetle *Tribolium castaneum*. We found that injections of an AVPL mimetic peptide (AVP1) prolonged the knockout time, implying that the AVPL causes longer suppression of the recovery from water shock.

Introduction

Tribolium castaneum, a notorious stored product pest, became a model genetic organism after the whole genome was sequenced. AVPL in this species of insect was previously identified, but the function is unknown yet. Suppression of the gene expression by using RNA interference (RNAi) of the AVPL gene found no significant developmental defects previously (Aikins et al., 2008). In this study, we used a new assay method that examined the activity of AVPL in the larval alert state by measuring recovery time after water submersion.

Question and Hypothesis

Question: Whether AVPL mimetics have effects on the alert state and on the survival of *T. castaneum*?

Hypotheses: Injections of AVPL mimetics into the larvae of *T. castaneum* will change the recovery time from the knockout induced by water submersion. The injections will also affect the survivorship.

Methods and Experimental Design

- Three injection solutions were prepared: Phosphate buffered saline (PBS), 10 μM AVP1, and 10 μM AVP2.
  - AVP1: A plant cyclotide that acts on the *Tribolium* AVPL receptor as partial (Emax~50%) biased agonist with an EC50 of ~5μM.
  - AVP2: An AVPL D-amino acid analogue which acts as full biased agonist with an EC50 ~80nM.
- Sixth instar larvae were immobilized on double sticky tape and injected with a 100 nL solution through abdominal segments (Figure 1A).
- Following injection, larvae were submerged in water for 2 minutes (Figure 1B).
- Larvae were then placed on a dry paper towel and monitored until they showed signs of movement. The recovery time was measured.
- For measuring the long term survivorship, the insects were placed in individual wells of a 96-well plate with food and placed in an incubator (30° C, ~40% relative humidity).
- Insect mortality was monitored for the 6 to 7 days following injection.

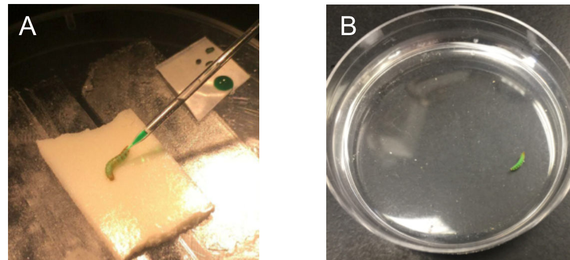

Figure 1. Micro injection and bioassay of *T. castaneum* larvae. (A) A larva was immobilized on a double sticky tape and AVPL mimetics (or PBS) was injected. Non-toxic green food dye was mixed in the injection solution to confirm the small volume (100 nL) injections. (B) The individual injected with AVPL mimetics were submerged in water in a petri dish for 2 min.

Results

1. Injections of AVP1 resulted in significant delay in the recovery time from the knockout induced by submersion in water (Figure 2).

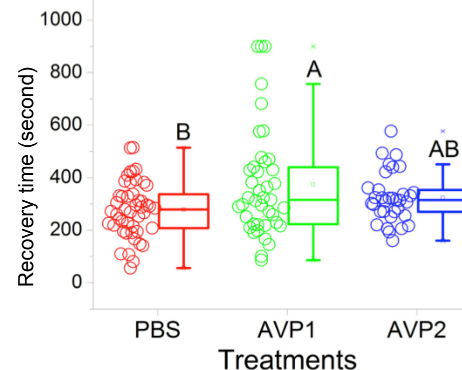

Figure 2. Comparison of revival times after water submersion. The boxes are for interquartile (25 and 75%). The short horizontal bars display the 95 and 5%. Statistics for the comparison was done by an ANOVA-Tukey-Kramer HSD test (P=0.05). n= 47, 41, and 34, respectively.

2. Natural mortalities (including the PBS injection) were too high to draw a conclusion.

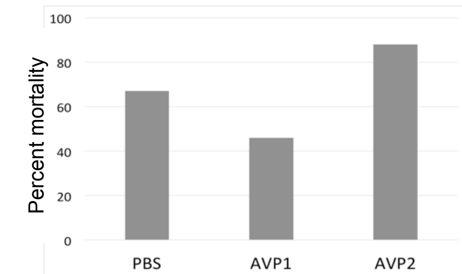

Figure 3. Percent mortality measured 6 to 7 days after the injections. N= 27, 26, and 34 for control, AVP1, and AVP2 injections, respectively.

Future Directions

- We need to repeat the assays to confirm the results in recovery time assay and mortality assay.
- We will include additional controls: uninjected control with and without immersion in water.
- We would like to examine the effects of AVPL mimetics in other pest arthropods, including varroa mite.

References

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