Identification and expression of PBAN/diapause hormone and GPCRs from *Aedes aegypti*

Man-Yeon Choi a,⇑, Alden Estep a,⇑, Neil Sanscraint a, James Becnel a, Robert K. Vander Meer a

⇑United States Department of Agriculture-Agriculture Research Service, Center of Medical, Agricultural and Veterinary Entomology (CMAVE), 1600 SW 23rd Drive, Gainesville, FL 32608, USA

⇑Navy Entomology Center of Excellence, Box 43, Naval Air Station, Jacksonville, FL 32212-0043, USA

**ABSTRACT**

Neuropeptides control various physiological functions and constitute more than 90% of insect hormones. The pheromone biosynthesis activating neuropeptide (PBAN)/pyrokinin family is a major group of insect neuropeptides and is well conserved in Insecta. This family of peptides has at least two closely related G-protein-coupled receptors (GPCRs) activated by PBAN and a diapause hormone (DH). They have been shown to control several biological activities including pheromone production and diapause induction in moths. However, beyond some moth species, the biological function(s) of PBAN/pyrokinin peptides are largely unknown although these peptides are found in all insects. In this study, we identified and characterized PBAN/pyrokinin peptides and corresponding GPCRs from the mosquito, *Aedes aegypti*. *Ae. aegypti* PBAN mRNA encodes four putative peptides including PBAN and DH, and is expressed in females and males during all life stages. The PBAN receptor (PBAN-R) and the DH receptor (DH-R) were functionally expressed and confirmed through binding assays with PBAN and DH peptides. These receptors are differentially expressed from eggs to adults with the relative gene expression of the PBAN-R significantly lower during the 4th instar larval (L4) and pupal (P1–P2) stages compared to the 2nd and 3rd instar larval stages (L2 and L3). However, DH-R expression level is consistently 4–10 times higher than the PBAN-R in the same period, suggesting that PBAN-R is downregulated in the late larval and pupal stages, whereas DH-R stays upregulated throughout all developmental stages. PBAN/pyrokinin mRNA expression remains high in all stages since it produces PBAN and DH peptides. This study provides the foundation for determining the function(s) of the PBAN/pyrokinin peptides in mosquitoes and establishes data critical to the development of methods for disruption of these hormone actions as a novel strategy for mosquito control.

© 2013 Published by Elsevier Ireland Ltd.

**1. Introduction**

Insect peptide hormones are involved in many important functions such as regulation of fat body homeostasis, feeding, digestion, excretion, circulation, reproduction, metamorphosis, and behavior (Gade and Goldsworthy, 2003; Clark et al., 2004). Neuropeptides, primarily produced in the nervous system, are the largest group of insect hormones and many families of neuropeptides have been identified from insects. A major family of neuropeptides is the pheromone biosynthesis activating neuropeptide (PBAN)/pyrokinin group, found in all insect orders to date. The first pyrokinin peptide was identified from the cockroach, *Leucophaea maderae*, stimulating the contraction of hindgut muscles (Holman et al., 1986). It was named leucopyrokinin due to a pyroglutamate residue at the N-terminal end. Independently, the first PBAN peptide was isolated from *Helicoverpa zea* (Raina et al., 1989), and since then many PBAN-like peptides have been identified from different insect groups based on their ability to stimulate sex pheromone biosynthesis in moths and/or through peptide sequence homology (Rafaeli, 2009; Choi et al., 2010; Jurenka and Rafaeli, 2011).

