

Pheromone Biosynthesis Activating Neuropeptide (PBAN) in Insects

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곤충의 페로몬 생합성 활성화 신경펩타이드(PBAN)

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ABSTRACT: Neuropeptides produced in neurosecretory cells are the largest group of insect hormones. They regulate various physiological functions, such as fat body homeostasis, feeding, digestion, excretion, circulation, reproduction, metamorphosis, and behavior throughout all life stages. The PRXamide peptide family (X, a variable amino acid) is a well-characterized neuropeptide component with a common amino acid sequence, PRXamide (NH₂), at the C-terminal end conserved across Insecta. The PRXamide peptides are classified into three subfamilies, each having diverse biological roles in insects: (1) pyrokinin (PK) includes the pheromone biosynthesis activating neuropeptide (PBAN) and the diapause hormone (DH), (2) the capability (CAPA) peptides, and (3) the ecdysis-triggering hormone (ETH). PBAN as a member of PK subfamily was first identified to stimulate pheromone biosynthesis in moths three decades ago. Since then, PBAN peptides have been extensively studied by various research groups from a broad spectrum of arthropods. In this paper, we briefly review insect PBAN molecules with emphasis on gene structure and expression, signal transduction, physiological mechanism in sex pheromone biosynthesis, and application for pest management.

Key words: Neuropeptide, PRXamide, Pyrokinin, PBAN, Sex pheromone biosynthesis, Pest control

초록: 신경펩타이드(Neuropeptide)는 신경세포에서 분비되는 단백질질성 물질로, 곤충 호르몬에서 가장 큰 그룹으로 차지한다. 이들은 곤충의 전 생육단계에 걸쳐 지방체의 항상성, 섭식, 소화, 배설, 순환, 번식, 탈피/변태 등 다양한 생리적 기능과 행동을 조절하는데 관여하고 있다. 신경호르몬 일종인 PRXamide (NH₂) 펩타이드 계열 호르몬은 카르복실기 끝에 PRX (X, 다양한 아미노산)라는 공통의 아미노산 서열이 특징적으로 존재하고 있으며, 곤충 전반에 걸쳐 발견된다. 곤충에서 PRX 신경호르몬은 다양한 생물학적 기능에 관련하고 있는데 호르몬구조와 기능에 따라 크게 3가지로 분류한다. Pyrokinin (PK) 계열의 호르몬은 페로몬 생합성 활성화 신경펩타이드(pheromone biosynthesis activating neuropeptide, PBAN) 및 휴면 호르몬(diapause hormone, DH)이 속하며, 카파(CAPA) 펩타이드 호르몬, 그리고 탈피촉진 호르몬(ecdysis triggering hormone, ETH)이 여기에 속한다. PK 계열의 PBAN 호르몬은 지금으로부터 약 30년전 나방에서 처음 밝혀졌으며, 성페로몬 생합성을 자극하는 신경호르몬으로 확인되었다. 그 이후, PBAN의 연구는 절지동물 전반에 걸쳐 다양한 연구자들에 의하여 광범위하게 이루어졌다. 본 종설은 PBAN의 유전자 구조와 발현, PBAN에 의한 세포신호 전달과 성페로몬 생합성에 관련된 생리적 기작, 그리고 신경호르몬과 PBAN을 이용한 새로운 해충 방제법 개발의 가능성과 예를 소개한다.

검색어: 신경호르몬, PRX펩타이드, Pyrokinin, PBAN, 성페로몬 생합성, 해충방제

PBAN is one of the neurohormones in insects. Insect neurohormones are produced in neurosecretory cells and are typically composed of neuropeptides (NPs) with < 50 amino acids and stable protein molecules. NPs occupy over 90% of total insect

hormones and nearly 50 NP families from over 400 different insect species have been identified (Boo, 2001; Garczynski et al., 2019; Nassel, 2002; Nassel and Homberg, 2006; Nassel and Winther, 2002, 2010; Nassel and Zandawala, 2019; Vanden Broeck, 2001; Veenstra, 2016; Yeoh et al., 2017). NPs regulate or modulate a variety of physiological actions, such as fat body homeostasis, feeding, digestion, excretion, circulation, repro-

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duction, metamorphosis, and behavior throughout all life stages (Altstein and Nassel, 2010; Bendena, 2010; Gade and Hoffmann, 2005; Nassel and Winther, 2010; Nassel and Zandawala, 2019; Schoofs et al., 2017).

