Insect Biochemistry and Molecular Biology 42 (2012) 816–825

Contents lists available at SciVerse ScienceDirect

Insect Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/ibmb

Calcitonin-like diuretic hormones in insects

Meet Zandawala*

Department of Biology, University of Toronto Mississauga, Room 3016A/B, William Davis Building, 3359 Mississauga Road N., Mississauga, ON, Canada L5L 1C6

ARTICLE INFO

Article history:
Received 8 May 2012
Received in revised form
19 June 2012
Accepted 24 June 2012

Keywords:
Neuropeptide
Neurohormone
Diuretic hormone 31 (DH_{31})
GPCR
Malpighian tubules
Diuresis
Visceral muscle
Excretory system

ABSTRACT

Insect neuropeptides control various biological processes including growth, development, homeostasis and reproduction. The calcitonin-like diuretic hormone (CT/DH) is one such neuropeptide that has been shown to affect salt and water transport by Malpighian tubules of several insects. With an increase in the number of sequenced insect genomes, CT/DHs have been predicted in several insect species, making it easier to characterize the gene encoding this hormone and determine its function in the species in question. This mini review summarizes the current knowledge on insect CT/DHs, focusing on mRNA and peptide structures, distribution patterns, physiological roles, and receptors in insects.

1. Introduction

Neuropeptides represent the largest class of signaling compounds amongst insects that control several critical biological processes including growth, development, homeostasis and reproduction. Based on the annotation of complete insect genomes, it is estimated that about 30–40 neuropeptide precursor-encoding genes are present in a given insect (see Roller et al., 2008). Calcitonin-like diuretic hormone (CT/DH) is one such neuropeptide that has been shown to affect salt and water transport by Malpighian tubules (MTs) of several insects. As the number of complete sequenced genomes has increased greatly in recent times, opportunities for genome mining are plentiful. Consequently, CT/DHs and their associated receptors have been predicted in several insect species, as well as crustaceans, chelicerates and other members of Arthropoda (Christie, 2008; Christie et al., 2010a,b, 2011; Gard et al., 2009; Hauser et al., 2006, 2008). This greatly facilitates the cloning and characterization of genes encoding this hormone and its receptor. For example, primers and probes can be generated using the predicted sequences to perform quantitative-PCR and *in situ* hybridization, respectively, to determine the expression pattern of the transcripts encoding these peptides and their receptors. RNAi can also be used to knockdown these transcripts and study their effect on whole animal physiology. More importantly, knowing the tissues in which the receptor is expressed allows one to perform bioassays using synthetic peptides (as opposed to crude tissue extracts or biochemically purified peptides) on actual target tissues. Hence knowing their gene sequence will aid in elucidating the function(s) of CT/DHs in the species in question. Since little is known about the biological activities of CT/DHs, it is worthwhile to summarize the molecular and physiological knowledge of these peptides in order to create avenues for future research to determine their function(s). In this mini review, the current knowledge on insect CT/DHs is summarized, particularly focusing on mRNA and peptide structures, as well as their distribution patterns, physiological roles, and associated receptors.

2. Discovery

The existence of insect hormones related to the vertebrate calcitonin (CT) has been known for quite some time. Their presence was first examined in the corpus cardiacum and corpus allatum of the insect *Leucophaea maderae* via immunohistochemistry using antisera raised against mammalian CT (Hansen et al., 1982). Similar immunohistochemical analyses were also performed in the tobacco hornworm moth, *Manduca sexta*, and the Colorado potato beetle, *Leptinotarsa decemlineata* (El-Salhy et al., 1983; Veenstra et al., 1985). However, it wasn't until 2000 that the first representative

* Corresponding author. Tel.: +1 905 828 5333; fax: +1 905 828 3792.
E-mail addresses: meet.zandawala@utoronto.ca, meetzandawala@hotmail.com.

0965-1748/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ibmb.2012.06.006

of the CT/DH family was isolated and functionally characterized in the Pacific beetle cockroach *Diploptera punctata* (Furuya et al., 2000). This peptide was originally referred to as diuretic hormone 31 (DH_{31}), owing to the fact that it increased fluid secretion in MTs of various insects and contained 31 amino acids (Furuya et al., 2000; Te Brugge et al., 2005). Although it has low sequence identity to the vertebrate CT, it has the conserved C-terminal Glycine-X-Proline-NH_2 and they are similar in length (see Table 1) (Furuya et al., 2000). These are both important features in terms of their bioactivity as has been shown using vertebrate CTs (see Andreotti et al., 2006). Residues 9–19 of salmon calcitonin are involved in forming a stable α-helix which interacts with the C-terminus (Amodeo et al., 1999). Furthermore, the α-helical region has been shown to directly interact with the N-terminus of the receptor and varying the length of the helix causes a reduction in the peptide's bioactivity (Andreotti et al., 2006; Stroop et al., 1996). Since their isolation from *D. punctata*, CT/DHs have been identified and cloned in several insect species (Table 1) and their role as diuretic hormones examined in few insects (see Section 5.1). Due to minimal sequence similarity between vertebrate CTs and insect CT/DHs, as well as their inability to act as "true" diuretic hormones (they are mostly only able to stimulate MT secretion) in most of the species examined so far (see Section 5.1), one might question the appropriateness of the name attributed to this peptide family. Due to the functional similarity between the vertebrate calcitonin receptors and insect CT/DH receptor(s) (see Section 6.1), the CT-like nature of this peptide family remains assured. However, their role as diuretic hormones in insects is still questionable.