The PBAN/pyrokinin peptide family is defined by FXPRL-NH₂ or a similar amino acid (AA) sequence in the C-terminal end, as this is the minimal sequence required for physiological activity (Nachman et al., 1986; Raina and Kempe, 1990; Fonagy et al., 1992; Kuniyoshi et al., 1992; Raina and Kempe, 1992). This motif has been identified in a variety of insect orders as well as some crustaceans, and has been shown to regulate a variety of insect functions: (1) stimulate hindgut muscle contraction in cockroaches (Holman et al., 1986); (2) stimulate sex pheromone biosynthesis in female moths (Raina et al., 1989); (3) induce melanization in moth larvae (Matsumoto et al., 1990); (4) induce embryonic diapause and seasonal polyphenism in moths (Suwan et al., 1994; Uehara et al., 2011); (5) accelerate puparium formation in the flesh fly...
Insect PBAN/pyrokinin family peptides are known to be trans-
ferred from two independent genes, PBAN/pyrokinin and capability
(=capa) genes. PBAN/pyrokinin (or DH-PBAN) genes encode four or
five FXPRL or similar sequence peptides, and capa genes, usually
code one DH-like peptide plus several capa family peptides
(Jurenka and Nusawardani, 2011). Thus far, about 200 PBAN/
pyrokinin family peptides have been reported from over 40 species
(GenBank, unpublished data), however, the physiological function
of most of these peptides is unclear. The physiological mechanism
of PBAN to stimulate pheromone biosynthesis in pheromone
molds of moths is well understood. It acts directly on these glands
by stimulating specific G-protein-coupled receptor (GPCR) to open
a ligand-gated calcium channel to allow the influx of extracellular
Ca\(^{2+}\), which is the critical second messenger for PBAN signal trans-
duction in moths (Jurenka et al., 1991; Choi and Jurenka, 2006).
PBAN receptors (or called pyrokinin2 receptors = PK2-Rs) have
been identified in about a dozen lepidopteran moth and other spe-
cies (reviewed by Jurenka and Nusawardani, 2011) since the first
PBAN receptor was identified from Helicoverpa zea (Choi et al.,
2003). DH receptors (or called pyrokinin1 receptors = PK1-Rs) have
been identified from only a few species: Bombyx mori (Honnma
et al., 2006); Anopheles gambiae (Olsen et al., 2007), and Rhodnius
prolixus (Paluzzi and O’Donnell, 2012).

Aedes aegypti is the most important vector of arboviruses that
cause dengue hemorrhagic fever, yellow fever, and chikungunya
primarily because of its preference for obtaining blood meals from
humans. As reported by the World Health Organization (WHO), the
global incidence of dengue has grown dramatically in recent dec-
ares. Over 40% of the world’s population is at risk for dengue
and there may be 50–100 million dengue infections worldwide
eyear. In Brazil alone there were more than 700,000 con-
firmed cases of dengue per year from 2002–2008 (Figueiredo
et al., 2010). There is currently no vaccine for dengue and the only
method to prevent and control dengue transmission is to combat
the mosquitoes of which Ae. aegypti is the primary vector. Control
for Ae. aegypti has relied primarily on conventional insecticides, but
development of resistance to commonly used insecticides has re-
sulted in a significant loss of efficacy. We are faced with the need
to develop new strategies of mosquito control using novel modes
of action.

We identified, characterized, and determined Ae. aegypti PBAN
and DH, their corresponding GPCRs, and investigated gene tran-
scriptions from egg to adult stages. The results from this study pro-
vide basic knowledge to assist in determining the physiological
function(s) of PBAN/pyrokinin peptides which might be important
for development of novel mosquito control methods.

2. Materials and methods

2.1. Mosquitoes

The Orlando strain of Ae. aegypti has been in continuous colony
in the Mosquito and Fly Research Unit insectary at the Center for
Medical, Agricultural, and Veterinary Entomology (CMAVE),
USDA-ARS (Gainesville, FL, USA), since initially colonized in 1952
from collected wildtype specimens. Standardized rearing and
hatching methods were utilized and have been described previ-
ously (Pridgeon et al., 2008). Adult female samples were collected
daily for examination of age related expression, as well as at vari-
ous time points after blood-feeding. Immature mosquito samples
were collected from eggs (2–3 mg), 1st instar (80 individuals),
2nd instar (40 individuals), 3rd instar (20 individuals), 4th instar
(10 individuals), and pupae (10 individuals). Samples were imme-
diately processed to isolate RNA or frozen at ~80 °C.

