Graphic abstract

Diapause hormone in the *Helicoverpa/Heliothis* complex: a review of gene expression, peptide structure and activity, analog and antagonist development, and the receptor ^δ

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^δ Dedicated to Prof. Abba J. Kastin for his magnificent scientific career and 35 years of excellent stewardship of *Peptides*. We are particularly grateful for his critical role in raising awareness of the field of invertebrate peptide research among the larger peptide science community.

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Abstract

This review summarizes recent studies focusing on diapause hormone (DH) in the *Helicoverpa/Heliothis* complex of agricultural pests. Moths in this complex overwinter in pupal diapause, a form of developmental arrest used to circumvent unfavorable seasons. DH was originally reported in the silkworm *Bombyx mori*, a species that relies on DH to induce an embryonic diapause. But, in the case of *Helicoverpa/Heliothis*, levels of *dh* transcripts and DH peptides are more abundant in nondiapausing pupae than in diapausing individuals, and DH effectively terminates diapause within a specific temperature range. A structure activity relationship study indicated that the active core of DH is the C-terminal hepta-peptide, LWFGPRLa. We designed and synthesized a first generation of DH agonists and identified two agonists (PK-2Abf and PK-Etz) that were nearly 50- and 13-fold more potent than the native hormone. These studies revealed two structural characteristics of DH and its agonists that are essential for interaction with the receptor: a trans-Pro configuration to form a type I β -turn and a hydrophobic moiety involved in ligand binding. Modification of DH at the active core yielded a potent DH antagonist (DH-Jo, **acetyl**-GLWA**[Jo]**RLa) as well as an agonist (DH-2Abf-K). Three compounds (Decyl-1963, Dodecyl-1967, Heptyl-1965) were identified as agents capable of penetrating the cuticle of young pupae and thereby preventing the entry into diapause. DH receptor cDNA was cloned and an effective *in vitro* high throughput screen system was established for future use. This work sets the stage for further development of DH analogs and antagonists that have the potential to disrupt insect diapause as a tool for pest management.

Key words: insect diapause, diapause hormone, gene expression, diapause termination, structure activity relationship, analogs development, diapause prevention, receptor

1. Introduction

Diapause is a developmental strategy used widely by insects to escape seasonal challenges such as winter in temperate latitudes (2, 3). This developmental arrest is programmed by subtle changes of daylength and temperature that accompany the transition from summer to autumn, and in preparation for the unfavorable environment most insects respond by reducing their metabolic rate, sequestering fat reserves, and arresting or slowing the whole developmental process. This development arrest is coordinated internally by the insect's endocrine system. The endocrine regulation is thus vulnerable to disruption and could offer new tools for insect pest management.

Our focus is on diapause hormone (DH), a neuropeptide hormone first identified from the commercial silkworm, *Bombyx mori*. In the silkworm, DH is secreted from the mother's subesophageal ganglion and is directly responsible for the induction of diapause in the eggs that she lays (21). This was the first identified neuropeptide reported to directly regulate insect diapause. Diapause in most other cases results from the absence of a hormonal signal (usually the absence of juvenile hormone in adults and ecdysone in larvae and pupae), but in the case of *B. mori* it is the presence of the hormone (DH) that causes the entry into diapause (21). DH in *B. mori* is encoded by the same gene that encodes pheromone biosynthesis activating neuropeptide (PBAN) and three other short neuropeptides (14). With the rapid development of modern molecular biology techniques, DH-PBAN homologous genes were characterized from various other species of Lepidoptera including moths in the *Helicoverpa/Heliothis* complex (20, 28, 30). The amino acid sequences shown in Fig. 1 show high homology for DH among the Lepidoptera. Although the sequences have been known for some time, the biological function for DH in species other than *B. mori* was not clear. Interestingly, our early research found that DH was not capable of inducing pupal diapause in the *Helicoverpa/Heliothis* complex, but instead it effectively terminates pupal diapause (20, 30), a surprising contrast to the results noted in *B. mori*.

