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FXPRL-amide peptides induce ecdysteroidogenesis through a G-protein coupled receptor expressed in the prothoracic gland of *Bombyx mori*

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Abstract

The FXPRL-amide peptide family (pyrokinin/PBAN family) consists of insect peptides that function broadly in insect life processes and are characterized by a conserved C-terminal motif. In the silkworm, *Bombyx mori*, sex pheromone biosynthesis and induction of embryonic diapause are regulated by peptides from this family. To elucidate other functions of *Bombyx* FXPRL-amide peptides, we analyzed the tissue expression patterns of two known *Bombyx* G-protein coupled receptors for these peptides. We found that the *Bombyx* diapause hormone receptor (BmDHR), is expressed in the prothoracic gland (PG), the organ which synthesizes and releases the insect molting hormones, ecdysteroids. Furthermore, diapause hormone (DH), a member of the *Bombyx* FXPRL-amide peptides, increases both intracellular Ca^{2+} and cAMP concentrations and induces ecdysteroidogenesis in late fifth instar PGs coincident with BmDHR expression in the PGs. DH also has the highest prothoracicotropic activity among the FXPRL-amide peptides, which corresponds well to the ligand specificity of heterologously expressed BmDHR. These results demonstrate that FXPRL-amide peptides can function as prothoracicotropic factors through the activation of BmDHR and may play an important role in controlling molting and metamorphosis.

Keywords: FXPRL-amide peptide; Diapause hormone; PBAN; Pyrokinin

1. Introduction

Most life processes of insects such as metamorphosis, reproduction, and diapause, as well as numerous other behaviors, are controlled by various neuropeptides. Several insect neuropeptides are multifunctional affecting numerous target organs, such as the FXPRL-amide peptide family (pyrokinin/PBAN family) that comprises several peptides from various insect species and which is characterized by a conserved C-terminal FXPRL-amide motif (Table 1). FXPRL-amide peptides mediate various functions such as muscle contractions (Holman et al., 1986), activation of sex pheromone biosynthesis (Kitamura et al., 1989; Raina et al., 1989), cuticle melanization (Matsumoto et al., 1990), induction of embryonic diapause (Imai et al., 1991), and breaking of pupal diapause (Zhang et al., 2004; Zhao et al., 2004).

In the silkworm, *Bombyx mori*, two FXPRL-amide peptides, diapause hormone (DH) and pheromone biosynthesis activating neuropeptide (PBAN) have been isolated (Kitamura et al., 1989; Imai et al., 1991). DH is synthesized in the female subesophageal ganglion (SOG) and induces embryonic diapause by acting on developing oocytes during pupal–adult development (Yamashita, 1996). PBAN is also synthesized in the female SOG and stimulates sex pheromone synthesis in the pheromone glands of adult females (Teal et al., 1996). These peptides are encoded by a single gene (*DH-PBAN* gene) along with three other FXPRL-amide peptides, α-, β- and γ-SGNP (Sato et al., 1993). All of these peptides, with the exception of α-SGNP, can activate pheromone biosynthesis as well as induce the production of diapause eggs; α-SGNP has no effect on diapause

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Table 1
Sequences of FXPRL-amide peptides

Species	Peptide	Peptide sequence
Bombyx mori	PBAN	LSEDMPATPADQEMYQPDPEEMESRTRY**FSPRLamide**
	DH	TDMKDESDRGAHSERGALW**FG**P**RLamide**
	α-SGNP	II**FTPKLamide**
	β-SGNP	SVAKPQTHESLE**F**I**PRLamide**
	γ-SGNP	TMS**FSPRLamide**
Helicoverpa zea	PBAN	LSDDMPATPADQEMYRQDPEQIDSRTKY**FSPRLamide**
	PGN-24	NDVKDGAASGAHSDRLGLW**FG**P**RLamide**
	PGN-7	VI**FTPKLamide**
	PGN-18	SLAYDDKS**F**ENVE**FTPRLamide**
	PGN-8	TMN**FSPRLamide**
Drosophila melanogaster	CAPA-3	TGPSASSGLW**FG**P**RLamide**
	DrmPK-2	SVP**FKPRLamide**
Leucophaea maderae	Leucopyrokinin	pETS**FTPRLamide**