2.2. RNA isolation and cDNA synthesis

Total RNA was isolated using the RNeasy®-4PCR Kit (Invitro-
gen/Life Technologies, Carlsbad, CA) from either fresh or frozen
samples from the different developmental stages described above.
Pooled samples were homogenized in cell lysis buffer using a
microcentrifuge pestle and tube and tissue was ground until no
large chunks were visible. The isolation continued following the
manufacturer’s protocol and included a DNase digestion step to re-
move contaminating DNA. The isolated total RNA samples were
analyzed on a Nanodrop 2000 (Thermo Scientific, Pittsburgh, PA)
to determine RNA concentration and immediately used for cDNA
synthesis. One hundred fifty ng of total RNA from each sample
was converted into first strand cDNA using the Cloned AMV
First-Strand CDNA Synthesis Kit as well as 150 ng of total RNA in
a ~RT step (Invitrogen/Life Technologies, Carlsbad, CA) and was
then frozen at ~20 °C.

2.3. Cloning genes for PBAN/pyrokinin, PBAN-R and DH-R

The synthesized cDNA from 1 to 2 day old adults was used to
amplify the PBAN/pyrokinin, PBAN-R and DH-R genes. The full
sequence of Ae. aegypti PBAN mRNA was amplified with a specific
primer set: sense primer, 5’-ATGGCCATGTTACCCCTTTAATGTA-3’
and antisense primer, 5’-CTACTATAAGAATAATGTTCAGCGG-GC-3’
based on the nucleotide sequence of VectorBase data (Vector-
base Gene transcription no. Aaeli012600-RA). PCR was performed
with the following temperature program: 5 cycles at 95 °C for
30 s, 63 °C for 30 s, and 72 °C for 1 min and 35 cycles at 95 °C
for 30 s, 60 °C for 30 s, and 72 °C for 1 min. The PCR product was gel
purified and cloned using the pGEM®-T-Easy Vector System per
manufacturer’s instructions (Promega Corporation, Madison, WI),
and sequenced at Macrogen USA (Rockville, MD). The obtained
full-length sequence information was aligned and sequences com-
pared with other known PBAN/pyrokinin sequences using Genetyx
DNA software (ver. 10, Genetyx Co. Tokyo).

To clone the PBAN receptor we amplified the sequence (1218
nucleotides) (GenBank accession No. XM_0016571610 with primer
MFLRYFFFNVICIFAILAIRSAIGEGVDPATEQKIN
NFPLASQMDSEDDS KR AAAMWGGKLR GRR
DH
TIAWHELIDEMEIDDNPLYESGESPQVARVEIA
GQFYYVULLTGRLL OPOPFYHSTAPEL
NP-β
GRR DASSSENNSRRPFAPRL GR NLPFESRPL
PBAN
GR SGAPVVDNFXA

Please cite this article in press as: Choi, M.-Y., et al. Identification and expression of PBAN/diapse hormone and GPCRs from Aedes aegypti. Molecular and
Cellular Endocrinology (2013), http://dx.doi.org/10.1016/j.mce.2013.05.019
set: sense primer, 5'-ATGTCAGTACAAACCTAACCAATGC-3' and anti-sense primer, 5'-TTA ACT TAC CCT GGA AGC GTC CCG A-3' for 35 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min. A search of *Ae. aegypti* genomic data from VectorBase (www.vectorbase.org) yielded a ~100 K-base pair (bp) long expressed sequencing tag (EST) containing six exons (1917 nucleotides) and five intron gaps (see Fig. 2). The entire exon domain nucleotides (deposited GenBank accession No. KC155994) were amplified with the primer set: sense primer, 5'-ATGATGGAGCTGCAGCAGGTGTCA-3' and anti-sense primer, 5'-TCAGCGAATCTCATTGTTGATTTCGGCC-3' under similar PCR conditions as above. The PCR product was cloned, sequenced and analyzed by the same method and procedure described above.

