Chapter 37

FXPRLamide (Pyrokinin/PBAN) Family

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ABSTRACT

The pyrokinin/pheromone biosynthesis-activating neuropeptide (PK/PBAN) family is one of the most studied neuropeptide (Np) families in insects and is one of the most important in insect physiology regulation. Physiological functions regulated by the PK/PBAN peptides include development, mating, muscle contraction, and tanning. Members of the family are found in many Lepidopteran and other insect species. In this chapter, we briefly present a historical perspective of the discovery of the PK/PBAN peptides, provide details on the structure of the PK/PBAN genes and the processing of their prohormone to bioactive peptides, describe the distribution of the mRNA and peptides in the insect nervous system, and summarize the current knowledge on the PK/PBAN receptors, their signaling mechanisms, and their biological activity. Employment of the PK/PBAN family of peptides as a basis for designing a novel generation of insect-control agents based on Np antagonists is also briefly discussed.

INTRODUCTION

The pyrokinin/pheromone biosynthesis-activating neuropeptide (PK/PBAN) family of peptides is one of the most studied neuropeptide (Np) families in insects and is one of the most important in regulating their physiology. Physiological functions regulated by the PK/PBAN peptides include development and mating, as well as muscle contraction activities and induction of cuticular melanization. The family is present in a wide variety of insects and comprises the following C-terminally amidated peptides: PKs; myotropins (MTs); PBANs; diapause hormone (DH); and β- and γ-subesophageal Nps (SGNPs), all of which share a common sequence (FXPRLa). PK/PBAN peptides have been studied for nearly 30 years, mainly in moths, during which many new members have been identified, the genes of the peptides and their receptors have been cloned and characterized, and their distribution in the nervous system studied in many moth species; their physiological roles have been determined through physiological and behavioral analyses at the whole-organism level, complemented by molecular and cellular studies. The PK/PBAN peptides also form a basis for the development of new-generation insect-control agents based on their antagonists.

In this chapter, we review the current knowledge of the PK/PBAN family of peptides, most of which is based on studies with PBAN and DH in Lepidopterans and describe the application of this knowledge to the generation of highly potent, selective, and readily bioavailable potential insect-control agents. The amount of information accumulated on this family is too large to be covered within the framework of this chapter, and the reader is referred to the reviews cited in the text. For the convenience of the reader, and to minimize confusion that might arise from the complexity of the peptide terminology, each of the main sections below is divided into subsections based on *pban* or *capa* gene-encoded peptides and their characteristics in Lepidopteran or non-Lepidopteran species.

DISCOVERY OF THE PK/PBAN FAMILY OF PEPTIDES: HISTORICAL PERSPECTIVE

PBAN

The first member of the family to be discovered was leucopyrokinin (LPK), identified in the cockroach *Leucophaea maderae* in 1986.[14] It was followed in 1989 by the pheromone biosynthesis activation neuropeptide (PBAN), first reported by Raina and Klun[29] as a Np that regulates sex pheromone production in the female moth *Helicoverpa zea*; Raina et al.[28] isolated and characterized it as a 33 amino acid, C-terminally amidated Np, which was designated *Hez*-PBAN. Since 1989, the primary sequences of 24 PBAN molecules from many other moth species have been determined[3] (Table 1). PBAN molecules were also found in insects belonging to orders other than Lepidoptera, for example, *Drosophila melanogaster*, *Anopheles*

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TABLE 1 Amino Acid Sequence of PBAN and Other Peptides of the PK/PBAN Family