This review summarizes the past decade of research on DH and its regulation of insect diapause, with special emphasis on the *Helicoverpa/Heliothis* complex. We provide an update

on DH gene cloning and expression analysis, the structure and activity relationship of DH on pupal diapause termination, the development of DH analogs and antagonists, and DH receptor studies. These studies have generated new chemical agents that we anticipate will be useful for developing new tools for future application in disrupting the critical diapause response.

2. DH gene cloning and expression analysis in the *Helicoverpa/Heliothis* complex

The DH gene and cDNA have been characterized and cloned from various species of Lepidoptera since the 1990s. The first partial *pban* gene was cloned from *Helicoverpa zea* by Davis et al. (1), and the full-length cDNA encoding both DH (initially referred to as '*H. zea* Asn²⁴-Leu⁴⁷') and PBAN in *H. zea* was reported two years later (11). At nearly the same time, DH cDNA was also cloned and reported from *B. mori* (9, 14). When tested in a *B. mori* bioassay, DH injection induced embryonic diapause, and synthetic DH, with the amino acid sequence deduced from *Bombyx dh* cDNA, showed the same biological activity as the native peptide isolated from the subesophageal ganglion (21). Many studies examining this gene focused on the function of PBAN, but initially DH was largely neglected. In 2003, Xu and Denlinger cloned *dh* cDNA from *H. virescens* and found that DH effectively terminates pupal diapause in both *H. virescens* and *H. armigera* at select temperatures (20). Zhang et al. (29) cloned *dh* from *H. armigera* and also demonstrated that DH cannot induce pupal diapause in *H. armigera*, but rather breaks diapause effectively. DH termination of diapause was further verified in *H. assulta* (30), thus similar diapause-terminating responses for DH are noted in all species of this complex that were examined.

DH gene expression studies were also carried out in all of these species. Northern blotting, semi-quantitative PCR, and quantitative PCR techniques consistently indicate lower expression of *dh* mRNA in diapausing pupae compared to their nondiapausing counterparts in *H. virescens*, *H. armigera*, *H. assulta*, and *H. zea* (20, 22, 28-30). Northern blotting results comparing *H. zea dh* mRNA levels between diapausing and nondiapausing pupae are shown in Fig. 2 (adapted from Zhang and Denlinger (22)). *dh* mRNA level were also verified by *in situ* hybridization at the cellular level: optical densities of *dh* mRNA in the neurosecretory somata of 3 or 5 days old

nondiapausing pupae is much higher than those in their diapausing counterparts (15). Furthermore, studies of DH at the protein level are consistent with data at the mRNA level. Using antiserum against synthetic DH, neurosecretory cells expressing DH are localized in the subesophageal ganglion. Expression patterns of both diapausing and nondiapausing pupae were examined by immunocytochemistry, and immunoreactive neurosecretory somata are consistently larger in nondiapausing individuals and the axons are clearer, suggesting that more DH peptide is synthesized and released in nondiapausing pupae (15). ELISA experiments to compare DH titers in diapausing and nondiapausing hemolymph indicate that much less DH peptide is circulating in diapausing individuals compared to their nondiapausing counterparts (15, 16, 30).

Briefly, the *dh* cDNA sequence information and the antibody generated against DH synthetic peptide demonstrate that both *dh* mRNA and DH peptide are much more abundant in nondiapausing pupae, an observation consistent with a function for DH in diapause termination (20).

3. Structure-activity relationship for DH on diapause termination in *H. zea*

Structure activity relationships (SAR) of peptide hormones provide both an understanding of functional mechanisms at the molecular level as well as a tool for developing new peptide mimics. One essential aspect of an SAR study is to establish an easy but effective bioassay method. In *Bombyx*, DH can be readily injected into 3-4 days old polyvoltine strain female pupae at room temperature, and DH activity can be determined by observing coloration of the deposited eggs as early as 3 days after oviposition. Both types of eggs are pale yellow when newly laid, but a bit later diapause eggs turn a dark purple color, while non-diapause eggs remain pale yellow (21). SAR studies of DH on diapause induction in *B. mori* (5, 12, 13, 17) indicate that the minimum structure for activity is PRLa (5), and the penta-peptide at the C-terminus is the active core required to elicit diapause (12, 17, 21). Since the diapause induction bioassay using *Bombyx* pupae is both easy and effective, and cross activity is also available for

the FXPRLa family peptides in other species, as for pyrokinin from *Locusta*, it has become one of the standard bioassays for determining activity of the FXPRLa family of peptides (12).