### 2.4. Functional expression of receptors and binding assay for peptides

The open reading frame (ORF) of *Ae. aegypti* PBAN-R cDNA was amplified using the sense primer 5'-AGAATGATGGAGCTGCAGCAGGTGTCA-3' and the anti-sense primer, 5'-TCAGCGAATCTCATTGTTGATTTCGGCC-3'. The ORF of the short receptor (GenBank Accession No, XM_001657160) was amplified using the sense primer, 5'-AGAATGATGGAGCTGCAGCAGGTGTCA-3' and the anti-sense primer, 5'-TTA ACT TAC CCT GGA AGC GTC CCG A-3'. Each PCR product was inserted into the same vector for functional expression as described above.
All peptides used in this study were synthesized or purchased from AnaSpec, Inc. (Fremont, CA). The preparation of cells, peptides, and Fluoro-4AM with lepidopteran saline followed a previously described method (Choi et al., 2003). Cell fluorescence intensity in a 96-well cell-culture plate was measured using a plate reader (BMG's NovoStar, Cary, NC) equipped with filters (excitation: 485 nm and emission: 520 nm), and one pipetter and two injectors. Fluorescence measurements from each well were taken every 10 s. After 30 s the peptide ligand (10 μL) was added by pipetter and changes of fluorescent intensity were measured for up to 3 min. Then, 5 μL of 1 μM ionomycin (Invitrogen) was added to the cells to obtain a maximum fluorescence reading. The effect of ligand-exposure was expressed relative to the maximum value obtained with ionomycin. Data were analyzed using Microsoft Excel as described previously (Choi et al., 2003) and measured EC50 values of ligands were determined using GraphPad Prism 4.03 (GraphPad Software, Inc., San Diego, CA, USA).

2.5. Quantitative (q) PCR procedure

All qPCR was carried out on an ABI7300 Real-Time PCR System (Applied Biosystems/Life Technologies, Carlsbad, CA) using Platinum SYBR Green qPCR SuperMix-UDG with ROX (Invitrogen). The following primer sets were used for amplification of the target genes: PBAN/pyrokinin, 5'-TTTCTGCGATATTAGGAGT-3' and 5'-GGCTCTGTACGATCA-3' (Pridgeon et al., 2009). Initial analysis (Pfaffl, 2001) to determine primer efficiencies. A minimum construct a standard curve for primer sets according to Pfaffl's method (Pfaffl, 2001). The full length of PBAN/pyrokinin mRNA (Fig. 1). Of the four peptides all except NP-PFFAPRLamide), are considered putative DH and PBAN homologs, and have a C-terminal amide group provided by glycine (G) (Veenstra, 2010). The amino acid sequence of AedaePBAN-R (638-AA) has a long nucleotide sequence containing six exons and five intron gaps. However, the AedaePBAN-R gene is consistent with a R2 fit of at least 0.98. The efficiency calculation of the three repetitions was averaged to give a final efficiency. The L24 reference gene has been demonstrated to be less variable than other standard reference genes for Ae. aegypti (Pridgeon et al., 2009). Initial analysis showed L24 expression to be relatively stable throughout the immature and adult sample time periods measured.

2.6. Data analysis

Using the qPCR methods described earlier, expression profiles were created for PBAN/PYROKININ, PBAN-R and DH-R in Ae. aegypti from all life stages. Relative expression of the gene of interest (GOI) to the reporter gene L24 was calculated by the equation \( \left( \frac{E_{\text{GOI}}}{E_{\text{L24}}} \right)^{\left( \frac{1}{Ct} \right)} \) where efficiency (E) was determined as described earlier and fractional cycle threshold (Ct) was determined by Sequence Detection Software v1.3.1 (Applied Biosystems/Life Technologies) using automatic baseline determination. The results were analyzed by non-parametric analysis as ranks (Fisher’s LSD, ANOVA) using GraphPad Prism 6.01 (GraphPad Software).