Code name	Insect species	Amino acid sequence
Hez-PBAN	*Helicoverpa zea*	LSDDMPATPADQEMYRQDPEQIDSRTKY**FS**PRLa
Bom-PBAN-I	*Bombyx mori*	LSEDMPATPADQEMYQPDPEEMESRTRY**FS**PRLa
Bom-PBAN-II	*Bombyx mori*	RLSEDMPATPADQEMYQPDPEEMESRTRY**FS**PRLa
Bma-PBAN	*Bombyx mandarina*	LSEDMPATPADQEIYQPDPEVMESRTRY**FS**PRLa
Lyd-PBAN	*Lymantria dispar*	LADDMPATMADQEVYRPEPEQIDSRNKY**FS**PRLa
Has-PBAN	*Helicoverpa assulta*	LSDDMPATPADQEMYRQDPEQIDSRTKY**FS**PRLa
Agi-PBAN	*Agrotis ipsilon*	LADDTPATPADQEMYRPDPEQIDSRTKY**FS**PRLa
Mab-PBAN	*Mamestra brassicae*	LADDMPATPADQEMYRPDPEQIDSRTKY**FS**PRLa
Spl-PBAN	*Spodoptera littoralis*	LADDMPATPADQELYRPDPDQIDSRTKY**FS**PRLa
Plx-PBAN	*Plutella xylostella*	RLKDSGLAPPDEYRTPELLDARAQY**FS**PRLa
Har-PBAN	*Helicoverpa armigera*	LSDDMPATPADQEMYRQDPEQIDSRTKY**FS**PRLa
Hev-PBAN	*Heliothis virescens*	ADDMPATPADQEMYRQDPEQIDSRRTKY**FS**PRLa
Spe-PBAN	*Spodoptera exigua*	LSDDMPATPADQELYRPDPDQIDSRTKY**FS**PRLa
Anp-PBAN	*Antheraea pernyi*	LSDDMPATPKDQEMYHQDPEQVDTRTRY**FS**PRLa
Scr-PBAN	*Samia cynthia ricini*	LTEDMPATPTDQEMFDQDPEQIDTRTRY**FS**PRLa
Ado-PBAN	*Adoxophyes sp*	QSEAVTSSDEQVYRQDMSPVDGRLKY**FS**PRLa
Mas-PBAN	*Manduca sexta*	ISEDMPATPSDQEYPMYHPDPEQIDTRTRY**FS**PRLa
Assc-PBAN	*Ascotis selenaria cretacea*	QLVDDVPQRQQIEEDRLGSRTRF**FS**PRLa
Cla-PBAN	*Clostera anastomosis*	LADDMPATPSDQEYYRQDPEQIDSRSNY**FS**PRLa
Ort-PBAN	*Orgyia thyellina*	LSDDMPATPPDQEYYRPDPEQIDSRTKY**FS**PRLa
Sol-PBAN	*Solenopsis invicta*	SYGDAYEVDEDDHPL**F**V**PRL**a
	Solenopsis richetri	SYGDAYEVDEDDHPL**F**V**PRL**a
	Solenopsis geminate	SYGDAYEVDEDDHPL**F**V**PRL**a
	Solenopsis pergandii	SYGDAYEVDEDDHPL**F**V**PRL**a
	Solenopsis carolinesis	SFGDAYEVDEDDHPL**F**V**PRL**a
Bom-DH	*Bombyx mori*	TDMKDESDRGAHSERGALW**FG**PRLa
Hez-DH	*Helicoverpa zea*	NDVKDGAASGAHSDRLGLW**FG**PRLa
Has-DH	*Helicoverpa assulta*	NDVKDGAASGAHSDRLGLW**FG**PRLa
Har-DH	*Helicoverpa armigera*	NDVKDGAASGAHSDRLGLW**FG**PRLa
Agi-DH	*Agrotis ipsilon*	NDVKDGGADRAHSDRGGMW**FG**PRLa
Spl-DH	*Spodoptera littoralis*	NEIKDGGSDRGAHSDRAGLW**FG**PRLa
Lom-PK-I	*Locusta migratoria*	pEDSGDGWPQQP**F**V**PRL**a
Lom-PK-II	*Locusta migratoria*	pESVPT**FT**PRLa
Lem-PK	*Leucophaea maderae*	pETS**FT**PRLa

TABLE 1 Amino Acid Sequence of PBAN and Other Peptides of the PK/PBAN Family—Cont'd

Pea-PK-5	*Periplaneta americana*	GGGGSGETSGMW**FG**PR**La**
Pea-PK-6	*Periplaneta americana*	SESEVPGMW**FG**PR**La**
Lom-MT-I	*Locusta migratoria*	GAVPAAQ**FS**PR**La**
Lom-MT-II	*Locusta migratoria*	EGD**FT**PR**La**
Lom-MT-III	*Locusta migratoria*	RQQP**FV**PR**La**
Lom-MT-VI	*Locusta migratoria*	RLHQNGMP**FS**PR**La**
Scg-MT-1	*Schistocerca gregaria*	GAAPAAQ**FS**PR**La**
Pss-β-SGNP (PT)	*Pseudaletia separata*	KLSYDDKVFENVE**FT**PR**La**
Hez-β-SGNP	*Helicoverpa zea*	SLAYDDKSFENVE**FT**PR**La**
Has-β-SGNP	*Helicoverpa assulta*	SLAYDDKSFENVE**FT**PR**La**
Har-β-SGNP	*Helicoverpa armigera*	SLAYDDKSFENVE**FT**PR**La**
Agi-β-SGNP	*Agrotis ipsilon*	SLSYEDKMFDNVE**FT**PR**La**
Mab-β-SGNP	*Mamestra brassicae*	SLAYDDKVFENVE**FT**PR**La**
Spl-β-SGNP	*Spodoptera littoralis*	SLAYDDKVFENVE**FT**PR**La**
Bom-β-SGNP*	*Bombyx mori*	SVAKPQTHESLE**FI**PR**La**
Drm-β-SGNP (PK-2)*	*Drosophila melanogaster*	SVP**FK**PR**La**
Hez-γ-SGNP	*Helicoverpa zea*	TMN**FS**PR**La**
Has-γ-SGNP	*Helicoverpa assulta*	TMN**FS**PR**La**
Har-γ-SGNP	*Helicoverpa armigera*	TMN**FS**PR**La**
Agi-γ-SGNP	*Agrotis ipsilon*	TMN**FS**PR**La**
Mab-γ-SGNP	*Mamestra brassicae*	TMN**FS**PR**La**
Spl-γ-SGNP	*Spodoptera littoralis*	TMN**FS**PR**La**
Bom-γ-SGNP	*Bombyx mori*	TMS**FS**PR**La**

Bold letters indicate conserved amino acid sequences. DH – diapause hormone; PBAN – pheromone biosynthesis-activating neuropeptide; PK – pyrokinin; MT – myotropin; PT – pheromonotropin; SGNP – subesophageal ganglion neuropeptide.
**A member of the family but with the difference that X = Lys or Ilu. The sequence of α-SGNP is the same in all Lepidopterans (VIFTPKLa). References for the listed peptides can be found in Ref. 3 Ort-PBAN and Sol-PBAN were published in Refs. 8, 34, respectively. Additional sequences of PBAN, DH, α-, β-, and γ-SGNP can be found in Ref. 16.*

gambiae, *Aedes aegypti*, *Tribolium castaneum*, and aphids. These peptides were not cloned but were predicted from the genome sequences of the insects.[16] Comparisons among the primary sequences of the PBAN molecules revealed that the Lepidopteran peptides share a high degree of homology (Table 1). Regardless of their length and degree of homology, all PBANs contain the C-terminal pentapeptide signature sequence FSPRLa.