The PRXamide family of neuropeptides is conserved across Insecta (Jurenka, 2015), is well-characterized, and has a common amino acid sequence: PRXamide (-NH₂) (X= a variable amino acid) at the C-terminal end. The PRXamide family peptides are classified into three subfamilies, each having diverse biological roles in insects: (1) pyrokinin (PK) includes the pheromone biosynthesis activating neuropeptide (PBAN) and the diapause hormone (DH) (peptides normally produced from the single *pk/pban/dh* gene), (2) the capability (CAPA) peptides (peptides produced from the *capa* gene), and (3) the ecdysis-triggering hormone (ETH) (peptides produced from the *eth* gene) (Ahn and Choi, 2018; Jurenka, 2015). Since the 1980s, the PK subfamily has been extensively studied by various research groups, the first peptide, leucopyrokinin, was isolated from the cockroach, *Leucophaea maderae*, to stimulate muscle contraction and myotropic activity in insects (Holman et al., 1986; Lajevardi and Paluzzi, 2020; Schoofs et al., 1993). Soon after then, many NPs of PK subfamily were found to stimulate pheromone biosynthesis by PBAN (Choi and Vander Meer, 2012a; Kitamura et al., 1989; Raina et al., 1989), and induce embryonic diapause by DH in the silkworm (Imai et al., 1991; Sato et al., 1993), terminate larval and pupal diapause in moths (Suang et al., 2017; Xu and Denlinger, 2003; Zhang et al., 2004), control molting and metamorphosis in the moth (Watanabe et al., 2007), modulate feeding behavior in the fly (Melcher and Pankratz, 2005), induce larval cuticular melanization in moths (Matsumoto et al., 1990; Matsumoto et al., 1992), and to accelerate fly pupariation (Zdarek et al., 1997, 1998). In this paper, we briefly review insect PBAN molecules with emphasis on gene structure and expression, signal transduction, physiological mechanism in sex pheromone biosynthesis, and application for pest management.

Molecular Structure of PBAN

The PK subfamily peptides are composed of two NP groups, PK1 and PK2 peptides, that are differentiated by variations in the C-terminal motif and have their own receptors for different

biological signals (Choi et al., 2017; Hull et al., 2021; Jurenka and Nusawardani, 2011; Jurenka, 2015). PK1 peptides, occasionally referred to as tryptoPKs or DH-like peptides, are characterized by a WFGPRLamide C-terminus. In contrast, PK2 peptides, such as PBANs, lack the Trp (W) residue and have C-terminal ends consisting of FXPRLamide or similar motifs (X = a variable amino acid) (Hull et al., 2021). To date, over 250 PK/PBAN/DH peptides have been identified from over 50 species in various insect groups (Choi et al., 2015; Choi and Vander Meer, 2012b; Garczynski et al., 2019; Jurenka, 2015), but the biological functions of these peptides are largely unknown.

In moths, the PK1 (= DH-like) and PK2 (= PBAN-like) peptides are usually transcribed from the same gene, called various names, *pk*, *pban*, *dh*, *dh/pban*, *pk/pban/dh* genes, depending on research groups. Number of mature peptides translated from these genes is estimated from one to five peptides. Most insect *pk/pban/dh* genes are encoding a single PK1 (WFGPRLamide) or no PK1 and multiple PK2 (FXPRLamide) peptides (Fig. 1). Lepidopteran species have been found to produce five NPs, one PK1 (= DH-like) and four PK2 (= PBAN-like) peptides including PBAN. The C-terminal pentapeptides are significant in stimulating pheromone biosynthesis in moths (Kuniyoshi et al., 1992a; Kuniyoshi et al., 1992b; Ma et al., 1996; Raina and Kempe, 1990) and binding to their receptors (Ahn et al., 2020; Kim et al., 2008). Beyond Lepidoptera, other insect groups (e.g., Hymenoptera, Coleoptera, Hemiptera, and Diptera) are expected to produce variable numbers of their mature peptides (1-4 NPs) (Ahn and Choi, 2018; Choi et al., 2015; Choi and Vander Meer, 2012b; Choi et al., 2011; Jurenka, 2015). Amino acids sequences of each mature peptide encoded from *pban* genes are usually estimated based on mono or dibasic cleavage sites in insect neuropeptides (Southey et al., 2008; Veenstra, 2000), or confirmed by a mass spectrometry analysis (Ma et al., 2000). However, preproPK peptides encoded from the *pk/pban/dh* transcripts are unclear whether their mature peptides are released or not (Choi et al., 2015). For example, the PBAN-like peptide (QPQPVFYHSTTPRLamide) of the mosquito *Aedes aegypti* was expected to be processed as a mature peptide based on the sequence (Bader et al., 2007b), however, the peptide has not been detected from the subesophageal ganglion (SEG) by the mass spectroscopy (Predel et al., 2010). Therefore, this peptide might not be translated and released