To investigate gene expression, total RNA from each life stage was synthesized and cDNA was described above. The cDNA (15 ng/2 μL) from each stage was used to amplify for full sequence with PBAN-R primer set (5'-AGA ATG TAG GAG CAT CAG AG TG TCA-3' and 5'-TCACGCAACT TCATTTGTGGTCGCCC-3') and DH-R primer set (5'-AGA ATG TTC AGT ACA AAC ATG GCA-3' and 5'-CTAATGACTCATTGCTGTCAAGCAGA-3'). PCR was performed as follows: 95 °C for 3 min, and 40 cycles at 95 °C for 30 s, 53 °C for 30 s, and 72 °C for 2 min. Then, 72 °C for 5 min with Taq polymerase (Bio-Rad, Hercules, CA, USA). A 796-bp fragment of the mosquito SNF7/MVB4b (AEL001698) gene was also amplified as a positive control using primers (5'-CGACGACGACTGCA CTGTTGA-3' and 5'-CCACCTTTAGCTGACCAAGCAGA-3'). Each, PCR product was checked for amplification using 1.5% agarose gel electrophoresis and visualized using GelRed™ (Biotium, Hayward, CA, USA) under a UV light. The expected PCR products were purified and cloned, then confirmed by DNA sequence (Interdisciplinary Center for Biotechnology Research, ICB, University of Florida).

3. Results

3.1. PBAN/pyrokinin mRNA and pre-propeptides

The full length of Aedes aegypti PBAN/pyrokinin (AedaePBAN/pyrokinin) mRNA encodes 171-AA and produces four putative peptides based on six possible endopeptidase cleavage sites (underlined with italics in Fig. 1). The cleaved peptides are predicted to have a C-terminal amide group provided by glycine (G) (Veenstra, 2010; Southey et al., 2008). The first domain, 10-AA (AAMWF GPRLamide) and the third domain, 18-AA (DASSSNENNSR PFPAPRlamide), are considered putative DH and PBAN homologs, respectively. There are two additional putative peptides proposed here, neuropeptide (NP)-β and NP-γ, deduced from the PBAN/pyrokinin mRNA (Fig. 1). Of the four peptides all except NP-β were previously identified by mass spectrometry from Ae. aegypti (Pree del et al., 2010). Major expression of the PBAN/pyrokinin mRNA was observed in male and female adult heads, probably from sub-esophageal ganglia (SG), since it was not detected from adults with their heads removed (Fig. 1).

3.2. Identification of PBAN and DH receptors

Putative receptors for PBAN and DH were amplified using a PCR-based cloning strategy. The amino acid sequence (638-AA) (Accession No. KC155994 deposited in GenBank) was then identified and confirmed as the Aedes aegypti PBAN receptor (AedaePBAN-R) through a combination of functional expression and peptide binding assay (Table 1). The part (N-terminal side) of amino acid sequence from the putative AedaePBAN-R (638-AA) is identical to a published sequence as Ae. aegypti PBAN/pyrokinin receptor (405-AA) registered in GenBank (XP_001657210) except for 3 AAs (Fig. 2). This receptor was expressed in SF9 cells, but almost no signal was detected when challenged with PBAN/pyrokinin peptides including AedaePBAN (data not shown). In this study, however, the AedaePBAN-R gene is consistent with a 110kb long nucleotide sequence containing six exons and five intron gaps. The amino acid sequence of AedaePBAN-R (638-AA) has a 21 Kbp
intron gap between the 5th and 6th exon, and the 6th exon contains an additional 236-AA region at the C-terminal end when compared to the previous receptor (405-AA) (Fig. 2).

The full sequence (374-AA) for *Aed*es aegypti DH receptor (*AedaeDH-R*) was identified and confirmed through functional expression and peptide binding assay (Fig. 2). The *AedaeDH-R* gene is composed of two exons between one intron gap, and a relatively simple structure compared to *AedaePBAN-R* (Fig. 2). The protein sequence homology of PBAN-R vs. DH-R is relatively low (31–47%), but the sequences of the 7 trans-membrane (TM) domains are conserved (Fig. 2).

### 3.3. Receptor binding affinity with peptides

The receptors were functionally expressed and maintained in Sf9 cells as described previously (Choi et al., 2003). We utilized a

intravenous injection of 100 μL of the PBS solution containing 500 ng of each receptor. The mosquitoes were then kept at 25°C in a 12:12 h light/dark cycle until they reached the stage 2. The adults at stage 2 were used for the experiment.