3. mRNA and prepropeptide structures

CT/DH mRNA and prepropeptide structures are well conserved across insects. CT/DH transcripts are composed of 4–6 exons (Fig. 1 and Table 2). In the sequences examined here, exon 1 represents most of the 5′ untranslated region (UTR) and the CT/DH mature peptide is encoded by the last exon, with the exception of *Anopheles gambiae* where the last two exons (exons 5 and 6) encode CT/DH (Fig. 1). More specifically, the region encoding the CT/DH mature peptide is at the 5′ end of the last exon (3′ end of exon 5 and 5′ end of exon 6 in *A. gambiae*). The majority of the last exon is the 3′ UTR which can be as large as 937 bp in *Rhodnius prolixus CT/DH-C* (*Rhopr-CT/DH-C*) (Fig. 1) (Zandawala et al., 2011). Intron lengths have not been examined as complete genomes are not available for all the species examined here. CT/DH prepropeptides range in size from 103 amino acid residues (aar) in *Bombus terrestris* CT/DH-A to 146 aar in Rhopr-CT/DH-C (Fig. 2A). CT/DH prepropeptides are predicted to produce four peptides following post-translational proteolytic processing: signal peptide, precursor peptide 1 (PP1), CT/DH and precursor peptide 2 (PP2) (Fig. 2A and B). Interestingly, PP1 in Rhopr-CT/DH-B and Rhopr-CT/DH-C are considerably longer than others due to the presence of exon 4 and exons 4 and 5, respectively. It has been previously suggested that Rhopr-CT/DH-B and Rhopr-CT/DH-C are more derived than Rhopr-CT/DH-A and could have resulted from a possible DNA insertion event in *R. prolixus* (Zandawala et al., 2011). Although PP1 is not similar to any other known peptides, its conservation at the C-terminus may suggest a biological role for this peptide. However, attempts to identify this peptide in *R. prolixus* central nervous system (CNS) extracts via mass spectrometry analysis have been unsuccessful (Zandawala and Orchard, unpublished). The multiple sequence alignment shows that CT/DH and its flanking cleavage sites are very well conserved across all insects (Fig. 2A and B). Lastly, PP2 is a small non-conserved peptide, ranging from 1 to 10 aar, which most-likely lacks any biological activity.

4. Distribution

4.1. mRNA distribution

CT/DH transcript distribution in insects has not been studied in great detail. FlyAtlas database (www.flyatlas.org) reports *Drome-CT/DH* expression in various tissues of both the larval and adult flies. As evident from the data, *Drome-CT/DH* is abundantly expressed in the CNS and midgut, which corroborates the presence of Drome-CT/DH-like immunoreactive cells in the CNS and midgut. In *R. prolixus*, at least three CT/DH-encoding transcripts are expressed within the CNS (Zandawala et al., 2011). In particular, all three variants are expressed in the brain and various ganglia (Zandawala and Orchard, unpublished). Moreover, all three variants are expressed in an individual *R. prolixus* with no apparent difference in expression between males and females (Zandawala and Orchard, unpublished). This begs the question as to why three CT/DH-encoding transcripts are present in *R. prolixus* CNS considering they all produce the mature CT/DH peptide. Perhaps the answer may lie in any potential roles for PP1 which is variable across the three variants. The lack of mutually-exclusive exons within the three transcripts makes it difficult to determine the cellular localization of the individual transcripts (see Section 3); however, cell-specific localization of *Rhopr-CT/DH* (all three transcripts) using fluorescent *in situ* hybridization (FISH) (Fig. 3) reveals a pattern which has good overlap with the immunohistochemical localization of the peptide (Te Brugge et al., 2005; Zandawala et al., 2011). Immunohistochemical analysis reveals a few more cells than are localized by FISH which might indicate low transcript expression in these cells. Interestingly, a fourth splice variant (referred to as *Rhopr-CT/DH-D* from here on) for this gene was recently discovered in ESTs derived from *R. prolixus* testes (Ons et al., 2011). *Rhopr-CT/DH-D* does not encode the CT/DH mature peptide but it may still encode a peptide similar to Rhopr-CT/DH-C PP1 (see Section 3). Additional investigations are needed to determine the presence of Rhopr-CT/DH-D peptide in *R. prolixus* testes and its physiological role(s), if any.

4.2. Peptide localization

Immunohistochemical analyses to localize CT/DH-like peptides have only been conducted in a handful of species following the isolation of the first peptide from *D. punctata* brain/corpus cardiacum extracts. As expected, CT/DH-like immunoreactivity is present in the CNS of all the insects in which it has been examined, including *Drosophila melanogaster*, *R. prolixus* and *Oncopeltus fasciatus* (Park et al., 2008; Te Brugge et al., 2005; Te Brugge and Orchard, 2008). In *D. melanogaster* larvae, Drome-CT/DH-like immunoreactivity is present in cells throughout the CNS, except in thoracic segment 1 (Park et al., 2008). Surprisingly, Drome-CT/DH distribution in the adult CNS has not been mapped yet. With regards to the gut, Drome-CT/DH-like immunoreactivity is observed in endocrine cells throughout the larval midgut, whereas CT/DH-expressing endocrine cells are only found in the last half of the posterior midgut of adult *D. melanogaster* (Veenstra, 2009). Within larvae, Drome-CT/DH endocrine cells are more abundant in the anterior midgut and the anterior-middle midgut junction compared to the middle and posterior midgut. Interestingly, some of the tachykinin-expressing endocrine cells in the posterior midgut also express Drome-CT/DH. This Drome-CT/DH distribution pattern in the midgut is similar in both feeding and wandering third instar larvae, but the staining is much reduced in the wandering larvae. It is believed that these CT/DH-expressing endocrine cells are responsible for stimulating fluid secretion by the MTs and the midgut in addition to their role in modulating peristalsis (LaJeunesse et al., 2010; Veenstra,

Table 1
Structures of mature CT/DHs (deduced or sequenced).