in the mosquito, but, further study is necessary to confirm the presence or absence of the mature peptide. Another case is QLQSNGEPAYRVRTPRamide peptide of the fruit fly *Drosophila melanogaster* that had been proposed as a *hugin* prepropeptide (Meng et al., 2002), but this peptide was also never detected from the fly central nervous system (CNS) or

ring gland by multiple peptidomic analyses (Baggerman et al., 2005; Baggerman et al., 2002; Wegener et al., 2006). In insects, *D. melanogaster* has the simplest *pk/pban/dh* gene, producing a single PK2 (= PBAN-like) peptide, followed by the sand fly *Phlebotomus papatasi* with two PK2 (= PBAN-like) peptides (Fig. 1).

Species	PK1 (= DH-like)	PK2 (= PBAN-like)
<i>H. zea</i>	NDVKDGAASGAHSDRLGLWFGPRL	VIFTPKL SLAYDDKSFENVEFTPRL LSDDMPATPADQEMYRQDPEQIDSRRTKYFSPRL TMNFSRPL
<i>H. assulta</i>	NDVKDGAASGAHSDRLGLWFGPRL	VIFTPKL SLAYDDKSFENVEFTPRL LSDDMPATPADQEMYRQDPEQIDSRRTKYFSPRL TMNFSRPL
<i>H. armigera</i>	NDVKDGAASGAHSDRLGLWFGPRL	VIFTPKL SLAYDDKSFENVEFTPRL LSDDMPATPADQEMYRQDPEQIDSRRTKYFSPRL TMNFSRPL
<i>H. virescens</i>	NDDKDGAASGAHSDRLGLWFGPRL	VIFTPKL SLSYDDKSFENVEFTPRL LADDMPATPADQEMYRQDPEQIDSRRTKYFSPRL TMNFSRPL
<i>A. ipsilon</i>	NDVKDGGADRGAHSDRGMWFGPRI	VIFTPKL SLSYEDKMFNDVEFTPRL LADDTPATPADQEMYRPDPEQIDSRRTKYFSPRL TMNFSRPL
<i>S. littoralis</i>	NEIKDGGSDRGAHSDRAGLWFGPRL	VIFTPKL SLAYDDKVFENVEFTPRL LADDMPATPADQELRYRPPDQIDSRRTKYFSPRL TMNFSRPL
<i>S. exigua</i>	NEIKDGGSDRGAHSDRAGLWFGPRL	VIFTPKL SLAYDDKVFENVEFTPRL LSDDMPATPADQELRYRPPDQIDSRRTKYFSPRL TMNFSRPL
<i>C. anastomosis</i>	NTMKDGGADRGAHSDRGLWFGPRL	VVFTPKL SMAYDDKSYENVEFTPRL LADDMPATPSDQEYRQDPEQIDSRSNYFSPRL TMLTSPRL
<i>O. thyellina</i>	NDVKDDGGDRVAHSDRGGQLWFGPRL	VIFTPKL SLSTYEEKLYDNVEFTPRL LSDDMPATPPDQEYRQDPEQIDSRRTKYFSPRL TMIFSPRL
<i>A. pernyi</i>	NDIKDEGDKGAHSDRGLWFGPRL	VIFTPKL SIGDIYQEKRTYENVEFTPRL LSDDMPATPKDQEMYHQDPEQVDRTRTRYFSPRL TITFSPRL
<i>B. mori</i>	TDMKDESDRGAHSEGRALWFGPRL	IIFTPKL SVAKPQTHESLEFIPRL LSEDMPATPADQEMYQDPEEMESRTRYFSPRL TMSFSRPL
