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Development of neuropeptide analogs capable of traversing the integument: A case study using diapause hormone analogs in *Helicoverpa zea*

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ABSTRACT

Diapause hormone and its analogs terminate pupal diapause in *Helicoverpa zea* when injected, but if such agents are to be used as effective diapause disruptors it will be essential to develop simple techniques for administering active compounds that can exert their effect by penetrating the insect epidermis. In the current study, we used two molecules previously shown to have high diapause-terminating activity as lead molecules to rationally design and synthesize new amphiphilic compounds with modified hydrophobic components. An assay for diapause termination identified 13 active compounds with EC_{50}'s ranging from 0.9 to 46.0 pmol per pupa. Three compounds, Decyl-1963, Dodecyl-1967, and Heptyl-1965, selected from the 13 compounds most active in breaking diapause following injection, also successfully prevented newly-formed pupae from entering diapause when applied topically. These compounds feature straight-chain, aliphatic hydrocarbons from 7 to 12 carbons in length; DH analogs with either a short-chain length of 4 or an aromatic phenethyl group failed to act topically. Compared to a high diapause incidence of 80–90% in controls, diapause incidence in pupae receiving a 10 nmole topical application of Decyl-1963, Dodecyl-1967, or Heptyl-1965 dropped to 30–45%. Decyl-1963 and Dodecyl-1967 also remained effective when topically applied at the 1 nmole level. These results suggest the feasibility of developing DH agonists that can be applied topically and suggest the identity of new lead molecules for development of additional topically-active DH analogs. The ability to penetrate the insect epidermis and/or midgut lining is critical if such agents are to be considered for future use as pest management tools.

1. Introduction

Diapause hormone (DH, sequence depicted in Fig. 4) is a member of the FXPRLamide family of peptides that share a conserved C-terminal sequence. This peptide family is present in diverse insects including moths, flies, mosquitoes, cockroaches, honeybees, beetles and others. Their biological functions are likewise diverse, having been implicated in regulation of diapause and development, muscle contraction, and stimulation of sex pheromone synthesis. DH, a 24-aa peptide, was originally discovered as an inducer of egg diapause in the silkworm, *Bombyx mori* (Yamashita, 1996; Denlinger et al., 2005), but it is also highly effective in terminating pupal diapause in the agriculturally-important Lepidoptera pests in the *Helicoverpa/Heliothis* complex in both North America and Asia (Xu and Denlinger, 2003; Zhang and Denlinger, 2012; Zhang et al., 2004, 2008; Zhao et al., 2004). Another member of this family, pheromone biosynthesis activating

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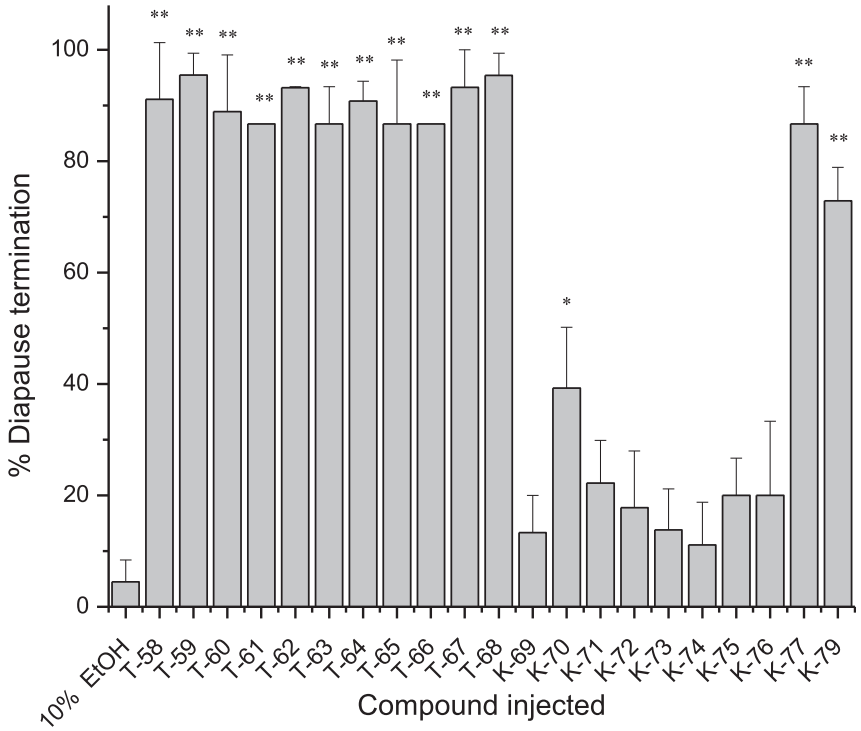