FXPRL motif sequences are indicated in boldface and "amide" indicates C-terminal amidation.

induction but can stimulate pheromone production (Sato et al., 1993). Interestingly, DH can be detected in the SOG throughout larval development in larvae destined to produce diapause eggs as well as non-diapause eggs (Kitagawa et al., 2005). Moreover, the *DH-PBAN* gene is also expressed in the SOG of male pupae (Sato et al., 1994) with no sexual differences in the levels of DH during the later stages of larval–pupal–adult development (Kitagawa et al., 2005). These findings indicate that *Bombyx* FXPRL-amide peptides may have functions during postembryonic development other than the induction of embryonic diapause and activation of pheromone biosynthesis.

Recently, several G-protein coupled receptors (GPCRs) for FXPRL-amide peptides have been identified in *Bombyx* (Hull et al., 2004; Homma et al., 2006) as well as in other species (Park et al., 2002; Choi et al., 2003; Rosenkilde et al., 2003; Cazzamali et al., 2005). The two *Bombyx* GPCRs, PBAN receptor (BmPBANR) and DH receptor (BmDHR), were cloned from pheromone glands and ovaries, respectively. BmPBANR and BmDHR are encoded on two different cDNAs and when conceptually translated are ∼60% homologous. In contrast, BmPBANR homology with putative PBAN receptors from other insect species (GenBank accession numbers DQ407742, AY319852, AY792036, AY974334) is >80%, a likely indication that BmDHR and BmPBANR mediate different biological functions. Indeed, disruption of gene function via RNA interference has shown that BmPBANR mediates sex pheromone biosynthesis in *B. mori* (Ohnishi et al., 2006) while RT-PCR analyses demonstrating that *BmDHR* mRNA expression in the ovaries coincides with the DH-sensitive stage suggest that BmDHR is involved in the induction of embryonic diapause (Homma et al., 2006). Despite mediating different biological functions, both receptors couple to calcium dependent pathways. *Xenopus* oocytes expressing BmDHR generated Ca^{2+} dependent Cl^- (Ca^{2+}-Cl^-) currents following application of DH (EC_{50} value of 70 nM) (Homma et al., 2006). Similarly, recombinant BmPBANR in Sf9 cells mobilized extracellular calcium in a PBAN dose-dependent manner (Hull et al., 2004). The identification and characterization of these FXPRL-amide peptide receptors not only makes it possible to better elucidate the mechanisms underlying pheromone biosynthesis and embryonic diapause, but also affords the chance to identify novel functions of these peptides. For example, analyses of the spatial and temporal expression patterns of the receptors would be expected to reveal new target organs for FXPRL-amide peptides.

In the present study, we performed reverse transcriptase (RT)-polymerase chain reaction (PCR) analyses of BmDHR and BmPBANR using various tissues from late fifth instar larvae in order to assess the possibility that *Bombyx* FXPRL-amide peptides mediate functions during the later stages of larval–pupal development. We found that *BmDHR* is expressed in the prothoracic gland (PG). The PG is the synthetic organ of ecdysteroids, which are essential for molting and metamorphosis in insects (Gilbert et al., 2002). While it has been established that prothoracicotropic hormone (PTTH) regulates ecdysteroidogenesis in the PG (Ishizaki and Suzuki, 1994; Gilbert et al., 2002), we show that activation of BmDHR by FXPRL-amide peptides during the later stage of the fifth instar also induces ecdysteroidogenesis in the PG. This report represents the first identification of a *Bombyx* prothoracicotropic factor other than PTTH and a corresponding GPCR that mediates ecdysteroidogenesis. These findings indicate that FXPRL-amide peptides may play an important role in insect molting and metamorphosis.

2. Materials and methods

2.1. Experimental animals

B. mori racial hybrids (Kinshu × Showa) were fed on an artificial diet at 25 °C under a 16 h light/8 h dark photoperiod, and staged after the final (fourth) larval ecdysis. Wandering behavior in most larvae started on day 6 of the fifth instar and pupation occurred on day 10.

2.2. Synthetic and recombinant peptides

PBAN, DH and the three types of SGNP were synthesized as described previously (Kitamura et al., 1989; Yamashita et al., 1998). Recombinant PTTH was prepared as described previously (Ishibashi et al., 1994).