<i>B. mandarina</i>	TDMKDESDRGAHSEGRALWFGPRL	IIFTPKL SVAKPENFEFIPRL LSEDMPATPADQEIYQDPEVMESTRTRYFSPRL TMSFSRPL
<i>M. sexta</i>	NDIKDEGDRGAHSDRGLWFGPRL	VIFTPKL SLDDSTQEKRFYENVEFTPRL ISEDMPATPSDQEYPMYHPDPEQIDTRTRYFSPRL THFSRPL
<i>S. c. ricini</i>	NDVKDEGDRGAHSDRGLWFGPRL	VIFTPKL ASNAYQEKRTYENVEFTPRL LTEDMPATPTDQEMFDQDPEQIDTRTRYFSPRL TMIFSPRL
<i>Adoxophyes sp.</i>	NFKEENFDRNIRSGRANVFKPIL	VIFTPKL SMEDPYEEKRSYDVFDPRL QSEAVTSSDEQVYRQDMSVPDGRGLKYFSPRL TVKLTPRL
<i>C. pomonella</i>	NYKDDTMDRTAKNGRARVFTPIR	VIFTPKL SSDDAFEKRGYENDFTPRL QADAVTASDEVYRQDASIDGRSKYFSPRL TVELTPRL
<i>P. xylostella</i>	DDLKDEDIQRDARDRASMWFGPRL	VIFTPKL NADEQQQSVDFTPRL RLKDSGLAPPDEYRIPPELLDARAQYFSPRL GGSMIFSPRL
<i>A. s. cretacea</i>	NDLKEDGEREANSRQGLWFGPRL	VIFTPKL SVDFTPRL QLVDVVPQRQIEEDRLGSRTRFFSPRL TTMNFSRPL
<i>M. vitrata</i>	LDDSKDEVDRGASDRGLWFGPRL	VVFTPKL SIGGVFQDKKYDNVEFTPRL IPDALPVTSPDDDDVYSFKPDSGEVDRRTSYFNPRL KVSFSRPL
<i>D. plexippus</i>	DDNLDRGAHSDRGGVWFGPRL	VIFTPKL ALDQYSERMAGNIDFTPRL LPERTPTSSDEDSIQDAIAANRRPSYFSPRL NLPFSRPL
<i>S. invicta</i>	TSQDIASGMWFGPRL	QPFQTPRL GSGEDLSYGDAYEVEDDDHPLFVPRL LPWIPSPRL
<i>S. richteri</i>	TSQDIASGMWFGPRL	QPFQTPRL GSGEDLSYGDAYEVEDDDHPLFVPRL LPWIPSPRL
<i>S. geminata</i>	TSQDIASGMWFGPRL	QPFQTPRL SGEDLFSYGDAYEVEDDDHPLFVPRL LPWIPSPRL
<i>S. pergandii</i>	TSQDIASGMWFGPRL	QPFQTPRL GSGEDLFSYGDAYEVEDDDHPLFVPRL LPWIPSPRL
<i>S. carolinensis</i>	TTQDIASGMWFGPRL	QPFQTPRL GSGEDLFSYGDAYEVEDDDHPLFVPRL LPWIPSPRL
<i>A. melifera</i>	TSQDITSGMWFGPRL	QITQTPRL ESGEDYFSYGFPKQDEELYTEEQIYLPLFASRL VPWTPSPRL
<i>T. castaneum</i>	NDDSKETYFWFGPRL	HVVNFTPRL SPPFAPRL HSSPFSRPL
<i>B. tabaci</i>	ASFNRADLWFGPRL	SMNYTPRL STPFVPRL NNQIFSPRL
<i>A. glycines</i>	GGTTQASNGGIWFGPRL	SPPYSPPFSRPL QAIMNQPVPRL
<i>A. pisum</i>	GGTTQSSNGGIWFGPRL	SPPYSPPFSRPL QAVMAQPQVPRL
<i>H. halys</i>		QLVSRPRL SPPFAPRL FYAPFSRPL
<i>A. gambiae</i>	AAMWFGPRL	PQPIFYHITSPRL DSVGENHQRPFPAPRL NLPFSRPL
<i>A. aegypti</i>	AAAMWFGPRL	QPQPVFYHSTTPRL DASSNENNSRPPFAPRL NLPFSRPL
<i>P. papatasi</i>		SNKYMTPRL ASGDNDSQRTRPPFAPRL SLPFSRPL
<i>D. melanogaster</i>		QLQSNGEPAYRVRTPRL SVPFKPRL