Other Peptides of the Family

Further studies of insect Nps revealed that other peptides also have a very similar C-terminal sequence: FXPRLa (X = S, T, G or V). To mention just a few, PKs (e.g. LPK,[14] Lom-PK-I and Lom-PK-II) and the myotropins (e.g., Lom-MT-I to IV), which are myotropic peptides isolated from the migratory locust, *Locusta migratoria*; PK

peptides of *Periplaneta americana* —Pea-PK-5 and Pea-PK-6—and the myotropic peptide of *Schistocerca gregaria* (Scg-MT-1). Additional peptides that were found to share the same consensus sequence are β- and γ-SGNPs; pheromonotropin (PT)—an 18 amino acid peptide isolated from *Pseudaletia (Mythimna) separate*—which is now included in the β-SGNP group; and DH, which induces embryonic diapause in *Bombyx mori* and terminates pupal diapause in *Helicoverpa/Heliothis* moths. For review, see Ref. 3 and Table 1. As the number of available sequences has increased, the *X* in the consensus sequence has been extended to include I (as in *Bom*-β-SGNP) and K (as in *Drm*-β-SGNP, see Table 1). All peptides sharing the above sequences were grouped into one family, which was designated the FXPRLa family or the PK/PBAN family, named after PBAN and Lem-PK, which was the first member of this family to be identified[14] and had myostimulatory ("kinin"-like) action and a pGlu residue at its N-terminus, (and thus termed pyrokinin).

More recently, in light of genomic database records, sequence similarities to previously annotated genes and actual gene sequencing, it became apparent that PK/PBAN peptides are encoded by two genes, which are found in many Lepidopteran and non-Lepidopteran insect species.[16,26] These findings help to categorize the FXPRLa peptides into two main groups that are processed from either the *pban* gene or the *capa* gene. To avoid terminological confusion, in this chapter, these peptides will be designated as *pban* gene-encoded peptides (PBAN, DH, β-, and γ-SGNPs) or *capa* gene-encoded peptides (CAPA-PKs), respectively. Exploration of the *capa* gene also reveals two additional periviscerikinin peptides sharing just the PRLa C-terminal sequence; they are termed CAPA-1 and 2 or PVK-1 and 2, and are not addressed in this chapter. For further information on the *pban* and *capa* gene peptides, the reader is referred to Refs. 16, 24, 26 and references therein.

STRUCTURE OF THE mRNA/GENE

PK/PBAN peptides are encoded by two genes: the *pban* gene (also termed *dh-pban* gene)[3,16] and the *capa* gene, which are present in almost all insects.[16,26]

pban Gene

The *pban* gene was first isolated from *H. zea* in 1989 by Raina[28] and encodes the PBAN preprohormone and three additional peptides—DH, β-SGNP and γ-SGNP—all of which contain the family's consensus sequence and one FXPKLa peptide (α-SGNP)[16] (Table 1). To date, the *pban* gene has been cloned from 19 species of Lepidopteran moths, in all of which it encodes all five PK/PBAN peptides. PBAN, DH, and β-SGNP are also produced in many other—non-Lepidopteran insects (e.g., the fire ant, the mosquito and the red flour beetle *Tribolium castaneum*), whereas α- and γ-SGNP are not always encoded.[16] In *Drosophila*, only two peptides are encoded by the *pban* gene (termed *hugin* gene).[16]

capa Gene

The *capa* gene was first identified in *D. melanogaster*. It encodes a CAPA-PK and two periviscerokinins—PVK-1 and PVK-2—which contain a PRLa sequence at their carboxy terminus.[16,26] CAPA-PK is also termed DH-like PK (because of the C-terminal-consensus WFGPRLa sequence typical of all DH Nps), CAPA-3 or myotropin-1 in *Drosophil*a, PK-1 in *Manduca sexta*, and PK-5 and PK-6 in *P. americana* (Table 1). The *capa* gene is present in many insect species, including Lepidopterans, and it encodes CAPA-PK in all tested insects other than *Apis mellifera.*[16] To the best of our knowledge, the *pban* or *capa* genes have not yet been cloned from *L. migratoria*, *S. gregaria* or *P. americana* although CAPA-PKs have been isolated, or predicted in light of sequence homologies, and localized in various parts of the nervous system.

PROCESSING OF THE PRECURSOR

pban Gene and *capa* Gene

The *pban* gene and the *capa* gene are translated into typical Np preprohormones, which contain, at their N-terminus, a signal sequence that is removed before their transfer to the Golgi apparatus. In both genes, all bioactive peptides in the prohormone are separated by single or pairs of basic amino acids at their N-terminus, and by a Gly followed by a single or pair of basic amino acids at the C-terminus, which serve as post-translational sites for processing and C-terminal amidation enzyme, respectively (e.g., see a *pban* gene structure and processing sites of *Orgyia thyellina*[34] and a *capa* gene of *M. sexta*).[19]

Peptides derived from the *capa* gene undergo cell-type-specific sorting and packaging and were the first insect Nps shown to be differentially processed. Differential processing has been shown in *D. melanogaster* and in *B. mori* and in *P. americana* in which it exhibits an interesting and uncommon phenomenon, that different peptides originating from the *capa* gene could be individually packaged and fuse with each other and with other peptide-containing vesicles in the cytoplasm, after leaving the trans-Golgi network. A detailed description of the *capa* gene prohormone processing, alternative-splicing mechanism, sorting, and packaging is presented in Refs. 26, 30.