Species	Peptide structure	Reference
Insects		
Rhodnius prolixus[1]	GLDLGLSRGFSGSQAAKHLMGLAAANYAGGP-NH_2	(Te Brugge et al., 2008)
Diploptera punctata[1]	GLDLGLSRGFSGSQAAKHLMGLAAANYAGGP-NH_2	(Furuya et al., 2000)
Apis mellifera[1]	GLDLGLSRGFSGSQAAKHLMGLAAANYAGGP-NH_2	(Schooley et al., 2005)
Solenopsis invicta[1]	GLDLGLSRGFSGSQAAKHLMGLAAANYAGGP-NH_2	Predicted
Camponotus floridanus[1]	GLDLGLSRGFSGSQAAKHLMGLAAANYAGGP-NH_2	(Gruber and Muttenthaler, 2012)
Apis florea[1]	GLDLGLSRGFSGSQAAKHLMGLAAANYAGGP-NH_2	Predicted
Megachile rotundata[1]	GLDLGLSRGFSGSQAAKHLMGLAAANYAGGP-NH_2	Predicted
Bombus terrestris[1]	GLDLGLSRGFSGSQAAKHLMGLAAANYAGGP-NH_2	Predicted
Bombus impatiens[1]	GLDLGLSRGFSGSQAAKHLMGLAAANYAGGP-NH_2	Predicted
Tribolium castaneum[2]	GLDLGLGRGFSGSQAAKHLMGLAAANFAGGP-NH_2	(Li et al., 2008)
Anopheles gambiae[3]	TVDFGLSRGYSGAQEAKHRMAMAVANFAGGP-NH_2	(Coast et al., 2005)
Aedes aegypti[3]	TVDFGLSRGYSGAQEAKHRMAMAVANFAGGP-NH_2	(Schooley et al., 2005)
Culex quinquefasciatus[3]	TVDFGLSRGYSGAQEAKHRMAMAVANFAGGP-NH_2	(see Zandawala et al., 2011)
Drosophila melanogaster[4]	TVDFGLARGYSGTQEAKHRMGLAAANFAGGP-NH_2	(Coast et al., 2001)
Drosophila virilis[5]	TVDFGLARGYSGTQEAKHRMGLAAANFPGGP-NH_2	(see Schooley et al., 2012)
Nasonia vitripennis[6]	GLDLGLNRGFSGSQAAKHLMGLAAANYAGGP-NH_2	(Hauser et al., 2010)
Nasonia longicornis[6]	GLDLGLNRGFSGSQAAKHLMGLAAANYAGGP-NH_2	Predicted
Nasonia giraulti[6]	GLDLGLNRGFSGSQAAKHLMGLAAANYAGGP-NH_2	Predicted
Pogonomyrmex barbatus[6]	GLDLGLNRGFSGSQAAKHLMGLAAANYAGGP-NH_2	Predicted
Acromyrmex echinatior[7]	GLDLGLNRGYSGSQAAKHMMGLAAANYAGGP-NH_2	Predicted
Atta cephalotes[7]	GLDLGLNRGYSGSQAAKHMMGLAAANYAGGP-NH_2	(Gruber and Muttenthaler, 2012)
Acyrthosiphon pisum	GLDLGLSRGYSGTQAAKHLMGMAAANFAGGP-NH_2	(see Zandawala et al., 2011)
Nilaparvata lugens	GLDLGLSRGFSGSQAAKHLMGLAAANYAAGP-NH_2	(see Zandawala et al., 2011)
Bombyx mori	AFDLGLGRGYSGALQAKHLMGLAAANFAGGP-NH_2	(Schooley et al., 2005)
Harpegnathos saltator	GLDLGLSRGFSGSQSAKHMMGLAAANYAGGP-NH_2	(Gruber and Muttenthaler, 2012)
Heliothis virescens	ALDLGLSRGYSGALQAKHLMGLAAAHYAGGP-NH_2	(see Schooley et al., 2012)
Manduca sexta	ALDLGLSRGYSGALQAKHLIGLAAANYAGGP-NH_2	(see Schooley et al., 2012)
Pediculus humanus corporis	GLDLGLSRGFSGSQAAKHLMGLAAANFAGGP-NH_2	(see Schooley et al., 2012)
Dendroctonus ponderosae	GIDLGLGRGFSGSQAAKHLMGLAAANFAGGP-NH_2	(see Schooley et al., 2012)
Linepithema humile	GLDLGINRGFSGSEAAKHLMGLAAANYAGGP-NH_2	Predicted
Crustaceans		
Daphnia pulex	GVDFGLGRGYSGSQAAKHLMGLAAANYAIGP-NH_2	(Gard et al., 2009)
Homarus americanus[2]	GLDLGLGRGFSGSQAAKHLMGLAAANFAGGP-NH_2	(Christie et al., 2010)
Lepeophtheirus salmonis	GLDFGLGRGFSGTQAAKHFMGLAAAKYAGGP-NH_2	(see Schooley et al., 2012)
Chelicerates		
Ixodes scapularis	AGGLLDFGLSRGASGAEAAKARLGLKLANDPYGP-NH_2	(Christie, 2008)
Varroa destructor	SNGMLDFGLARGMSGVDAAKARLGLKYANDPYGP-NH_2	(see Zandawala et al., 2011)
Arachnids		
Tetranychus urticae	GLDLGLRRGLSGQRAAKHLVGLANAEFAGGP-NH_2	(see Schooley et al., 2012)
Vertebrates		
Gallus gallus domesticus	CASLSTCVLGKLSQELHKLQTYPRTDVGAGTP-NH_2	(see Furuya et al., 2000)
Homo sapiens	CGNLSTCMLGTYTQDFNKFHTFPQTAIGVGAP-NH_2	(see Furuya et al., 2000)

Amino acids that are shared by all sequences are highlighted in black.
Amino acids that are shared by all arthropod sequences are highlighted in gray.
Amino acids that are shared by all insect sequences are highlighted in yellow.
[1,2,3,6,7] Identical sequences.
[4] *Drosophila melanogaster* sequence is identical to *Drosophila pseudoobscura pseudoobscura, Drosophila yakuba, Drosophila rhopaloa, Drosophila persimilis, Drosophila elegans, Drosophila simulans, Drosophila sechellia, Drosophila ficusphila, Drosophila takahashii, Drosophila erecta, Drosophila eugracilis* and *Drosophila biarmipes* sequences.
[5] *Drosophila virilis* sequence is identical to *Drosophila grimshawi, Drosophila willistoni, Drosophila mojavensis, Drosophila ananassae, Drosophila kikkawai* and *Drosophila bipectinata sequences*.