Fig. 1. Comparison of PK1 (= DH-like) and PK2 (= PBAN-like) neuropeptides encoded from selected *pk/pban* genes in Insecta. Proposed NP sequences shaded in mosquitoes and flies are not expected to be functional peptides. Full amino acid sequence comparisons are from lepidopteran species, *Helicoverpa zea* (U08109), *Helicoverpa assulta* (U96761), *Helicoverpa armigera* (AY043222), *Heliothis virescens* (AY173075), *Agrotis ipsilon* (AJ009674), *Spodoptera littoralis* (AF401480), *Spodoptera exigua* (AY628764), *Clostera anastomosis* (AY786305), *Orgyia thyellina* (AB259122), *Antheraea pernyi* (AY445658), *Bombyx mori* (NM_001043856), *B. mandarina* (Xu et al., 1999), *Manduca sexta* (AY172672), *Samia cynthia ricini* (AY286544), *Adoxophyes sp.* (AF395670), *Cydia pomonella* (QDK59915), *Plutella xylostella* (AY904342), *Ascotis selenaria cretacea* (AB308061), *Maruca vitrata* (AFX71575), *Danaus plexippus* (EHJ67284); hymenopteran species, *Solenopsis invicta* (ACL35348), *Solenopsis richteri* (ADI88481), *Solenopsis geminata* (ADI88478), *Solenopsis pergandii* (ADI88479), *Solenopsis carolinensis* (ADI88480), *Apis melifera* (NP_001104182); coleopteran species, *Tribolium castaneum* (EFA11568); hemipteran species, *Bemisia tabaci* (XP_018895833), *Aphis glycines* (KAE9544772), *Acyrtosiphon pisum* (XP_003245653), *Halyomorpha halys* (XP_014289936); and dipteran species, *Anopheles gambiae* (XM_307885), *Aedes aegypti* (Q16N80), *Phlebotomus papatasi* (AIY99907), *Drosophila melanogaster* (AAF54833). Genebank accession numbers are in parenthesis (updated from Choi et al., 2015).

Expression and Synthesis of PBAN

PBAN is synthesized in SEG (Raina et al., 1989), which is now referred to as the gnathal ganglia (GNG), located near the brain (Ito et al., 2014), and released into the hemolymph, where it travels to the pheromone glands to activate pheromone biosynthesis in moths (Altstein, 2004b; Jurenka, 2017; Rafaeli, 2009). In Noctuidae moths, female adults typically produce and release their sex pheromones during the scotophase only, not photophase (Choi et al., 1998b). However, expressions of *pk/pban/dh* transcripts and mature peptides are found in the neurosecretory cells regardless of the photoperiods. In addition, sex pheromone components can be biosynthesized in the pheromone gland, when PBAN molecules are injected into female moths during the daytime, indicating the PBAN receptor is always active (Choi and Jurenka, 2004; 2006b). These results suggest that female adults will synthesize PBAN molecules all the time but not release them into hemolymph during the daytime; the endocrinal control could be under the circadian regulation for releasing PBAN into the hemolymph from the GNG (Bloch et al., 2013). Also, the *pban* transcripts were clearly expressed in all the developmental stages of the silkworm and corn earworm (Ma et al., 1994; Ma et al., 1998; Xu et al., 1995) and in the male and female moths (Choi et al., 1998a; Ma et al., 1998; Rafaeli et al., 2007). Expressions of *pban/dh* transcripts using mRNA detection methods (Choi et al., 2017; Choi et al., 2013; Choi et al., 2014; Choi et al., 2015; Choi and Vander Meer, 2012a; Choi et al., 2012; Lee and Boo, 2005; Senthilkumar and Srinivasan, 2019; Wei et al., 2004b; Xu and Denlinger, 2003) or PBAN-like peptides in CNS using immunocytochemistry and/or mass spectrometry were confirmed in various insect groups (Ahn and Choi, 2018; Chen et al., 2019; Choi et al., 2004; Choi et al., 2001; Choi and Vander Meer, 2009; Hull et al., 2021; Ma et al., 2000; Ma and Roelofs, 1995b; Sun et al., 2003; Wei et al., 2008). The ubiquitous presence of PBAN-like molecules during different life stages indicates that the role of PBAN-like could be involved in multiple biological functions such as feeding activity (Audsley and Weaver, 2009; Bader et al., 2007a; Bader et al., 2007b; Melcher et al., 2007; Melcher and Pankratz, 2005) and molting process (Watanabe et al., 2007).