DISTRIBUTION OF THE PEPTIDE/mRNA

Peptides

pban Gene-Derived Peptides

PBAN in Lepidoptera

PBAN distribution was studied by immunochemical methods (radioimmunoassay, enzyme-linked immunosorbent assays (ELISA) and immunocytochemical (ICC) methods) in adult females, males and larvae of various Lepidopteran species (e.g., *H. zea*, *M. sexta*, *B. mori*). These studies found PBAN-immunoreactive (IR) neurons in the brain and subesophageal ganglion (SOG) that projects to the corpora cardiaca (CC) and in ganglia of the ventral nerve cord (VNC). In the SOG, PBAN is found in seven pairs of neurosecretory cells. PBAN-IR is also found in the CC and in a pair of cells in the thoracic and abdominal ganglia. The ICC studies also reveal two pairs of axons originating from cell bodies in the SOG, which extend the entire length of the VNC and terminate in the terminal abdominal ganglia. ELISA studies indicate that the brain–SOG complex contains the highest level of PBAN-like IR, followed by the CC and the thoracic ganglia, and that abdominal ganglia contains low levels of the peptide. A more detailed analysis of PBAN-IR-containing cells, by means of matrix-associated laser desorption/ionization combined with mass spectrometry (MALDI/MS), reveal a basically similar distribution. For review, see Refs. 11, 27.

DH in Lepidoptera

DH distribution was studied by ICC analyses in *H. armigera* and *B. mori*. In *H. armigera*, DH is localized in the mandibular, maxillary, and labial cell clusters of the SOG, in a pair of ventral midline lateral neurons, and in each thoracic ganglion at all the developmental stages (embryo, larva, pupa, and adults).[33] In *B. mori*, immunoreactive neural processes with varicosities are in the CC and the corpus allatum (CA); as in *H. armigera*, IR is detected at all developmental stages.[21]

PBAN in Non-Lepidopteran Species

ICC testing of the distribution of PBAN in the fire ant *Solenopsis invicta* found that the peptide-producing neurons are located in the SOG and CC in all adult sexual forms. PBAN-IR is also detected in two segmented thoracic and four segmented abdominal ganglia, which project to the neurohemal perisympathetic organs (PSOs), indicating release of PBAN to the hemolymph.[9]

Other FXPRLa-Containing Peptides in Non-Lepidopteran Species

More recently, detailed bioinformatic studies were combined with MALDI-TOF and electrospray ionization quadrupole (ESI-Q)-TOF MS to make an inventory of Nps containing the PK/PBAN consensus sequence in different parts (ganglia and nerves) of the central nervous systems (CNSs) of *L. migratoria* and *S. gregaria*. In both species, the peptides are detected in the SOG, in the retrocerebral complex, which comprises the CC and the CA, and in the hypocerebral ganglion, which is part of the stomatogastric nervous system. In *S. gregaria* FXPRLa-containing peptides are also detected in the recurrent and esophageal nerves (which are also part of the stomatogastric nervous system).[10] Because the *pban* gene and *capa* gene have not yet been cloned from locusts, it is difficult to relate these peptides (termed PKs in the above study) to any of the PK/PBAN family gene products.

capa Gene-Derived Peptides

CAPA-PKs in Lepidopteran and Non-Lepidopteran Species

CAPA-PK peptides are the characteristic and most abundant Nps in the abdominal neurohemal system. The location and projection of CAPA neurons was studied in D. melanogaster, P. americana and M. sexta. So far, CAPA-PKs are expressed only in the CNS and not in any other tissue. The peptides are synthesized in median neurosecretory neurons of the abdominal ganglia and are released from the PSOs into the hemolymph. In Drosophila, a truncated form of CAPA-PK was detected in the neurosecretory cells of the SOG, in the retrocerebral complex, and in the larval ring gland, it was also found in the SOG of M. sexta. In P. americana, CAPA-PKs also occur in interneurons, from which they may be released into the CNS. A detailed review on the distribution of CAPA peptides by MS is presented in Ref. 26.

Gene/mRNA

pban Gene Expression in Lepidopteran and Non-Lepidopteran Species

pban gene distribution and expression were determined by Northern blotting, real-time polymerase chain reaction (RT-PCR), and in situ hybridization, and the results are similar to those obtained by immunochemistry. PBAN mRNA is expressed in both male and female moths and is detected in the SOG as well as in the brain, thoracic ganglia, and abdominal ganglia of larvae, pupae, and adults of several moth species (*H. zea*, *H. armigera*, *M. sexta*, *and B. mori*). Expression is predominantly in the SOG, where gene transcripts are located in the same DH-PBAN-producing neurosecretory cells as detailed above (Ref. 27 and references therein).

pban gene transcripts are also found in the head, thorax, and abdominal ganglia of the female fire ant, *S. invicta*. The strongest transcriptional signal and highest number of *pban* gene copies are detected in the head, and much weaker and

fewer ones in the thoracic and abdominal ganglia, where the numbers of copies were almost negligible.[9]

capa Gene Transcripts in Lepidopteran and Non-Lepidopteran Species

In situ hybridization of CAPA mRNA was performed in Lepidopteran and non-Lepidopteran species. In *M. sexta*, about 15 pairs of CAPA interneurons were detected in the brain of first-instar larvae. The number of neurons decreased during postembryonic development and only one pair expressed CAPA mRNA in the adult brain.[19]