Similarly, we established a diapause termination activity assay using *H. zea* as a model system (27). We first demonstrated that the optimal temperature for DH diapause termination activity was 21°C in the strain of *H. zea* that we used. The temperature optimum we observed for *H. zea* was slightly different from that reported for *H. armigera* (25-27 °C) (29) and *H. assulta* (25 °C) (30). These differences are likely due to adaptations to different geographic regions of origin. The *H. zea* pupal diapause termination assay consists of injecting compounds into 7 day-old diapausing pupae and examining their diapause status, using the location of their eyespots as a diapause biomarker. At 7 days, eyespots of diapausing pupae are still clearly visible in the middle of the pupal eye, while the eyespots of pupae that have terminated diapause move to the corner of the pupal eye and then disappear (27).

We investigated the active core of DH for pupal diapause termination in *H. zea* using two series of DH analogs (27). First, thirteen DH analogs of different lengths were examined for diapause termination activity (Fig. 3a), and from this we concluded that the hepta-peptide, LWFGPRLa, is the active core. Elongated analogs at the N-terminus enhanced activity, but shortened analogs from the core sequence completely abolished diapause termination activity. The presence of an amide at the C-terminus is an essential feature: analogs lacking the amide are inactive. These results are consistent with results from previous experiments in *Helicoverpa/Heliothis* (20, 28) and *Bombyx* (21). The second group of analogs evaluated were Alanine-substituted analogs in which each amino acid residue of the hepta-peptide was replaced with an Alanine to examine the importance of each amino acid in the active core, as shown in Fig. 3b. The only replaceable amino acid residue is the Glycine in the middle of the sequence; this is consistent with previous conclusions about the FXPRLa family of peptides, in which X is the only amino acid residue that can be substituted (12). Leucine and Tryptophan at the N-terminus of the hepta-peptide cannot be exchanged, as indicated by the fact that an Alanine substitution at either of those positions completely abolished activity. This is different from DH in *Bombyx*; in that species these two amino acid residues are not essential for embryonic diapause induction activity, and the penta-peptide forms the active core (12, 21). It

is likely that the three amino acid residues at the N-terminus (Leucine, Tryptophan, Phenylalanine) form a hydrophobic binding region that interacts with the DH receptor in *H. zea*.

In a summary, the SAS of DH on diapause termination indicates that the active core of DH is the C-terminal hepta-peptide. Though C-terminus amidation and flexibility of Glycine are common features noted among FXPRLa family peptides, a special feature for DH in terminating pupal diapause is the necessity of Leucine and Tryptophan in the active core.

4. DH analog development

After determining the active core of DH for diapause termination in *H. zea*, we attempted to develop DH analogs that would be more potent in terminating diapause. For the first generation of DH analog development, we focused on structural simulation of the active core, the C-terminal amidated hepta-peptide. Thirteen DH agonist candidates (Table 1) including PK-Etz, PK-2Abf, *cyclo*[Asn¹]LPK, 901, and 904, were tested for their efficacy on diapause termination, and we then focused on four analogs that showed the most significant diapause termination activity. Our results revealed much lower ED₅₀s for several of these compounds. Compared to an ED₅₀ of 100 pmol per pupa for the native DH, the ED₅₀s of PK-2Abf and PK-Etz were 2 and 7 pmol per pupa, respectively, as shown in Table 2 (25), indicating that they are approximately 50- and 13-fold more potent than the native hormone. A locked trans-Pro mimetic component is incorporated in PK-Etz, forming a type I β -turn. Acetylation at the N-terminus with Tyrosine (Y) forms a similar hydrophobic group as the Leucine and Tryptophan in the hepta-peptide activity core for receptor anchoring. We deduce that these structures in PK-Etz significantly enhance its binding capacity with the receptor and subsequently elevate diapause termination activity. In PK-2Abf, a hydrophobic, polycyclic, aromatic moiety was appended to the N-terminus of a penta-peptide FTPRLa via a succinic acid linker. This compound exhibits the highest potency for diapause termination. The penta-peptide with 2 Abf is similar to the active core in that it not only retains the essential C-terminus, FTPRLa, but also amends the N-terminal with the large hydrophobic moiety 2Abf to substitute for Leucine and Tryptophan in the active core. Likely this moiety fits the hydrophobic pocket in the DH receptor