2009). However, stress caused by starvation and desiccation has been shown to induce the hormonal release of tachykinin, possibly from the endocrine cells in the midgut, which in turn acts on MTs to regulate *Drosophila* insulin-like peptide 5 signaling (Soderberg et al., 2011). Thus Drome-CT/DH present in these endocrine cells may also be released along with tachykinin during starvation and osmotic stress. This observation is in stark contrast to the role of Drome-CT/DH in fluid secretion.

Within the *R. prolixus* CNS, Rhopr-CT/DH-like immunoreactivity is consistently observed in two lateral neurosecretory cells in the dorsal brain and six cells on the posterior ventral surface of the mesothoracic ganglionic mass (MTGM) (Te Brugge et al., 2005). Interestingly, 5-hydroxytryptamine (serotonin) is co-localized with Rhopr-CT/DH in the 5 dorsal unpaired median (DUM) neurons of the MTGM (Te Brugge et al., 2005). The presence of serotonin in these 5 DUM neurons is unique as all the insect DUM neurons examined so far contain octopamine (see Orchard et al., 1989). These neurons project axons through their corresponding abdominal nerves, where they produce neurohemal sites. Moreover, the staining in DUM neurons is completely abolished in *R. prolixus* 1 h after feeding. The neurohemal sites are also much reduced in fed insects compared to unfed. Although serotonin and Rhopr-CT/DH are co-localized, and possibly co-released, they have different actions. Unlike serotonin, Rhopr-CT/DH neither stimulates absorption across the anterior midgut nor does it stimulate MT secretion to the same extent (see Section 5.1) (Te Brugge et al., 2002, 2009). In addition to

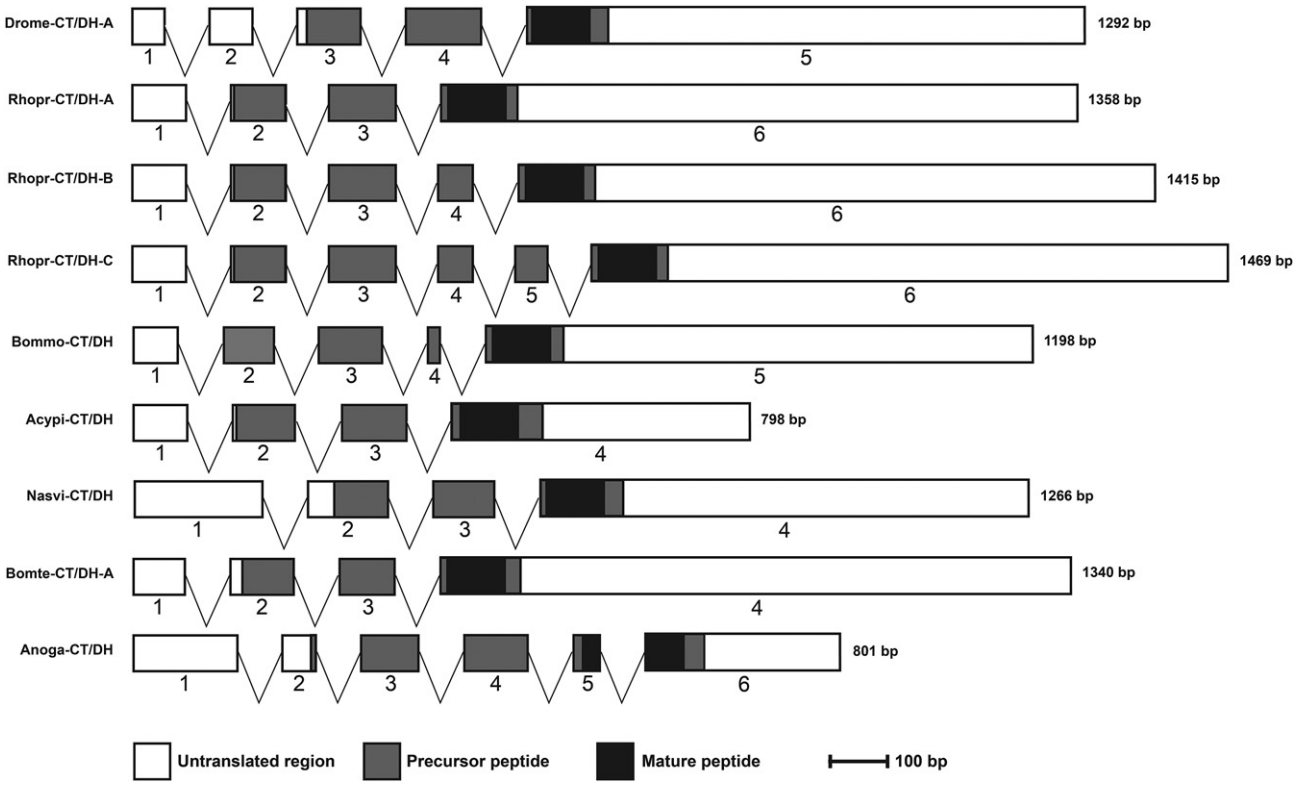

Fig. 1. Molecular organization of insect CT/DHs based on BLAST analysis and gene structure prediction using Webscipio 2.0 (Hatje et al., 2011). CT/DH mRNA sequences from the following insects were used for the analysis: *Drosophila melanogaster* (variant A – NM_078790.3), *Rhodnius prolixus* (variant A – HM030716.1; variant B – HM030715.1; variant C – HM030714.1), *Bombyx mori* (NM_001130907.1), *Acyrthosiphon pisum* (XM_001945866.2), *Nasonia vitripennis* (XM_001599898.2), *Bombus terrestris* (variant A - XM_003395571.1) and *Anopheles gambiae* (XM_321755.5). The boxes represent exons which are numbered sequentially. Introns are not included.