RECEPTORS AND SIGNALING CASCADE

PK/PBAN Receptors

A variety of techniques—bioinformatic, biochemical, molecular, physiological, pharmacological, and histochemical—were used to predict, clone, isolate, locate, and characterize the PK/PBAN receptors in various insects. The great majority of the studies focused on the receptors that mediate the pheromonotropic activity in moths. Early studies had suggested that PBAN might act on a target other than the pheromone gland (see Ref. 3 and references therein), but since the late 1990s, it became clear, beyond any doubt, that in most moth species, PBAN acts directly on the pheromone gland via the PK/PBAN receptors. A histochemical study of the pheromone gland cells of *H. peltigera* yielded the first direct indication of the presence of the PK/PBAN receptors in the pheromone gland.[1]

Similarly to Nps of other vertebrates and invertebrates, the PK/PBAN Nps activate cellular processes via G-protein-coupled receptors (GPCRs). At the turn of the century, many studies on cloning of insect Np GPCRs, including those of the PK/PBAN family, were stimulated by the *Drosophila* Genome Project,[13] and by completion of genome sequences of other insects. In light of these data, it is now apparent that all the PK/PBAN receptors fall into two groups: receptors for *capa* gene peptides—herein termed PK-1/DH-receptors (PK-1/DH-R); and receptors for the *pban* gene peptides—herein termed PK-2/PBAN-R. Both receptor types are found in a wide variety of insects and are activated by PK/PBAN/DH peptides, although at differing affinities. In general, PK-1/DH-R exhibits high affinity (at low nM doses) toward WFPRLa-containing peptides (DH signature sequence), whereas other PK/PBAN peptides activate the receptors at much higher concentrations; the PK-2/PBAN-R exhibit high (nanomolar) affinity to PBAN and lower affinity toward other FXPRLa peptides.

So far, 13 PK-2/PBAN-Rs from moths, *Drosophila* and mosquitoes and 2 PK-1/DH-Rs (from *B. mori* and from *Drosophila*) were cloned and expressed in cell lines. Another 12 were annotated according to sequence homologies with already-cloned PK-2/PBAN-R or PK-1/DH-R genes (Table 2). The first receptors were cloned in light of the assumption of Hewes and Taghert[13] that PK/PBAN-R might be homologous to the mammalian neuromedin U receptor (NmU-R) because their ligands—NmU and the PK/PBAN Nps—contain similar motifs at their C-termini, that is, FRPRNa and FSPRLa, respectively, and PBAN was found to activate an NmU-R in *Drosophila*. Primers based on the consensus sequences of NmU and on sequences of genes CG8784 and CG8795, CG9918 and CG14575—which, in the *Drosophila* Genome Project were predicted as coding for GPCRs were designed and were successfully used to clone two *D. melanogaster* receptors (PK-2-1/PBAN and PK-2-2/PBAN, originally termed CG8784 and CG8795, respectively), two *Anopheles* receptors (PK-1/DH and PK-2/PBAN), the *D. melanogaster* PK-1 receptor (PK-1/DH-R also termed, CG9918), and the *B. mori* and *H. zea* PBAN-Rs (Table 2). A few additional PBAN receptors from pheromone glands of adult female moths were cloned later, based on the already published PK/PBAN-R sequences (for example, *H. virescens*, *H. peltigera*, *H. armigera*, *Plutella xylostella*, and *Spodoptera exigua*), and from several different larval instars (for example, *S. littoralis*, *H. virescens*). A DH-R was cloned from pupal ovaries of *B. mori* (Table 2). Interestingly, multiple isoforms were identified in *H. virescens* and *M. sexta* and, most recently, an isoform of the *H. zea* PBAN-R, which contains a long C-terminal tail similar to that of *B. mori* was annotated (*Hez*-PBAN-R-iso; Table 2). In all of the studies, other than that on the *B. mori* pupal ovary, expression was monitored by observing Ca^{2+} influx; in the case of the *B. mori* pupal ovary receptor, it was monitored by voltage clamping. The occurrence of PBAN-R in adult male moths has also been reported.[4] Receptor characterization is detailed in Refs. 3, 16–18, 20 and references therein.

A detailed evolutionary trace analysis, based on sequence alignments of the actually cloned and the predicted PK-2/PBAN-Rs and PK-1/DH-Rs, aimed to discover highly conserved and group-specific amino acids that might predict global and group-specific putative ligand recognition and docking sites was conducted. In both groups, the study reveals several highly conserved trans-membrane (TM) amino acid residues that form, most probably, the binding sites that activate the receptors and some group-specific sequences in the extracellular domains, which could indicate specificity toward different *pban* gene and *capa* gene peptide ligands.[16] A preliminary verification of this hypothesis already was obtained from computer-modeled structure analysis of mutated and/or modified chimeric receptors. All of the studies add important information on the receptor's structure/activity relationship (SAR) and reveal the extracellular and intracellular domains responsible for receptor stability, ligand recognition, and binding.[6,7,17]

