Ecdysteroid Analog Feedback Regulation on Y-Organs in the European Green Crab, Carcinus maenas

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Introduction

Crustaceans periodically molt in order to grow

- The molting cycle is controlled by hormones
- Ecdysone, which is released from the Y-organ and hydroxylated to its primary form of 20-hydroxyecdysone (20E), stimulates molting
- Molting inhibiting hormone (MIH) is released from the X-organ-sinus gland complex and inhibits Y-organ production of ecdysone

Methods:

1. RH 70345 Crossreactivity with Antisera

- Methods: I conducted a radioimmunoassay with a range of concentrations of RH 70345.
- Results: For each dosage of RH 70345, over 100% of the labeled hormone bound to the antibodies in the antisera (data not shown). This indicates that the unlabeled hormone, the RH 70345, did not compete for binding and therefore, did not react with the antisera.

2. Effects of RH 70435 on Ecdysone Production In Vitro

- Methods: Incubated left and right Y-organs in crab saline for two hours and then RH 70345 solutions (100 or 200 pg/µL) for two hours.
- Results:
  - RH 70345 stimulates Y-organ production of ecdysone. However, the amount of ecdysone secreted demonstrates logarithmic growth over time. The Y-organ has a decreasing rate of ecdysone production, indicating that 20E may have an inhibitory effect on the Y-organ.

Time Course of Y-Organ Secretion of Ecdysone

- Methods: Incubated 3 crabs' left and right Y-organs in crab saline.
- Results: The amount of ecdysone secreted demonstrates logarithmic growth over time. The Y-organ has a decreasing rate of ecdysone production, indicating that 20E may have an inhibitory effect on the Y-organ.

- Methods: Took hemolymph samples and injected again after 18 and 24 hours. Took final hemolymph sample after 52 hours.

Summary

- RH 70345 did not crossreact with the antisera. Therefore, Y-organ incubations with the hormone analog could potentially produce more accurate data.

- Y-organ time course indicated that 20E may have an inhibitory effect on ecdysone production.

- In vitro trials indicated that RH 70345 at higher concentrations may stimulate Y-organ production of ecdysone. However, in vitro trials indicated that RH 70345 did not affect ecdysone production.

- More trials should be conducted in vitro to corroborate these results, specifically looking at effects on ecdysone production when the Y-organ is transfected from a hormone analog solution to crab saline.

- The effects of RH 70345 in vivo is ambiguous, possibly indicating an initial inhibitory effect of RH 70345 on ecdysone production regardless of concentration.

- More in vivo trials should be conducted. A wider range of RH 70345 concentrations should be used and hemolymph samples should be taken in between injections to better understand fluctuations of circulating ecdysone levels.

Research Questions

I. Does the hormone analog RH 70345 react with the antisera used during radioimmunoassay?

II. How does the RH 70345 affect the Y-organ in vitro? Does it stimulate or inhibit ecdysone production?

III. How does the RH 70345 affect the Y-organ in vivo? Does it stimulate or inhibit ecdysone production?