Fig. 3. Relative expression of *Ae. aegypti* PBAN/pyrokinin mRNA during the indicated developmental stages (top) and different ages of adults (bottom). Bars represent the means SEM of at least 3 replications. Bars with the same letters are not statistically different by analysis of Fisher's LSD (ANOVA) ($P < 0.05$). E = embryonic egg; L1 = first instar; L2 = second instar; L3 = third instar; L4 = fourth instar; P1 = early pupa; P2 = late pupa.

Fig. 4. Relative expression of *Ae. aegypti* PBAN-R mRNA during developmental stages (top) and different ages of adults (bottom). Bars represent the means SEM of at least 3 replications. Bars with the same letters are not statistically different by analysis of Fisher's LSD (ANOVA) ($P < 0.05$). E = embryonic egg; L1 = first instar; L2 = second instar; L3 = third instar; L4 = fourth instar; P1 = early pupa; P2 = late pupa.

Fig. 5. Relative expression of *Ae. aegypti* DH-R mRNA during developmental stages (top) and different ages of adults (bottom). E = embryonic egg; L1 = first instar; L2 = second instar; L3 = third instar; L4 = fourth instar; P1 = early pupa; P2 = late pupa. 

Fig. 6. Expression profiles of *Ae. aegypti* PBAN-R and DH-R during developmental stages (top) and adults at the indicated ages. Mosquito SNF7 gene was used for the positive control. E = embryonic egg; L1 = first instar; L2 = second instar; L3 = third instar; L4 = fourth instar; P1 = early pupa; P2 = late pupa.
calcium assay method to determine peptide affinity using half-maximal effective concentration (EC50) values for expressed receptors using a fluorescence plate reader to detect changes in fluorescence intensity. As shown in Table 1 and Fig. S1, the Aedes aegypti PBAN-R was activated by PBAN or PK2 peptides and not by AedaeDH. The PBAN-R had the lowest EC50 value (58 nM) with AedaePBAN. AedaeDH was also detected in all stages and is expressed more in early larval stages. The similar result was also observed from qPCR analysis (Figs. 4 and 6). DH-R expression showed a relatively high level compared to PBAN-R in all stages (Fig. 6) which was similar to results from the qPCR analysis (Figs. 5 and 6) although the shorter DH-R PCR product is usually expected to be amplified more than the longer PBAN-R product under the same condition.

4. Discussion

In some moth species, it is well understood that PBANs are synthesized in the subesophageal ganglion (SG), released into the hemolymph, and act on sex pheromone production in the pheromone gland (Raina et al., 1989). PBAN, DH and two or three additional neuropeptides (NPs) are translated from the PBAN/pyrokinin mRNAs in all insects investigated thus far (Choi et al., 2011). These peptides all share a common C-terminal FXPRPLamide or similar motif. The mosquito PBAN/pyrokinin mRNA produces four NPs rather than five NPs identified from moths, which is similar to hymenopteran and coleopteran insects (Choi et al., 2011). Interestingly, the corresponding Drosophila PK2 (+hugin) mRNA produces only two peptides (Choi et al., 2001).

In this study PBAN mRNA expression was not detected in the body without the head. However, a strong PBAN-like immunoreactivity by PBAN-antiserum was observed in the ventral nerve cord (VNC) of Aedes aegypti (Hellmich, 2010). Similar contradictory results from PBAN gene transcription and PBAN-like immunocytochemistry have been observed in the fire ant (Choi et al., 2009, 2011). Thus far, two genes, PBAN and copa mRNAs, are known to translate the FXPRPLamide (≈PBAN/pyrokinin family) peptides. Therefore, a PBAN-like peptide(s) existing in the VNC could be from the copa rather than PBAN mRNA. The copa gene produces a WFGPRL peptide at the C-termini which would be cross-reactive with PBAN-antiserum. PBAN mRNA is expressed in both female and male mosquitoes, which is similar to results for moths even though the physiological function is unclear (Choi et al., 1998). Recently, PBAN was determined to regulate production of free fatty acids in the male moth (Bober and Rafaeli, 2010). In the mosquito, qPCR profiles showed PBAN mRNA is expressed in all life stages although the expression levels vary among stages. These results are similar to the findings that PBAN mRNA was expressed in moth larvae and pupae (Jing et al., 2007).