TABLE 2 Summary of PK-1/DH and PK-2/PBAN Cloned Receptors

Insect	Code	Tissue	GenBank Acc. #	Expression sys.
Actually Cloned and Expressed				
Pheromone gland				
Helicoverpa zea	*Hez*-PBAN-R	Pheromone gland	AY319852	Sf-9
Bombyx mori	*Bom*-PBAN-R	Pheromone gland	AB181298	Sf-9
Heliothis virescens	*Hev*-PBAN-R (C)	Pheromone gland	EU000527	CHO
Heliothis peltigera	*Hep*-PBAN-R	Pheromone gland	JN648826	Sf-9
Plutella xylostella	*Plx*-PBAN-R	Pheromone gland	AY974334	HeLa
Spodoptera exigua	*Spe*-PBAN-R	Pheromone gland	ABY62317	–
Helicoverpa armigera	*Har*-PBAN-R	Pheromone gland	AY792036	–
Larvae				
Drosophila melanogaster	*Drm*-PK-1/DH-R (CG9918)	Whole larvae (3rd instar)	AF368273	CHO
Spodoptera littoralis	*Spl*-PBAN-R	Whole larvae (5th instar)	DQ407742	NIH3T3, HEK293 Sf-9
Heliothis virescens	*Hev*-PBAN-R (A)	Larvae CNS (5th instar)	EU000525	CHO
Heliothis virescens	*Hev*-PBAN-R (B)	Larvae CNS (5th instar)	EU000526	CHO
Pupae				
Bombyx mori	*Bom*-DH-R	Developing ovary (pupa)	AB164386	Xenopus oocytes
Whole insect				
Drosophila melanogaster	*Drm*-PK-2-1/PBAN-R (CG8784)	Whole insect	AY277898	CHO
Drosophila melanogaster	*Drm*-PK-2-2/PBAN-R (CG8795)	Whole insect	AY277899	CHO
Anopheles gambiae	*Ang*-PK-1/DH-R	Adult whole insect	AY900218	CHO
Anopheles gambiae	*Ang*-PK-2/PBAN-R	Adult whole insect	AY900219	CHO
Annotated Based on Predicted Sequence Homology				
Aedes aegypti	*Aea* PK-2/PBAN-R	–	XP_001657210	–
Aedes aegypti	*Aea* PK-1/DH-R		XP_001662936	–
Culex quinquefasciatus	*Cug* PK-2/PBAN-R	–	XP_001861460	–
Culex quinquefasciatus	*Cug* PK-1/DH-R	–	XP_001864163	–

(Continued)

TABLE 2 Summary of PK-1/DH and PK-2/PBAN Cloned Receptors—Cont'd

Insect	Code	Tissue	GenBank Acc. #	Expression sys.
Apis mellifera	*Apm* PK-2/PBAN-R	–	NP_001091688 XP_001122475	–
Apis mellifera	*Apm* PK-1/DH-Ra	–	NP_001157480 XP_623966	–
Tribolium castaneum	*Trb* PK-2/PBAN-R	–	XP_968501	–
Tribolium castaneum	*Trb* PK-1/DH-R	–	XP_968729 XP_968803	–
Acyrthosiphon pisum	*Acp* PK-2/PBAN-R	–	XP_001950091	–
Orgyia thyellina	*Ort*-DH-R	–	AB283041	–
Manduca sexta	*Mas*-PBAN-R-(A)	–	ACQ90219	–
Manduca sexta	*Mas*-PBAN-R-(B)	–	ACQ90220	–
Manduca sexta	*Mas*-PBAN-R-(C)	–	ACQ90221	–
Manduca sexta	*Mas*-PBAN-R-(D)	–	ACQ90222	–
Helicoverpa zea (isoform)	*Hez*-PBAN-R-iso	–	JN206677	–

CHO – Chinese hamster ovary cells; HEK293 – Human embryonic kidney cells.

SIGNALING CASCADE

PK-2/PBAN-R in Lepidopterans

Response to PBAN in Sex Pheromone Biosynthesis

In all moth species tested so far, Ca^{2+} is necessary for the biological response to PBAN and activation of the PBAN-R by the ligand results in an influx of extracellular calcium and a subsequent increase in cytosolic Ca^{2+}. Depletion of Ca^{2+} causes loss of pheromonotropic activity (for review, see Ref. 20).

Although extracellular Ca^{2+} has proved to be absolutely necessary for the bioactivity of PBAN in all moth species tested so far, the intracellular signal transduction cascade stimulated by the Np differs among moth species. In some moths, for example, *Heliothinae* species and *Argyrotaenia velutinana*, the cyclic nucleotide second messenger, cAMP, is a critical component in PBAN signaling, whereas in others, (e.g., *B. mori* and *S. litura*), cAMP do not seem to be involved, hinting at a possible correlation between the steps in the sex pheromone biosynthetic pathway affected by PBAN (early or late) and its ability to activate cAMP. The signaling pathways affected by PBAN in the pheromone gland were extensively studied in *B. mori* and several *Heliothinae* species; detailed reviews on PBAN signal transduction and its downstream cascade of events in biosynthesis of bombykol (the *B. mori* sex pheromone) and other sex pheromones of other moth species can be found in Refs. 17, 20.