better than the original structure formed in the hepta-peptide by Leucine and Tryptophan. This binding greatly enhances receptor activation and signal transduction. In this way, a low level of PK-2Abf can produce significant diapause termination activity. PK-2Abf is the most potent DH agonist we identified so far, and it will become the lead molecule for developing our next generation of analogs. Based on these results, we also proposed two significant features of DH binding to its receptor as shown in Fig. 4. First the ligand adopts the transPro configuration to form a type I β -turn structure in the active core. Secondly the hydrophobic moiety near the active core is also vital, as evidenced by hyperpotent activity of PK-Etz and PK-2Abf. This suggests that a hydrophobic pocket is present near the DH receptor active core and serves as an anchoring region at the binding site. The deduced model proposed in Zhang et al. (25) is shown in Fig. 4.

We also used PK-2Abf as a lead molecule to generate DH antagonists and new compounds that can effectively prevent larvae from entering pupal diapause. Our approach to convert agonists into antagonists was to modify the configuration of the active core, especially the trans-Pro structure in DH and its agonists into a similar but nonfunctional structure. We screened 13 potential compounds (Table 1) and identified only one compound, *acetyl*-GLWA[Jo]RLa (DH-Jo), as a DH antagonist (Fig. 5). When DH-Jo is co-injected with DH, it significantly inhibits diapause termination activity caused by DH (24). A dihydroimidazole motif (Jones motif) was incorporated to serve as a surrogate of the trans-Pro configuration. Compared to the active core LWFGPRLa, substituting GP with a Jones motif and replacing F with A, resulted in a DH antagonist. DH-Jo may still be able to bind to the DH receptor, but this binding is unable to elicit a downstream response. DH-Jo represents a novel type of peptide antagonist, and it offers a new strategy for generating antagonists for members of the FXPRLa family and possibly other neuropeptides families as well.

Using the hyperpotent agonist PK-2Abf as a template, we proposed to synthesize similar or more effective compounds that can prevent *H. zea* from entering pupal diapause when applied to the larval stage. Ten structurally different compounds were synthesized and tested for diapause prevention activity. Interestingly, only **2Abf-Suc**-FTPRLa (PK-2Abf) and **2Abf-Suc**-FKPRLa (DH-2Abf-K) showed significant activity. PK-2Abf not only successfully prevented larvae

from entering diapause, but also caused severe mortality when injected into late-stage larvae (24). The other compound, DH-2Abf-K, exhibited similar diapause prevention activity as PK-2Abf, but it did not cause significant mortality. The ED50 for DH-2Abf-K on diapause prevention was approximately 7 pmole per larva, a value similar to the ED50 for PK-2Abf on pupal diapause termination. Thus DH-2Abf-K is a good candidate molecule for further study on diapause prevention, while PK-2Abf may be considered for practical usage both because it can prevent diapause as well as cause mortality, two attractive features for pest management.

One of our long-term goals is to manipulate the critical diapause response by using DH-related compounds to manage field populations of these pest species of moths. Injection is a direct and efficient method to deliver compounds for laboratory studies as we have shown, but for practical applications in green houses and in the field, it is obviously not a realistic method of application. Thus, we have also attempted to design and synthesize new generations of compounds that can be applied to the insect topically or orally. Our recent study reported that three special compounds, Decyl-1963, Dodecyl-1967 and Heptyl-1965, can effectively penetrate the cuticle of young pupae and prevent *H. zea* from entering diapause (23). Substituting the hydrophobic group 2Abf with a straight-chain hydrocarbon consisting of 7-12 carbons, increased the capability of these compounds to penetrate the cuticle of young pupae. Decyl-1963, containing a straight-chain formed by 10 carbons, showed the greatest activity, while other compounds containing short chain lengths or larger aromatic groups could not effectively penetrate the insect cuticle and prevent pupae from entering diapause. These three compounds will be used as lead molecules for designing a new generation of more effective and potent compounds capable of penetrating the insect cuticle or gut.