We expected the registered Aedes aegypti pyrokinin receptor (405-AA; Genbank accession No. XM_001657160) to be the PBAN receptor; however, when this potential receptor was expressed in insect cells it responded poorly to PBAN/pyrokinin peptides. A new PBAN receptor search of the Aedes aegypti genomic data yielded a ~100 Kbp long EST containing six exons for PBAN-R (Fig. 2) and determined Aedes aegypti PBAN receptor (AedaePBAN-R: Accession No. KC155994). PBAN-R was found in all life stages, actively expressed from qPCR/RT-PCR analysis and confirmed the full sequence. The short receptor could be because the protein sequence was annotated incorrectly with splicing, or it is a PBAN-R variant. As expected, the mosquito PBAN-R was highly activated by Drosophila PK2 followed by PBANs from the fire ant, the cockroach and the moth.

Like the PBAN gene, two receptors are also clearly expressed in all developmental stages of Aedes aegypti, indicating that specific ligands, PBAN or DH, should activate the corresponding receptor. In moth species, PBAN-R expression has been detected during developmental and adult stages (Zheng et al., 2007; Nusawardani et al., in press), but not for all species (Lee et al., 2011). Variation could be a result of different detection methods or species-specificity. PBAN-R expression patterns are similar to PBAN in the adult stage, but it is very different in the late larval and pupal periods. The PBAN mRNA was expressed at moderate or high levels in the 4th (last) instar, early (P1) and late (P2) pupal stages, but PBAN-R transcription was detected only at a minimal level in these species.
stages. The disparate expression levels of the mRNAs for PBAN and PBAN-R indicate that another receptor other than PBAN-R could be activated during L4–P2 periods.

Ae. aegypti DH-R is expressed in larval, pupal and adult stages at higher levels than PBAN-R in the same periods. The difference is most evident in the L4–P2 stages, where DH-R expression is 4–10 times higher than PBAN-R, suggesting that DH-R is upregulated during this period while PBAN-R is downregulated. PBAN mRNA is expressed in all stages; thus, NPs including DH and PBAN are continuously synthesized in SC. Therefore, during the L4–P2 stages DH is available to activate DH-R, which is relatively insensitive to PBAN. These expression patterns suggest that the DH might be involved in the pupal development or eclosion to adults, which is supported by reports of accelerated puparium formation mediated by PBAN/pyrokinin peptides in the flesh fly, Sarcophaga bullata (Zdekare et al., 1997, 2004).

In recent years new strategies for Ae. aegypti control have been investigated. One of these strategies is RNA interference (RNAi) technology to selectively silence critical gene transcripts in order to debilitate or kill the mosquito and prevent disease transmission (Pridgeon et al., 2008; Coy et al., 2012). Identification of critical proteins in physiological pathways (targets) is key to the success of this strategy. Insect neuropeptide systems are prime targets for developing new strategies of insect pest control because they regulate critical physiological, metabolic, behavioral or reproductive processes (Geary and Maule, 2010; Choi et al., 2012). Specific peptides were first determined based on their regulation of single physiological functions; these peptides now appear to be involved in more than one function, and more than one peptide can regulate a single physiological process. The RNAi suppression of the PBAN/pyrokinin gene family could be a good target where silencing a single gene interferes with the production of multiple neuropeptides that have been shown to regulate multiple physiological processes. In this study, we identified and characterized the Ae. aegypti PBAN/pyrokinin and two receptors, and expression profiles in eggs, larvae, pupae and adults This is the first step toward identification of the physiological function(s) of PBAN/pyrokinin peptides and the development of methods to disrupt their production for applications in mosquito control.

Acknowledgements

We thank Drs. Valles and Allan for valuable comments to an earlier version. We also thank D. Milne and K. Chalaire for technical support.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.mce.2013.05.019.

References


Please cite this article in press as: Choi, M.-Y., et al. Identification and expression of PBAN/diapause hormone and GPCRs from Aedes aegypti. Molecular and Cellular Endocrinology (2013), http://dx.doi.org/10.1016/j.mce.2013.05.019