Response to PBAN in Cuticular Melanization

PBAN signaling was also examined in a larval receptor of *S. littoralis*, where it induces activation of a matrix-associated protein (MAP) kinase via a signaling mechanism that was protein kinase C (PKC)-dependent but Gαi-independent. As with the pheromone gland receptors, the larval receptor is dependent on Ca^{2+} and responds to PBAN analogs and PT (β-SGNP) by MAP kinase activation.[35]

PK-2/PBAN-R in Non-Lepidopterans

Response to pban Gene–Derived Peptides

Ca^{2+} is also involved in the activation of the PK-2/PBAN-R in *D. melanogaster* and *A. gambiae*, as indicated by Ca^{2+} elevation in CHO cells that express the cloned *Drm*- or *Ang*-PK-2/PBAN-R in response to nanomolar concentrations of *Drm*- or *Ang-pban gene*-derived peptides.[25,31]

PK-1/DH-R in Lepidopterans

Response to DH in Embryonic Diapause

PK-1/DH-R signaling was tested with a *B. mori* pupal ovary receptor, which regulates embryonic diapause. The receptor is also dependent on Ca^{2+} and is coupled to a Gq protein with downstream signaling action involving protein kinase C.[15]

PK-1/DH-R in Non-Lepidopterans

Response to CAPA-PK

Although the biological function of the CAPA-PK peptides is still unknown, similar results were obtained regarding involvement of Ca^{2+} in activation of the PK-1/DH-R by nanomolar concentrations of *Drm*- or *Ang*-CAPA-PK.[5,25]

BIOLOGICAL ACTIONS

The PK/PBAN family of peptides is a ubiquitous, multifunctional family that plays a major physiological role in regulating a wide range of developmental processes in insects: pupariation, diapause, cuticular melanization, feeding, that is, gut muscle contraction and mating behavior, that is, sex pheromone production. For a review, see Ref. 3. The wide distribution of these peptides and their receptors in all insects hints at the possibility that additional functions of the *pban* gene and *capa* gene peptides may be found in the future.

pban Gene-Derived Peptides

PBAN in Sex Pheromone Biosynthesis in Females

The first function that was discovered to be mediated by a member of the PK/PBAN family was stimulation of sex pheromone biosynthesis in female moths.[29] Now, after nearly 30 years of research, it is well established that PBAN regulates sex pheromone production in a circadian manner in many, but not all, moth species. PBAN is released to the hemolymph via the CC and reaches its target organ, the pheromone gland, where it exerts its activity. PBAN regulates either the initial or the terminal steps in the pheromone biosynthetic pathway, as indicated in the signaling cascade section. The cellular mechanisms by which PBAN regulates sex pheromone production were studied in detail and are reviewed in Refs. 17, 20.

PBAN in Sex Pheromone Biosynthesis in Males

A putative role of PBAN in the regulation of free fatty acids in male moths is hypothesized to be involved in chemical communication between the two sexes.[4] The mechanism by which PBAN affects male attractants is still unclear.

PBAN in Melanization

PBAN peptides are involved in cuticular melanization—the last step in the molting processes—in many noctuid moths. The possible involvement of this family of Nps in the control of larval cuticular melanization was first raised by Ogura and coworkers in studies of the common army worm, *Leucania separata*. The hormone—termed melanization and reddish coloration hormone (MRCH)—was later found to be identical with *Bom*-PBAN, which initiates melanization of the integument of many moth larvae. For a detailed review, see Ref. 3 and references therein.

DH in Embryonic and Pupal Diapause

As studies on the bioactivity of the *pban* gene peptides progressed, it became apparent that a *pban gene*-encoded peptide, DH, plays a major role in development processes. Studies since the late 1990s have found that DH induces embryonic diapause in *B. mori* and terminates pupal diapause in *Heliothinae* species. The effect of DH on embryonic and pupal diapause is reviewed in Ref. 32. DH is an important inducer of seasonal reproductive polyphenism in *O. thyellina*, inducing diapause that might be orchestrated via seasonal signaling pathways to accomplish seasonal adaptation.[34] Other functions of *pban* gene-encoded peptides are summarized in Ref. 3.

Bioactivity of Other FXPRLa-Containing Peptides in Lepidopteran and Non-Lepidopteran Insects

PK/PBAN Nps also accelerate pupariation in wandering larvae of the fleshfly (*Sarcophaga bullata*). Lem-PK (LPK) accelerate the switch from wandering behavior to immobilization/retraction behavior, and subsequently affect puparial tanning. By accelerating both aspects of puparium formation, Lem-PK mimick the effects of the pupariation factors by affecting the central motor neurons.[22] Studies by Altstien et al.[2] reveal that LPK and Lom-MT-II can also stimulate sex pheromone biosynthesis in *H. peltigera* adult females and cuticular melanization in *S. littoralis* larvae.

capa Gene-Derived Peptides

In contrast to the *pban gene*-encoded peptides, the physiological effects of the CAPA-PKs have not been investigated in detail and their role(s) in insect physiology is/are currently unknown.

The involvement of *pban*-gene-encoded Nps in the above functions, with the exception of myotropic activity, was determined by means of a variety of *in vivo* bioassays, for example, pheromonotropic, melanotropic, diapause, and pupariation assays. The myotropic activity was performed using an isolated cockroach hindgut. These studies revealed that most of the above functions could be stimulated by more than one member of this peptide family, sometimes at differing doses, and that the peptides are not species specific; this subject is reviewed in Ref. 3. Gene silencing was used to study the role of PBAN in sex pheromone biosynthesis in *P. xylostella*,[18]

PBAN signaling pathways regulating sex pheromone synthesis in *B. mori*,[20] and the role of PBAN in male moths.[4]

THE PK/PBAN PEPTIDES FAMILY AS POTENTIAL TARGETS FOR PEST MANAGEMENT

As is evident from the studies described above, PK/PBAN Nps regulate many physiological and behavioral processes during development and mating and maintain visceral activities. Peptides involved in the regulation of vital functions are prime targets for advancement of the understanding of insect physiology and, therefore, are also targets for the development of novel insect-control strategies based on interference with their activity by means of receptor-selective antagonists.