Signal Transduction of PBAN

Since the first PBAN in a moth (Raina et al., 1989), PBAN signaling to sex pheromone biosynthesis has been well understood in various moths (Jurenka, 2017). To date, no other insect groups have been reported to regulate pheromone biosynthesis using PBAN except the fire ant trail pheromone (Choi and Vander Meer, 2012a). A general PBAN mode of action model is that PBAN is synthesized in GNG and released into the hemolymph to circulate and reach the target pheromone gland. Early immunocytochemical and physiological evidence suggested PBAN travels to the pheromone gland through humoral, neural, or both routes, which suggests that sex pheromone biosynthesis can be regulated through circulation in the hemolymph (Choi et al., 1998b; Jacquin et al., 1994; Jurenka et al., 1993; Jurenka et al., 1991a; Raina, 1993; Raina and Klun, 1984; Ramaswamy et al., 1995; Ramaswamy et al., 1994; Zhu et al., 1995), or through the ventral nerve cord (Christensen and Hildebrand, 1995; Christensen et al., 1991; Christensen et al., 1994; Iglesias et al., 1998; Marco et al., 1996; Teal et al., 1989; Teal et al., 1999b; Thyagaraja and Raina, 1994). Now, PBAN signaling through the hemolymph as a humoral route is most likely involved in pheromone production in moths.

PBAN acts directly on pheromone glands by stimulating specific receptor linked G proteins to open a ligand-gated calcium channel to allow the influx of extracellular calcium ions (Ca^{2+}), which is the critical second messenger for PBAN signal transduction (Fonagy et al., 1992; Jurenka, 1996; Jurenka et al., 1994; Jurenka et al., 1991b; Ma and Roelofs, 1995a; Matsumoto et al., 1995a; Matsumoto et al., 1995b; Rafaeli and Soroker, 1994). The increased cytosolic calcium ions activate the adenylate cyclase to produce cAMP, which activates various enzymes involved in sex pheromone biosynthesis (Jurenka, 2017; Rafaeli and Gileadi, 1996). In other moths, like *Bombyx mori* and *Ostrinia nubilalis*, Ca^{2+} ions directly activate specific enzymes involved in sex pheromone biosynthesis (Fonagy et al., 1992; Ma and Roelofs, 1995a). Once PBAN binds to the receptor, a signal coupled to G proteins is initiated that induces the opening of a plasma membrane calcium channel (Choi et al., 2003; Choi and Jurenka, 2004, 2006b; Jurenka, 2017).

The signal transduction mechanism for enzyme activation is turned on quickly once PBAN binds with its receptor, PBAN

receptor (PBAN-R), and the pathway stays activated for a period of time. It is possible that some PBAN remains bound to the receptor, and may stay activated and continuously keep the calcium channel open. In moth species, maximum pheromone production occurs about 45 min after the initial exposure to PBAN using *in vivo* incubations (Choi and Jurenka, 2004; Choi et al., 1998b; Gazit et al., 1990; Rafaeli and Gileadi, 1995). Therefore, the signal transduction of PBAN in the pheromone gland is initiated by PBAN binding to PBAN-R, which is the first step for the functional PBAN; it has been a long-standing question until the identification of insect PBAN-R. The first G protein-coupled receptor (GPCR) for PBAN has been identified from the pheromone glands of the corn earworm *Helicoverpa zea* (Choi et al., 2003), followed by the silkworm *B. mori* (Hull et al., 2004), and identified many GPCRs for insect PBANs (Jurenka, 2015). Identifying additional GPCRs for PK peptides (= PBAN and DH-like) is necessary to clarify the signal transduction of unknown physiological roles of these peptides in insect development beyond pheromone regulation because PK peptides could activate their receptors associated with target organs/tissues.

Sex Pheromone Biosynthesis by PBAN

Sex pheromone components in moths are generally linear fatty acid-derived compounds, C10–18 hydrocarbon chains, with one to three double bonds, and alcohol, acetate ester, aldehyde, or epoxide as a functional group (Choi et al., 2016; Jurenka, 2003, 2004; Tillman et al., 1999). Specific enzymes are present in the pheromone glands of female moths and are involved in key steps of the pheromone biosynthetic pathway. These enzymes include fatty acid synthetases, desaturases, limited chain-shortening enzymes, and functional modification of the carbonyl carbon to make species-specific pheromone blends (Choi et al., 2016; Choi et al., 2005; Choi et al., 2002; Choi and Jurenka, 2006a; Choi et al., 2007; Dou et al., 2019; Jurenka, 2003, 2004). Female sex pheromone glands in Heliothine moths are typically located on the intersegmental cuticle between the 8th and 9th abdominal segments just anterior to the ovipositor (Li et al., 2015; Ma and Ramaswamy, 2003; Raina et al., 2000). The biosynthesis of sex pheromones in moths, not all moth species, is regulated by PBAN, which binds to the PBAN receptor on pheromone glands to activate specific enzymes related to specific pheromone biosynthetic pathway (Jurenka, 2017;