5. DH receptor

Cloning of the DH receptor makes it possible to develop a high-throughput *in vitro* assay system to screen agonist candidates before these analogs are examined in laborious *in vivo* bioassays. The PBAN receptor was well studied in different species (10), but studies on the DH receptor (DHR) were more limited. As with DH, the first DH receptor was identified from *Bombyx* (4).

Based on homology of this receptor with a mammalian neuromedin U receptor, DH receptor was cloned and identified as a G-protein coupled receptor with seven transmembrane domains. The ED50 of DH binding to the DH receptor was approximately 70 nM, while the ED50 of PBAN binding to the DH receptor was 1500 nM (4). The DH receptor is abundantly expressed in the ovary (19), an observation consistent with DH's function on the ovary to induce embryonic diapause in *Bombyx*. Vogel et al. (18) identified a partial sequence of the DH receptor in a transcriptome study of the sex gland in *H. virescens*. This DH receptor sequence information enabled us to quickly clone the DH receptor in *H. zea* (26). There are at least two variants of *dhr* mRNA, and a classic seven- transmembrane domain and a highly conserved C-terminal region are present. We noted that DHR mRNA is abundant in the ovary but is present in several other organs as well. We successfully expressed *H.zea* DHR in the *Xenopus laevis* oocyte system, but we failed to obtain a ligand-receptor interaction signal at reasonable physiological concentration range. However, a slightly different isoform of *dhr* mRNA (See Ref. 26 for details) was expressed in a CHO cell line, and the functional characterization was established (8). The CHO cell line offers a better system for high-throughput screening of a large number of analogs, and it also has the advantage of not requiring the task of oocyte injection and individual oocyte culturing.

Accordingly, over 30 mimetic analogs of the pyrokinin/DH peptide class featuring enhanced biostability were evaluated on the expressed HzDHR receptor CHO cell line. An intriguing D-amino acid containing analog, **2Abf-Suc-F[dA]PRLa**, was identified that elicited a super-agonist response, featuring 144% of the maximal response of the native DH hormone. Another biostable analog incorporating unnatural β -amino acids (**Ac-W[β^3 F]G[β^3 P]RLa**) featured an antagonist response against DH on the same receptor cell line (6). An *in vivo* evaluation of these two biostable analogs will be carried out in the future to determine their ability to disrupt diapause in pest heliothine insects.

In other experiments, five pyrokinin-like PRXamide receptors were cloned from the red flour beetle *Tribolium castaneum* and expressed in CHO cells. Functional characterization revealed that among these five receptors were not one, but two, that were DH-like; labeled TcPKr-A and TcPKr-B (6). The set of approximately 30 mimetic analogs of the pyrokinin/DH

peptide class mentioned above were also evaluated on these two DH-like TcPKr receptors. Interestingly, biostable analog **Hex-Suc-A[dF]**PRLa proved to be a selective agonist for TcPKr-A (maximal responses: 106% on TcPKr-A; 4% on TcPKr-B), whereas a related biostable analog **2Abf-Suc-AAAA[dF]**PRLa, extended by an additional 3 more Ala residues, proved to be a selective agonist for TcPKr-B (maximal responses: 104% on TcPKr-B; 3% on TcPKr-A). Furthermore, analog **2Abf-Suc-FSTRLa** demonstrated a selective antagonist response on TcPKr-A (maximal response of DH inhibited by 64% on TcPKr-A; but only 1% on TcPKr-B) (7). This latter analog contains a key replacement of Thr for Pro, which is evidently close enough to bind to the TcPKr-A DH-like receptor but not close enough to activate it, thereby acting as an antagonist. These selective biostable analogs provide intriguing tools for neuroendocrinologists in future efforts to determine the respective, and perhaps differential, roles that these two related DH-like receptors play in the regulation of diapause and/or other physiological systems mediated by pyrokinin peptides. These CHO cell lines expressing dhr from both *H. zea* and *T. castaneum* can in future be used as platforms to screen small molecule libraries to identify non-peptide agonists and/or antagonists capable of manipulating diapause to the detriment of pest insects.