the CNS, Rhopr-CT/DH-like immunoreactivity is observed in processes over the hindgut and on salivary glands, specifically the salivary nerve, accessory gland and salivary duct (Te Brugge et al., 2005). Unlike *D. melanogaster*, no CT/DH-containing endocrine cells were detected in *R. prolixus* midgut. Immunohistochemical analysis using *O. fasciatus* CNS reveals a pattern similar to that observed in *R. prolixus* (Te Brugge and Orchard, 2008). In particular, Rhopr-CT/DH-like immunoreactivity is localized to lateral neurosecretory cells in the brain and posterior ventral cells in the MTGM. However, instead of localizing to the DUM neurons, CT/DH-like immunoreactivity is localized to four pairs of dorsal medial neurosecretory cells in the MTGM of *O. fasciatus*. Staining is also observed in the processes originating from these cells that extend to the abdominal nerves. Rhopr-CT/DH-like immunoreactivity in the *O. fasciatus* gut presents an interesting situation. Similar to that seen in *R. prolixus*, Rhopr-CT/DH-like immunoreactivity is observed in processes over the hindgut (Te Brugge and Orchard, 2008); however, as seen in *D. melanogaster* (Veenstra, 2009), staining is observed in endocrine-like cells of midgut, specifically the fourth ventricle (Te Brugge and Orchard, 2008). Hence there is a possibility that endocrine-like cells containing CT/DH could be present in *R. prolixus* but were not detected at the feeding stages examined. Alternatively, this may reflect an inherent difference in peptide expression between herbivorous insects such as *D. melanogaster* and *O. fasciatus* and hematophagous insects such as *R. prolixus*. Additional analyses in other insects are needed to determine the presence of CT/DH-containing endocrine cells in insect midguts.

5. Biological activity

5.1. Diuresis

As mentioned earlier, CT/DH was originally named based on its ability to stimulate MT secretion in *D. punctata* (Furuya et al., 2000). Since then its role in diuresis has been demonstrated in various other insects including *D. melanogaster*, *A. gambiae* and *R. prolixus*

Table 2
Summary of CT/DH transcripts in insects.

Species	Variant	Accession number	No. of exons	Exons encoding prepropeptide	Exons encoding mature peptide	Reference
Drosophila melanogaster	A	NM_078790	5	3–5	5	(see Coast, 2006)
Drosophila melanogaster	C	NM_164825	5	3–5	5	
Rhodnius prolixus	A	HM030716	4	2, 3 & 6	6	(Zandawala et al., 2011)
Rhodnius prolixus	B	HM030715	5	2–4 & 6	6	(Zandawala et al., 2011)
Rhodnius prolixus	C	HM030714	6	2–6	6	(Zandawala et al., 2011)
Anopheles gambiae	–	XM_321755	6	2–6	5 & 6	
Bombyx mori	–	NM_001130907	5	2–5	5	(Roller et al., 2008)
Acyrthosiphon pisum	–	XM_001945866	4	2–4	4	
Nasonia vitripennis	–	XM_001599898	4	2–4	4	
Bombus terrestris	A	XM_003395571	4	2–4	4	