Despite the vast scientific and insecticidal/insect-control potential of antagonists and, to some extent, of agonists, their application in insects has not been widely implemented so far. This is mainly because of lack of defined methods for obtaining antagonists based on known Np agonists, and because of the inability to predict which conformation will lead to a highly potent inhibitory or stimulatory receptor-selective activity. In addition, peptides are highly susceptible to proteolytic degradation and have poor bioavailability. Therefore, their conversion into an insecticide prototype necessitates rendering them resistant to peptidase degradation, and designing them with high bioavailability. For many years, the approach most commonly used by the agrochemical industry for insecticide discovery was based on random screening of large chemical or natural libraries of nonpeptide compounds and then optimizing a lead molecule with respect to the desired properties. In the past decade, a parallel approach, based on rational drug design—or structure-based design—has evolved which integrates and implements the vast amount of information on the genes encoding the peptides and their receptors, as well as their SARs.

In the past years, we have applied an integrated strategy, termed **I**nsect **N**p **A**ntagonist **I**nsecticide (INAI), based on a rational design approach of small-molecule antagonists based on a known Np agonist for the design of antagonists and, especially, to determine the properties that give them high stability and bioavailability. We have used the PK/PBAN Np family as a model system. A large variety of cyclic and linear peptides have been designed, synthesized, and tested for antagonistic activity on PK/PBAN-mediated functions, and their metabolic stability and bioavailability, that is, ability to penetrate the insect cuticle and to reach and activate the target organ, have been extensively examined.

Highly potent and highly bioavailable antagonists that can penetrate the insects cuticle and effectively inhibit several key functions in moths were discovered; also—most important—a large amount of information has been gained on the agonistic and antagonistic structural requirements of the PK/PBAN peptides. For instance, experiments with restricted-conformation analogs provide compelling evidence that PK/PBAN peptides adopt a *trans*Pro, type I β-turn within the shared C-terminal pentapeptide region to achieve an agonist receptor response.[23] Information has also been gleaned on the requirements needed to obtain high metabolic stability and cuticular and digestive tract penetrability. For example, PK/PBAN analogs featuring enhanced biostability and bioavailability efficiently penetrate the insect cuticle[3,12,23] and gut and have demonstrated pheromonotropic activity when fed to *H. virescens*.[23] In other studies, potent biostable PK/PBAN agonists[23] have been shown to prevent pupal diapause in treated *H. zea* larvae that had been programmed for diapause, and a novel antagonist with a dihydroimidazole *trans*Pro motif[23] inhibited the termination of *H. zea* pupal diapause initiated by the DH. The results suggest potential for the development of novel control strategies that derail the success of overwintering in pest insects.[23] In addition, two PK/PBAN-Rs—one from *S. littoralis* larvae and one from the pheromone gland of *H. peltigera* adult females—have been cloned and stably expressed in a cell line. These receptors are currently being characterized and used in the development of microplate high-throughput binding assays for screening libraries obtained from various sources for occurrence of potential insect-control agents capable of binding to these receptors.

Knowledge on the conformational requirements needed to acquire highly potent, metabolically stable, and bioavailable antagonists, and the availability of stably expressed PK/PBAN cloned receptors, together with the many *in vivo* and *in vitro* assays that we have developed to monitor bioactivity and bioavailability, enable to proceed toward the design and synthesis of insecticide prototypes based on already available antagonists. In this context, the overall know-how, together with the generic nature of the strategy, will facilitate application to other insect Np families, in the development of Np-based insect-control agents. For detailed reviews on these topics, see Refs. 3, 12, 23 and references therein.

SUMMARY AND FUTURE PROSPECTS

Intensive studies of the PK/PBAN peptides have yielded a vast amount of important information on the chemical and molecular nature of the peptides in Lepidopteran and non-Lepidopteran insects: their genes, post-translational processing, distribution, receptors, signaling mechanisms, and bioactivity. Nevertheless, many questions about these peptides are still unresolved, especially regarding their mode(s) of action, and much remains to be learned about the structural, chemical, and cellular basis of their activity and also about downstream cellular events, species specificity, receptor heterogeneity, and the mechanisms that underlie their functional diversity.

The investigation of the transcriptional factors that regulate the *pban* gene and *capa* gene expression, which might be influenced by a circadian clock, is still in its infancy, as is that of the mechanisms of Np biosynthesis. The finding of the differential processing of the CAPA prohormone has provided a good opportunity to study the still poorly understood mechanism of packaging and sorting of Nps in the insect, and of the stimuli that regulate their release. The large number of cloned receptors provides a good basis for further studies of their SARs, and their stable expression in different cell lines invites further studies on the signaling pathways of the *pban*- and *capa* gene-encoded peptides—studies that might provide insights into the full range of cellular mechanisms that underlie the bioactivity of this multifunctional family in Lepidopteran and non-Lepidopteran insects. The vast number of insects—from diverse species and taxa—that all contain FXPRLa peptides and exhibit differential expression of the gene products calls for further studies on the bioactivity of the peptides, especially in non-Lepidopteran species, where knowledge is currently very scarce. Such studies could improve our understanding of their physiological role and shed light on their phylogenetic relationships during the course of evolution. The information that has already been accumulated, together with the currently available and developing bioinformatic, peptidomic, molecular, biological, and chemical analytical tools offer great potential for further exploration of the above issues. Apart from—and beyond—the high scientific value of the above findings, the strategies and approaches that were developed in the course of PK/PBAN research also offer great potential for practical application, by providing a basis for generation of insect Np antagonist-based insect-control agents, as described in this chapter.

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