6. Conclusions and future directions

In this review we highlight recent studies on DH and its role in regulating pupal diapause in the *Helicoverpa/Heliothis* complex. Expression of the *dh* gene is higher in nondiapausing pupae than in diapausing individuals at both the mRNA and protein levels, a feature that is consistent with the biological function of DH in terminating pupal diapause in *Helicoverpa/Heliothis*. This result is thus in marked contrast to the response in *B. mori*, where DH acts to induce embryonic diapause. Structure activity studies using truncated DH analogs and Alanine substitutions reveal that the hepta-peptide LWFGPRLa is the active core of this 24-amino acid DH. Two essential structural characteristics for DH and its analogs include a trans-Pro configuration with a type I β -turn at the active core and a hydrophobic moiety near the active core that is involved in ligand anchoring. Two DH agonists, PK-2Abf and PK-Etz, are considerably more potent than the native hormone. Using PK-2Abf as a lead molecule for development of second generation

analogs, we identified **acetyl**-GLWA**[Jo]**RLa (DH-Jo), as a DH antagonist and DH-2Abf-K as a DH agonist capable of preventing the entry into diapause when applied to late-stage larvae. Development of active compounds capable of penetrating the cuticle or midgut is a high priority for future application of this technology, and thus far we have identified three compounds (Decyl-1963, Dodecyl-1967, Heptyl-1965) that can penetrate the cuticle of young pupae and prevent the entry into diapause. Cloning of the *H. zea* DH receptor has facilitated the development of high throughput screening platforms. Several mimetic, biostable DH analogs have been identified on DH receptor platforms from *H. zea* and *T. castaneum*; these new agents feature superagonism, selective agonism, as well as antagonist responses. Future studies are planned to determine the *in vivo* effects of these intriguing biostable DH analogs on diapause in pest insects. The CHO cell line expressing systems will also be used for future large scale compound screens to identify nonpeptide analogs capable of disrupting insect diapause.

Our findings and the compounds we identified may prove useful in studies of other peptide analogs, especially for other members of the FXPRLa family of peptides such as PBAN in moths and pyrokinin in *Drosophila* and *Locusta*. Future work to identify additional components involved in the signaling pathway of DH and deciphering how DH interacts with other hormones that prompt insect growth and development will be critical for understanding the fine-tuning of the network regulating diapause. Availability of the DH receptor and high affinity analogs now enables us to explore more structural biology features of DHR binding to its ligand, thus making it possible to generate more potent and efficient small molecules that can be used to disrupt insect diapause. Based on structures now on hand, it should be possible to design and characterize new compounds with superior capacity to penetrate the insect cuticle and the lining of the midgut. Active compounds with the capacity to penetrate the epidermis will be most desired for translating this technology to future field application.

Acknowledgements

The authors gratefully acknowledge funding from National Institute of Food and Agriculture (NIFA), United States Department of Agriculture, most recently USDA-NIFA Grant No. 2015-???.

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Table 1

Sequences of agonist and antagonist candidates tested in diapause termination bioassays in *Helicoverpa zea*. Adapted from (24, 25).

Ac: acetyl-; Etz: *trans*Pro Etzkorn mimetic component; 2Abf: 2-amino-7-bromo-fluorene; Suc: succinoyl; Hex: hexanoyl; Hyp: Hydroxyproline; Oic: octahydroindole-2-carboxyl.