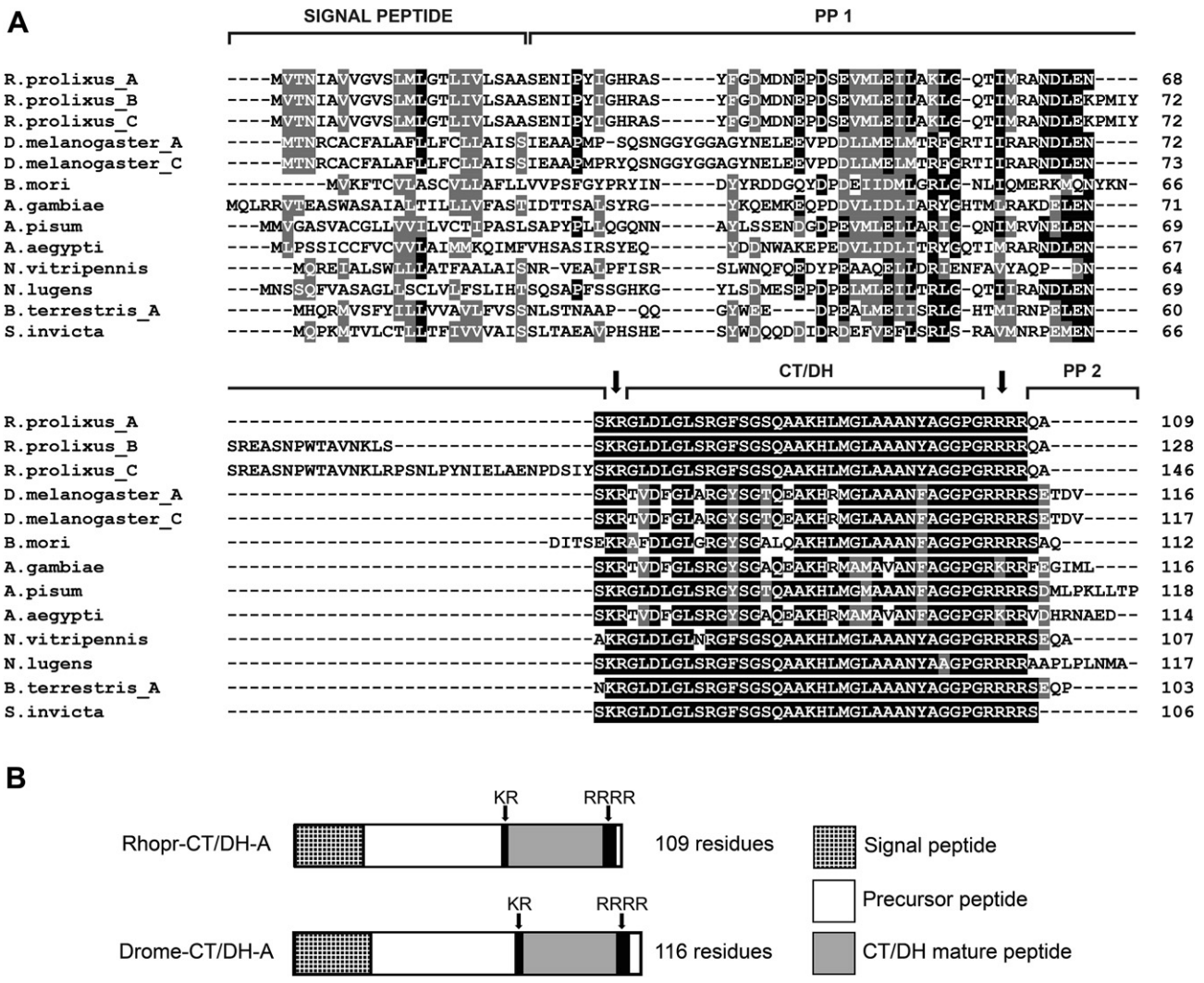

Fig. 2. CT/DH prepropeptides in insects. A) Multiple sequence alignment of insect CT/DH prepropeptides: *Rhodnius prolixus* (variant A – AEA51302.1; variant B – AEA51301.1; variant C – AEA51300.1), *Drosophila melanogaster* (variant A – NP_523514.1; variant C – NP_723401.1), *Bombyx mori* (NP_001124379.1), *Anopheles gambiae* (XP_321755.3), *Acyrthosiphon pisum* (XP_001945901.1), *Aedes aegypti* (EAT40182.1), *Nasonia vitripennis* (XP_001599948.1), *Nilaparvata lugens* (DB826761), *Bombus terrestris* (variant A – XP_003395619.1) and *Solenopsis invicta* (EFZ22024.1). PP – precursor peptide. The positions of the various peptides are based on their prediction in *Rhodnius prolixus* (redrawn from Zandawala et al., 2011). B) Schematic representations (drawn to scale) of *R. prolixus* and *Drosophila melanogaster* CT/DH prepropeptides. Arginine and lysine propeptide cleavage sites are indicated by arrows.

(Coast et al., 2001, 2005; Te Brugge et al., 2005) (Table 3). However, there are also examples of species such as *Acrosternum hilare* and *Podisus maculiventris* in which CT/DHs do not stimulate diuresis (Coast et al., 2011). Caution must still be exercised in discarding CT/DHs role in diuresis in these insects as only the non-native CT/DHs were tested; the native CT/DHs from these species may still be diuretic. Although CT/DHs and their role as a diuretic factor are somewhat conserved across insects, there are several differences in their underlying mechanisms (Table 3). Firstly, CT/DH employs different secondary messengers in MTs of different insects; calcium signaling is involved in *Locusta migratoria* while cAMP is used across various dipterans (Coast et al., 2001, 2005; Furuya et al., 2000). It is still unclear as to what the secondary messengers are in *D. punctata* or *R. prolixus* (Furuya et al., 2000; Te Brugge et al., 2005; Tobe et al., 2005). Secondly, the resulting ion composition of the secreted fluid in MTs stimulated by CT/DHs is dependent upon the species in question. For example, Rhopr-CT/DH has no effect on ion composition of the secreted fluid but mosquito CT/DHs stimulate increased Na^+ secretion (i.e. natriuresis) (Coast et al., 2005; Donini et al., 2008). Indeed the *Aedes aegypti* CT/DH was previously referred to as the Mosquito Natriuretic Peptide (Petzel et al., 1985, 1986, 1987). Lastly, the relative potencies of CT/DHs compared to other diuretic factors are also variable. For instance, CT/DHs stimulate maximum secretion rates in mosquitoes but only up to 50% of maximum rates in *D. punctata*, *L. migratoria* and *D. melanogaster* (Coast et al., 2001, 2005; Furuya et al., 2000). Rhopr-CT/DH is an extreme exception as the secretion rate is only 1.48% of maximum secretion with serotonin. Although this increase in secretion is small relative to that of serotonin, it is still 17-fold higher than basal secretion rates which are minimal in *R. prolixus* (Te Brugge et al., 2005).

CT/DH is not the only family of diuretic hormones (DHs) in insects. Other DHs include biogenic amines such as serotonin and tyramine, as well as various peptide families such as the kinins, corticotropin-releasing factor-like DHs (CRF/DHs) and CAP_{2b}-like DHs (see Schooley et al., 2012). The different diuretic factors could work in concert to control MTs and other feeding-related tissues. This may bring about synergism which results in a steeper response at lower concentrations of DHs. Synergism between various diuretic factors has been examined in a few species (Table 4). Not

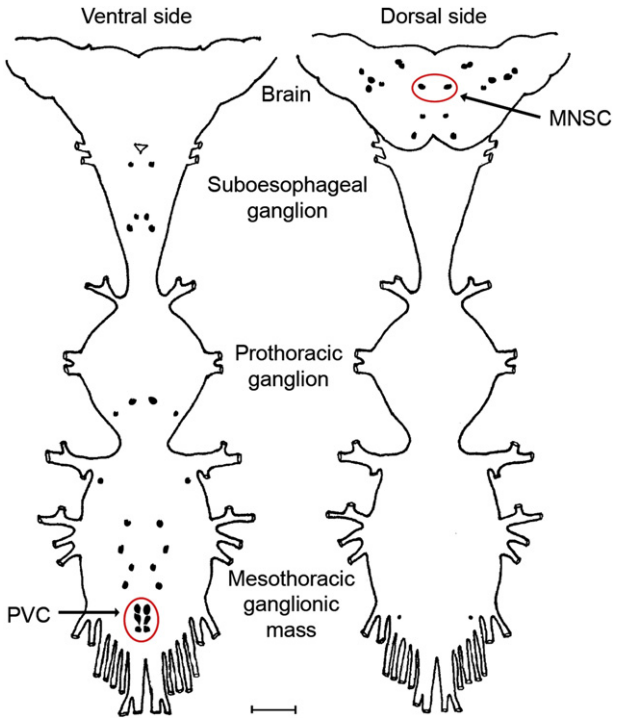

Fig. 3. *Rhopr-CT/DH* expression pattern determined using fluorescent *in situ* hybridization in fifth instar *R. prolixus* CNS (redrawn from Zandawala et al., 2011). Putative medial neurosecretory cells (MNSC) and posterior ventral cells (PVC) have been labeled. Scale bar: 200 μm.

surprisingly, the results are quite variable. For instance, Dippu-CT/DH and Dippu-CRF/DH are synergistic in *D. punctata* but only additive in *L. migratoria* (Furuya et al., 2000); however, when Dippu-CRF/DH is replaced with the native Locmi-CRF/DH, synergism is observed. On the other hand, Rhopr-CT/DH and serotonin are only additive in *R. prolixus* whereas Bommo-CT/DH and *Tenebrio molitor* CRF/DH are neither synergistic nor additive in *T. molitor*. Needless to say, synergism between CT/DHs and other diuretic factors needs to be tested in detail before its role as a diuretic can be discarded in any species.

