Bioactivities of Proctolin Mimetic in *Drosophila melanogaster*

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Abstract

The insect neuropeptide proctolin was originally purified for its myotropic actions on insect hindguts, however it has been shown to be distributed widely throughout arthropods. This pentapeptide, RYLPT or Arg-Tyr-Leu-Pro-Thr, is highly conserved across arthropod species. We were interested in whether observable bioactivity, physiological and behavioral changes, occurs upon the injections of proctolin or proctolin mimetic peptides. We found strong activities of proctolin and proctolin mimetic in immediate inductions of proboscis behavior and defecation. Peptidomimetics showed strong activities opening a new revenue for development of new class of insecticidal compound.

Purpose

The purpose of this experiment was to investigate the bioactivities of proctolin and its mimetic peptides in the fruit fly *Drosophila melanogaster*.

Questions and Hypotheses

Question: What is the activity of the proctolin and its mimetics once injected into *D. melanogaster*?

Hypothesis: Our hypothesis is that once injected with proctolin or one of its mimetics, we will be able to obtain observable phenotypes.

Study System

The neuropeptide proctolin was discovered originally in cockroaches (Brown and Starratt, 1975) and was sequenced because of its ability to contract skeletal muscles. It has since been found in multiple insect species as well as some arthropods, where it induces the contractions of both somatic and visceral muscles, by causing an increase in calcium through ion channels. For this study we focused on its ability to cause contractions in the proboscis of *D. melanogaster* after the insects were injected in the thorax with either proctolin or one of its mimetics.

Methods and Experimental Design

As this study was centered around proctolin and its effect on fruit flies, there were three different chemicals injected alongside a control of distilled water. The first chemical used was proctolin (RYLPT), with one mimetic 2334 (RYL[HyP]T) replacing proline with a Hydroxyprolin, and mimetic 2336 (RYL[Oic]T) replacing proline with a octahydroindole-2-carboxylic acid. The proctolin and its mimetics were colored with food coloring to better assess whether the injection was a success, and were injected and observed using a table top electronic injection machine. The needle of the equipment was filled with a mineral oil to better seal the glass needle to the machine, and once equipped, had the glass tip cut off to make injection possible. The flies were anesthetized as needed with CO_2 on a surface that allowed the movement of the gas to the flies which were placed on the surface under a microscope to allow for injection. Once injected, the flies were observed by a camcorder attached to the microscope to allow videos to be taken of the reactions and better observation of the proboscis movement. For each of the injections, control, proctolin, 2334, and 2336, five individuals were injected and observed.

Results

Along with the four different materials injected into the insects, there were three different volumes used, 30nl, 40nl, and 50nl. For the control, there was no response noted until the flies were injected with 50nl of distilled water. For proctolin, with 30nl injected 2/5 showed a response, as well as with 40nl, and 3/5 responded to the 50nl injection. 2334 showed 3/5 response at 30nl, 4/5 response at 40nl, and 5/5 response at 50nl. 2336 showed similar responses, with the exception of 4/5 response at 50nl, and the addition of excretion behavior exhibited at this concentration.

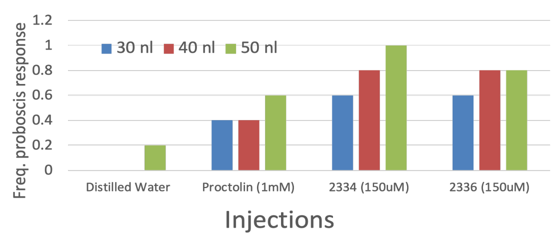

Fig. 1. Frequency proboscis responses in injections of different compounds at varied concentrations.

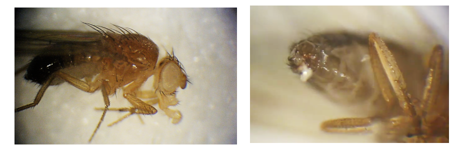

Fig. 2. Typical proboscis response (left) and defecation (right) upon injections of proctolin or proctolin mimetics. Video recording is available upon request.

Conclusions

One conclusion that can be made from this study is that there is an obvious response of the proboscis of *D. melanogaster* after injection of proctolin or one of its mimetics. Though this study only focused on the injection of these compounds, we had hoped to test the ability of proctolin on just the muscles of the proboscis to test whether it targets the muscle itself or causes the muscle to contract by using neural networks in the brain, we were unable to find a way to properly test just the muscles of the proboscis. We had hoped to remove the mouthparts just below the brain of the insect with a laser and place them on the neuropeptide and its mimetics to observe any response, however none was observed and we hypothesize this to be because the proboscis requires the hemolytic pressure of being attached to the head to extend.

One future direction of this study is the possibility of proctolin to be used in an insecticide to target only insects with proctolin receptors, as not all insects have the proctolin receptors, and mammals don't seem to have any receptors for proctolin. If proctolin can be shown to target the proboscis of different insects with proctolin receptors, it could prove to be an effective specialized insecticide.

References

Brown, B.E.,and A.N.Starratt (1975) Isolation ofproctolin, amy- otropic peptide from Periplaneta americana. J. Insect. Physiol. 21: 1879-1881.

Acknowledgements and Announcement

I would like to thank Dr. Jeremy Marshall for providing the research opportunity. Dr. Park is looking for a motivated undergraduate student who would continue this research. Contact @ ypark@ksu.edu