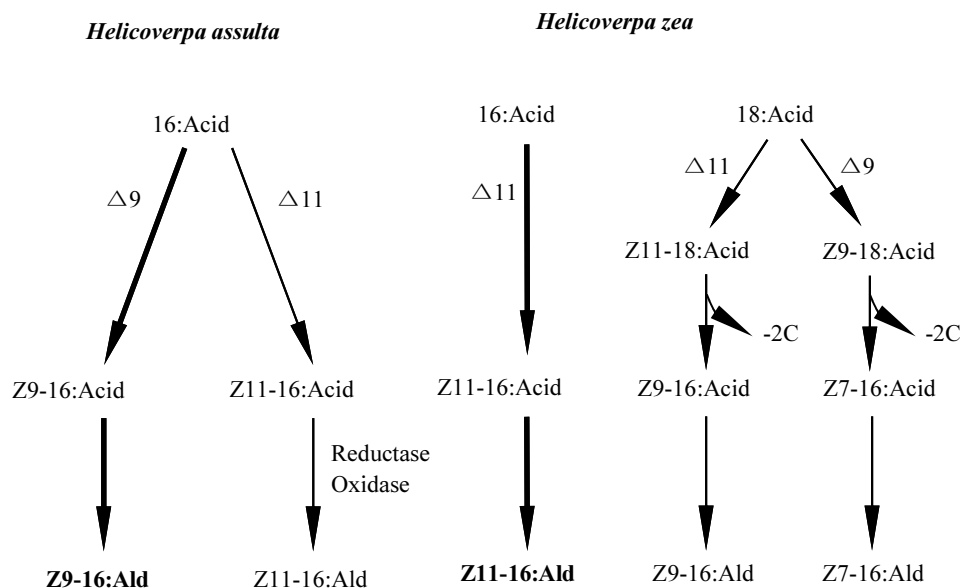


Fig. 2. Biosynthetic pathways of sex pheromone components produced by females of the corn earworm, *Helicoverpa zea* and the oriental tobacco budworm, *Helicoverpa assulta*. The major pathways are indicated with thick arrows. Hexadecanoic acid (16:Acid), Octadecanoic acid (18:Acid), (Z)-7-hexadecanoic acid (Z7-16:Acid), (Z)-9-hexadecanoic acid (Z9-16:Acid), (Z)-11-hexadecanoic acid (Z11-16:Acid), (Z)-9-octadecanoic acid (Z9-18:Acid), (Z)-11-octadecanoic acid (Z11-18:Acid), (Z)-7-hexadecenal (Z7-16:Ald), (Z)-9-hexadecenal (Z9-16:Ald), (Z)-11-hexadecenal (Z11-16:Ald), $\Delta 11 = \Delta 11$ desaturase, $\Delta 9 = \Delta 9$ desaturase (modified from Choi et al., 2002).

Jurenka and Rafaei, 2011). Examples of biosynthetic pathways in Heliothine moths are shown in Fig. 2: 1) biosynthesize saturated fatty acids as sex pheromone precursors; 2) introduce double bonds by fatty acyl-CoA desaturases into the fatty acyl chains; 3) occur one or two rounds of chain shortening steps by limited β -oxidation enzymes; 4) modify functional groups by oxidases, reductases, or acetyltransferases (Blomquist and Vogt, 2020; Choi et al., 2002; Jurenka, 2004).

For the biosynthesis of the hydrocarbon moth pheromones, the PBAN action is different in that acts in the final biosynthetic step. In the gypsy moth *Lymantria dispar*, as a case study, PBAN is involved in the final step which is the enzyme epoxidation process in the pheromone gland (Jurenka, 2003). Most pheromone biosynthetic steps occur in the oenocyte cells associated with the abdominal epidermis. After the hydrocarbon pheromone precursor is biosynthesized in oenocyte cells, it is then transported to the pheromone gland through hemolymph by lipophorin. The alkene precursor is taken up by pheromone gland cells, where it is acted on by an epoxidase to produce the sex pheromone, disparlure; PBAN activates the epoxidation enzyme in the gypsy moth pheromone gland (Jurenka et al., 2003; Miyamoto et al., 1999; Wei et al., 2003; Wei et al., 2004a).