5.2. Feeding-related physiological events

CT/DHs could also play an important role in feeding-related physiological events in various insects. In *R. prolixus*, CT/DH causes an increase in frequency of salivary gland muscle contractions (see Orchard, 2009). This may aid in the mixing of salivary gland contents and to propel the saliva out of the principal gland during feeding. Moreover, Rhopr-CT/DH causes an increase in frequency of anterior midgut, hindgut and dorsal vessel contractions, with cAMP possibly acting as the secondary messenger (Te Brugge et al., 2008, 2009). Interestingly, Rhopr-CT/DH and Rhopr-CRF/DH both increase anterior midgut contractions, but only Rhopr-CRF/DH increases water absorption from the anterior midgut (Orchard, personal communication; Te Brugge et al., 2009, 2011). Thus, Rhopr-CT/DH may play an indirect role in diuresis by increasing haemolymph circulation and mixing of the gut contents, which aids in reducing unstirred layers and facilitating ion transport. Moreover a peptide from this family was also isolated and partially sequenced from the Belgian forest ant, *Formica polyctena*, before the identification of Dippu-CT/DH in 2000 (see Schooley et al., 2012). Interestingly, the peptide was isolated based on its ability to stimulate the spontaneous writhing movements of *L. migratoria* MTs, which could indirectly contribute to diuresis. In *D. melanogaster*, CT/DH produced by enteroendocrine cells is required for peristalsis in the junction region between the anterior portion and acidic region of the larval midgut (LaJeunesse et al., 2010). Hence CT/DH may regulate digestion in *D. melanogaster*. On the other hand, Dippu-CT/DH linear and cyclic analogs have been shown to modulate appetitive behavior in food-deprived nymphs of *L. migratoria* (Kaskani et al., 2012). It acts as an anorexigenic by reducing the duration of the first meal and increasing the latency to feed.

Table 3
Summary of CT/DH effects on Malpighian tubule fluid secretion.

Factor	Species	Diuretic	EC_{50} (nM)	% of maximum (relative to)	Secondary messenger	Cellular effects	Reference
Dippu-CT/DH	*Diploptera punctata*	Yes	9.8	41% (Dippu-CRF/DH)	Not cAMP or cGMP	–	(Furuya et al., 2000; Tobe et al., 2005)
Dippu-CT/DH	*Locusta migratoria*	Yes	0.56	50% (Dippu-CRF/DH)	Ca^{2+}	No effect on Na^+/K^+ ratio of secreted fluid	(Furuya et al., 2000)
Drome-CT/DH	*Drosophila melanogaster*	Yes	4.3	35% (Musdo-Kinin)	cAMP	Stimulates an apical membrane V-type H^+ ATPase in principal cells; equal effect on K^+ and Na^+ transport across the basolateral membrane	(Coast et al., 2001)
Anoga-CT/DH	*Anopheles gambiae*	Yes	50	100%	cAMP	Natriuretic – selectively stimulates transepithelial Na^+ transport	(Coast et al., 2005)
Aedae-CT/DH	*Aedes aegypti*	Yes	–	100%	cAMP	Natriuretic – selectively stimulates transepithelial Na^+ transport	(Coast et al., 2005)
Rhopr-CT/DH	*Rhodnius prolixus*	Yes	<10	1.48% (Serotonin)	Not cAMP	Small lumen-positive shift in transepithelial potential of the upper tubule; No effect on ion composition of secreted fluid	(Donini et al., 2008; Te Brugge et al., 2005)
Rhopr-CT/DH	*Oncopeltus fasciatus*	Yes	–	–	–	–	(Te Brugge and Orchard, 2008)
Bommo-CT/DH	*Tenebrio molitor*	Yes	0.61	50% (cAMP)	–	–	(Holtzhausen and Nicolson, 2007)
Anoga-CT/DH	*Tenebrio molitor*	Yes	14	50% (cAMP)	–	–	(Holtzhausen and Nicolson, 2007)
Dippu-CT/DH	*Tenebrio molitor*	No	–	0%	–	–	(Holtzhausen and Nicolson, 2007)
Dippu-CT/DH	*Acrosternum hilare*	No	–	0%	–	–	(Coast et al., 2011)
Tenmo-CT/DH	*Podisus maculiventris*	No	–	0%	–	–	(Coast et al., 2011)

Table 4
Studies examining synergistic or additive effects between CT/DHs and other diuretic factors on Malpighian tubule secretion (modified from Holtzhausen and Nicolson, 2007).

Factor 1	Factor 2	Species	Combined effect	Reference
Dippu-CT/DH	Dippu-CRF/DH	*Diploptera punctata*	Synergistic	(Furuya et al., 2000)
Dippu-CT/DH	Dippu-CRF/DH	*Locusta migratoria*	Additive	(Furuya et al., 2000)
Dippu-CT/DH	Locmi-CRF/DH	*Locusta migratoria*	Synergistic	(Furuya et al., 2000)
Dippu-CT/DH	Locmi-Kinin	*Locusta migratoria*	Synergistic	(Furuya et al., 2000)
Drome-CT/DH	Musdo-Kinin	*Drosophila melanogaster*	Additive	(Coast et al., 2001)
Rhopr-CT/DH	Serotonin (5-HT)	*Rhodnius prolixus*	Additive	(Te Brugge et al., 2005)
Bommo-CT/DH	Tenmo-CRF/DH	*Tenebrio molitor*	Not synergistic or additive	(Holtzhausen and Nicolson, 2007)

5.3. Other effects

Recent work in *M. sexta* suggests that CT/DH may also be involved in ecdysis (Kim et al., 2006). A subset of ecdysis-triggering hormone receptor (ETHR)-expressing cells in the abdominal ganglia produce CT/DH. However, their precise physiological role during ecdysis has not yet been examined. Perhaps CT/DH stimulates ecdysis-associated diuresis in *M. sexta* as such a diuresis has been demonstrated using crude tissue extracts in another lepidopteran, *Pieris brassicae* (Nicolson, 1976). On a separate note, CT/DH may also be involved in modulating the neuronal clock network in *D. melanogaster*. Drome-CT/DH activates the *D. melanogaster* pigment-dispersing factor (PDF) receptor (PDFR) *in vitro* and also causes a large increase in cAMP levels in all PDF-expressing clock neurons (Mertens et al., 2005; Shafer et al., 2008).