Fig. 1. Diapause termination in pupae of *H. zea* in response to injection of a range of newly-designed compounds in a T- and K-series of DH analogs. The compounds were generated by modifying the N-terminus of two active lead molecules reported previously (Zhang et al., 2011). The testing dose was 0.1 nmole/pupa, and at this dose nearly all 11 of the T-series compounds were highly active, but only 3 of the 10 compounds in the K-series were active. Ten percent ethanol was used as the solvent, and pupae injected with 10% ethanol served as controls. Each column represents mean ± S.D. incidence of diapause termination in three replicates. 15–16 pupae were tested in each replicate. Single asterisk and double asterisks indicate significant and very significant differences between the test compounds and ethanol controls, respectively (*P < 0.05, **P < 0.01, Student's *t*-test).

neuropeptide (PBAN), is a 33-aa peptide that stimulates pheromone synthesis in adult female moths (Ma et al., 1994). Members of the family with shorter lengths, such as pyrokinins, stimulate contraction in the hind gut and other visceral muscles in the cockroach *Leucophaea maderae* (Holman et al., 1986) and accelerate pupariation in the flesh fly *Sarcophaga bullata* (Zdarek et al., 1997). It is still a bit unclear how members of this family that share a conserved structure exhibit such versatile and distinct physiological functions.

We propose that DH and its analogs could potentially be employed as tools to manage populations of the notorious heliothine agricultural pests by manipulating their overwintering pupal

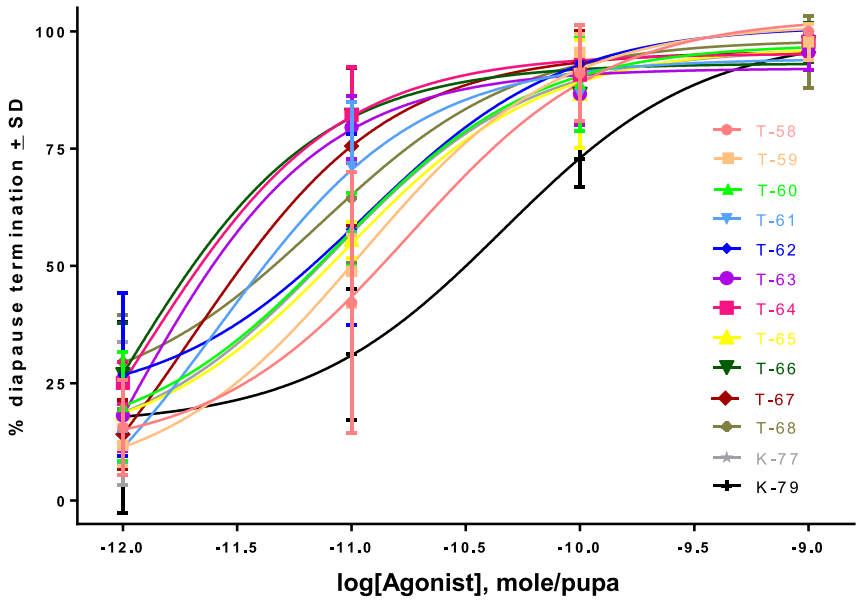

Fig. 2. Dose-response curves for 11 compounds in the T-series and 2 compounds in the K-series that showed the highest diapause terminating activity in *H. zea* following injection into pupae. Each point represents the mean ± S.D. incidence of diapause termination in 3 replicates of 12–15 pupae. The most potent compounds were T-66, T-64, T-63, T-67 and T-61, and the least active one was K-79.

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