Applications for Pest Management

Over two decades, the PRXamide peptides/receptors have been considered promising targets for pest control, because they are involved in various key biological processes described above (Altstein, 2001, 2004b; Altstein et al., 2000; Altstein and Nassel, 2010; Audsley and Down, 2015; Caers et al., 2012; Gade and Goldsworthy, 2003; Liu et al., 2021; Masler et al., 1993; Nachman et al., 1993; Pietrantonio et al., 2018; Scherckenbeck and Zdobinsky, 2009; Van Hiel et al., 2010). Disruption of an essential function(s) of PK/PBAN/DH peptides leads to novel pest management. In early years, although there were significant efforts to utilize insect NPs/GPCRs as insecticides, little progress was made. New genomic and proteomic technology and tools have advanced and facilitated the development of new approaches to insecticide discovery (Du et al., 2017; Fleites et al., 2020; Grimmelikhuijzen et al., 2007). Currently, three biological tools using the PRXamide peptides and their GPCRs are under development. First, the most popular and conventional approach

by peptide biochemists uses NP analogs and mimics to identify biological functions in insects (Nachman et al., 1986). Recently, many peptide mimics and analogs as agonists (PBAN-R activators) or antagonists (PBAN-R inhibitors) have been isolated or identified from a broad spectrum of arthropod pests (Abernathy et al., 1996; Alford et al., 2019; Altstein, 2004a; Altstein et al., 1999; Altstein et al., 2007; Ben-Aziz et al., 2005; Ben-Aziz et al., 2006; Gui et al., 2020; Hariton et al., 2010; Hariton et al., 2009a; Hariton et al., 2009b; Marciniak et al., 2011; Nachman et al., 2009a; Nachman et al., 2009b; Nachman et al., 2009c; Nachman et al., 1996; Nachman et al., 2001; Nachman et al., 2009d; Plech et al., 2004; Raina et al., 1994; Shi et al., 2021; Teal et al., 1999a; Teal and Nachman, 1997; Xiong et al., 2021; Zeltser et al., 2001; Zeltser et al., 2000; Zhang et al., 2009; Zhang et al., 2011; Zubrzak et al., 2007). Various structural modifications of PK/PBAN/DH peptides, such as backbone cyclization, enable to increase hydrophobicity and stability from the natural peptide ligands and delivery into insects.

Second, genes of the PBAN-like peptides and their GPCRs from insect pests were selected as RNA interference (RNAi) target. A handful of reports for the RNAi application have been demonstrated from a few insect pests (Choi and Vander Meer, 2012a, 2019; Choi et al., 2012; Lee et al., 2011; Lu et al., 2015; Ohnishi et al., 2006). For example, PBAN RNAi decreased sex pheromone production, increased larval mortality or delayed pupal development in the moths and ant by injection of the PBAN dsRNA (double-stranded RNA) (Choi et al., 2012; Lee et al., 2011; Lu et al., 2015).

Lastly and most recently, PK/PBAN/DH GPCR-based agonists and antagonists have been isolated to develop pest control methods that is the newest approach (Ahn et al., 2020; Choi and Vander Meer, 2021; Jiang et al., 2015; Jiang et al., 2014). This development of new methods is available with advanced genomic/proteomic tools. The fundamental mechanism of these strategies is to screen active peptides or analogs that strongly bind to the target receptor, thus blocking the specific physiological function triggered by the natural NP and GPCR binding. Recently, GPCR-based screening technology for the isolation and identification of bioactive peptides using an insect cell expression system has been demonstrated for insecticide discovery (Choi and Vander Meer, 2021). This mode of action is called as Receptor interference (RECEPTORi). This new

technology can rapidly screen bioactive peptides through millions of short peptides for blocking a specific insect GPCR system. These short peptides are not like natural ligands, but they can function as agonists or antagonists to the target GPCR.

Conclusions

PK/PBAN/DH family peptides and their GPCRs are found across Insecta and other invertebrates and are involved in various important biological processes during all developmental stages and in adults. With research continuing through the last three decades, these neuropeptides have been well characterized, and this research has been an integral part in understanding how their signals function and modulate a broad spectrum of physiological activities including pheromone biosynthesis and diapause in insects. The manipulation of insect neuropeptides to control pest population has great potential to be developed as target-specific, and biologically based tool in next-generation pest management.

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Statements for Authorship Position & Contribution

Choi, M.-Y.: Research Entomologist in USDA-ARS, analyzed data and wrote the manuscript.

The author read and approved the manuscript.

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