Agonist candidates		Antagonist candidates	
Name	Sequence	Name	Sequence
PK-2Abf	7Abf-Suc -FTPRLa	DH-Jo	Ac -GLWA[Jo]RLa
PK-Etz	Ac -YF[Etz]RLa	1605	Hex - Suc - A[dF] PRLa
901	Hex -FT[Hyp]RLa	1373	2Abf - Suc - A[dF] PRLa
cyclo[Asn ¹]LPK	<i>Cyclo</i> -[NTSFTPRL]	1374	2Abf - Suc - F[dF] PRLa
03661	<i>Cyclo</i> -[QQPFVPRLa]	1604	2Abf - Suc - F[dF] PRAa
1780	Ac -W[β ³ F]G[β ³ P]RLa	1607	2Abf - Suc -[β ³ F][dF]PRLa
1782	Ac -LW[β ³ F]G[β ³ P]RLa	1525	2Abf - Suc - AAAA[dF] PRLa
1783	Ac -LWFG[β ³ P]RLa	1478	2Abf - Suc - A[dF] PRAa
1784	Ac -LW[β ³ F]GPRLa	35-191	<i>Cyclo</i> [GLWFGPRL]
904	Hex -FT[Oic]RLa	1608-2	2Abf - Suc - F[dF] [β ³ P]RLa
1823	Hex - Suc -FTPRLa	1790A	2Abf - Suc -LWA[dF]PRLa
1824	Hex - Suc -FT[Hyp]RLa	1798A	<i>Cyclo</i> [Suc RWF[dF][a4G]]RLa
017112	2Abf - Suc -FT[Oic]RLa	1935	2Abf - Suc - A[dK] PRLa

Table 2

Comparison of the ED50s of DH and its analogs on terminating diapause in *H. zea*. From (25).

Material	~ED50 (pmol)
DH	100
PK-2Abf	2
PK-Etz	7
901	17
<i>cyclo</i> [Asn ¹]LPK	878

Figures legends

Figure 1 Sequence alignment of DHs from *Bombyx mori* (GenBank# D13437), *Helicoverpa zea* (GenBank# AAA20661), *Heliothis virescens* (GenBank# AAO20095), *Helicoverpa armigera* (GenBank# AAL05596), *Helicoverpa assulta* (GenBank# AAC64293), *Orgyia thyellina* (GenBank# BAE94185), *Manduca sexta* (GenBank# AAO18192), *Samia ricini* (GenBank# AAX99220), *Spodoptera littoralis* (GenBank# AAK84160), *Spodoptera exigua* (GenBank# AAT64424), *Antheraea pernyi* (GenBank# AAR17699), and *Clostera anastomosis* (GenBank# ABR04093). Identical amino acid residues are shaded in gray.

Figure 2 Northern blots comparing abundance of *dh* transcripts in diapausing pupae and nondiapausing individuals at different days after pupation. Adapted from (22). D: diapausing pupae at 18 °C; ND: nondiapausing individuals (pupae and pharate adults) at 25°C.

Figure 3 Structure activity relationship study of DH terminating diapause using (a) truncated analogs and (b) Alanine substitution revealed that the active core is the C-terminal heptapeptide, LWFGPRLa. The amount of each peptide injected is 1 nmole per pupa. From (27).

Figure 4 A deduced model to emphasize two characteristics of DH interaction with the DH receptor. First, within the active core, Proline adopts a transPro configuration to form a

type I β -turn. Second, a hydrophobic moiety is located near the active core and likely anchors the ligand to the receptor. Adapted from (25).

Figure 5 DH-Jo (Ac-GLWA[Jo]RLa) shown in the upper panel, acts as a DH antagonist. From (24).

Figure 1

Hezea-DH	NDV K DG-AASGAHSDRLG-LWFGPRL-NH ₂
Hearm-DH	NDV K DG-AASGAHSDRLG-LWFGPRL-NH ₂
Heass-DH	NDV K DG-AASGAHSDRLG-LWFRPRL-NH ₂
Hevir-DH	NDD K DG-AASGAHSDRLG-LWFGPRL-NH ₂
Bomor-DH	TDM K DE-SDRGHSE R GA-LWFGPRL-NH ₂
Masex-DH	NDI K DE-GDRGAHSD R GA-LWFGPRL-NH ₂
Saric-DH	NDV K DE-GDRGAHSD R GS-LWFGPRL-NH ₂
Anper-DH	NDI K DE-GDKGAHSD R GS-LWFGPRL-NH ₂
Split-DH	NEI K DGGSDRG A HSD R AG-LWFGPRL-NH ₂
Spexi-DH	NEI K DGGSDRG A HSD R AG-LWFGPRL-NH ₂
Orthy-DH	NDV K DDGQDRVAHSD R GGQLWFGPRL-NH ₂
Clana-DH	TNND N TM K DGGADRG A HSD R GG-LWFGPRL-NH ₂

Figure 2

Figure 3

a

b

Figure 4