6. Receptors

6.1. CT/DH receptor in D. melanogaster

Insect CT/DH receptors belong to the family of secretin-like (family B) G-protein coupled receptors (GPCRs) (Hewes and Taghert, 2001). The first insect CT/DH receptor was functionally characterized in *D. melanogaster* (CG17415/CG32843) (Johnson et al., 2005). Mammalian CT receptor-like receptor (CLR) signaling is dependent on two types of accessory proteins: receptor activity modifying proteins (RAMPs) and receptor component protein (RCP) (Evans et al., 2000; McLatchie et al., 1998). Likewise, the Drome-CT/DH receptor is activated by Drome-CT/DH ($EC_{50} = 5219$ nM) when co-expressed in HEK293 cells with the *D. melanogaster* RCP. However, an improved sensitivity is observed when the human RCP (hRCP) was co-expressed instead ($EC_{50} = 116$ nM) and this sensitivity is further improved when the receptor is co-expressed with hRCP and human RAMPs ($EC_{50} = 82$ nM). Unlike mammals, where RCP expression mirrors CLR expression, FlyAtlas data indicates that the *D. melanogaster* RCP is expressed in almost all tissues which suggests that it may also interact with other receptors (Chintapalli et al., 2007; Ma et al., 2003). Nonetheless, this is the most compelling evidence for the functional conservation between signaling from mammalian CT receptor-like receptors and insect CT/DH receptor.

Immunohistochemical analysis shows that the Drome-CT/DH receptor is expressed in principal cells of *D. melanogaster* MTs (Johnson et al., 2005). FlyAtlas data indicates that the Drome-CT/DH receptor transcript is also highly expressed in the adult CNS, crop, midgut, hindgut, and heart (Chintapalli et al., 2007). The relative expression of Drome-CT/DH receptor in the larval MTs is only about 3% compared to that in adults. Nonetheless, Drome-CT/DH receptor expression in these tissues is consistent with the biological function of this hormone in controlling water and salt homeostasis in *D. melanogaster* and other insects. In addition, Drome-CT/DH and Drome-CRF/DH receptors are expressed in neurons that also express the neuropeptide corazonin (Johnson et al., 2005). Corazonin transcript expression is inhibited during starvation and osmotic stress (Harbison et al., 2005; Zhao et al., 2010) and it has been suggested that this inhibition may be caused by signaling through Drome-CT/DH and/or Drome-CRF/DH (Zhao et al., 2010). Assuming that Drome-CT/DH levels are elevated following feeding and/or during over hydration, it may then cause increased corazonin expression and its subsequent release from the neurons. Drome-CT/DH and corazonin might then increase heart contractions (transcripts for their respective receptors are highly expressed in the heart) to increase circulation of other hormones, including diuretic hormones, that may be present in the haemolymph (Chintapalli et al., 2007). Hence CT/DHs may also have an indirect effect on water and salt balance.

6.2. CT/DH receptor in other insects

CT/DH receptors have also been previously predicted in *A. gambiae* (Hill et al., 2002), *Tribolium castaneum* (*Tc 70*) (Hauser et al., 2008) and *Apis mellifera* (*Am 55*) (Hauser et al., 2006) through genome-wide annotation of neuropeptide GPCRs. Phylogenetic analysis of family B GPCRs (Fig. 4) reveals that CT/DH receptors form a monophyletic clade. This clade is sister to another monophyletic clade that contains receptors orthologous to the *D. melanogaster* CG4395 GPCR (see Section 6.3). Interestingly, the *Acyrthosiphon pisum* genome contains two putative CT/DH receptors (receptor 1 [XP_001943752.2] and receptor 2 [XP_001945278.2]), unlike other insects examined which all have one CT/DH receptor. It is unclear whether both the receptors are true CT/DH receptors. This clarification must await functional characterization of these receptors.

6.3. Classification of family B receptors

There has been a lack of consensus on the classification of family B GPCRs and their ligands (Hill et al., 2002; Lovejoy et al., 2006; Price et al., 2004). However, recent studies on *D. melanogaster* family B GPCRs have helped clarify this. The *D. melanogaster* genome contains 5 peptide GPCRs (CG4395, CG17415, CG13758, CG8422 and CG12370) that belong to family B (Hewes and Taghert, 2001). Out of these 5 receptors, CG4395 and CG17415 (also known as CG32843) are most closely related to CT receptor and CT gene related peptide receptor of vertebrates. CG17415 has been functionally characterized as the CT/DH receptor, as mentioned earlier (Johnson et al., 2005). CG4395, which is expressed in a subset of *fruitless* neurons, is critical for male courtship behavior (Li et al., 2011). FlyAtlas database also reports CG4395 transcript expression in the brain and male accessory glands, which is consistent with this role (Chintapalli et al., 2007). Three independent reports have characterized CG13758 as the PDFR (Hyun et al., 2005; Lear et al., 2005; Mertens et al., 2005). Interestingly, despite the lack of sequence similarity between Drome-CT/DH and Drome-PDF, Drome-CT/DH activates the Drome-PDFR ($EC_{50} = 218.6$ nM) when functional ligand-receptor interactions are analyzed in HEK293 cells (Mertens et al., 2005). However, the receptor activation by 10^{-6} M Drome-CT/DH is less than 40% compared to the response by

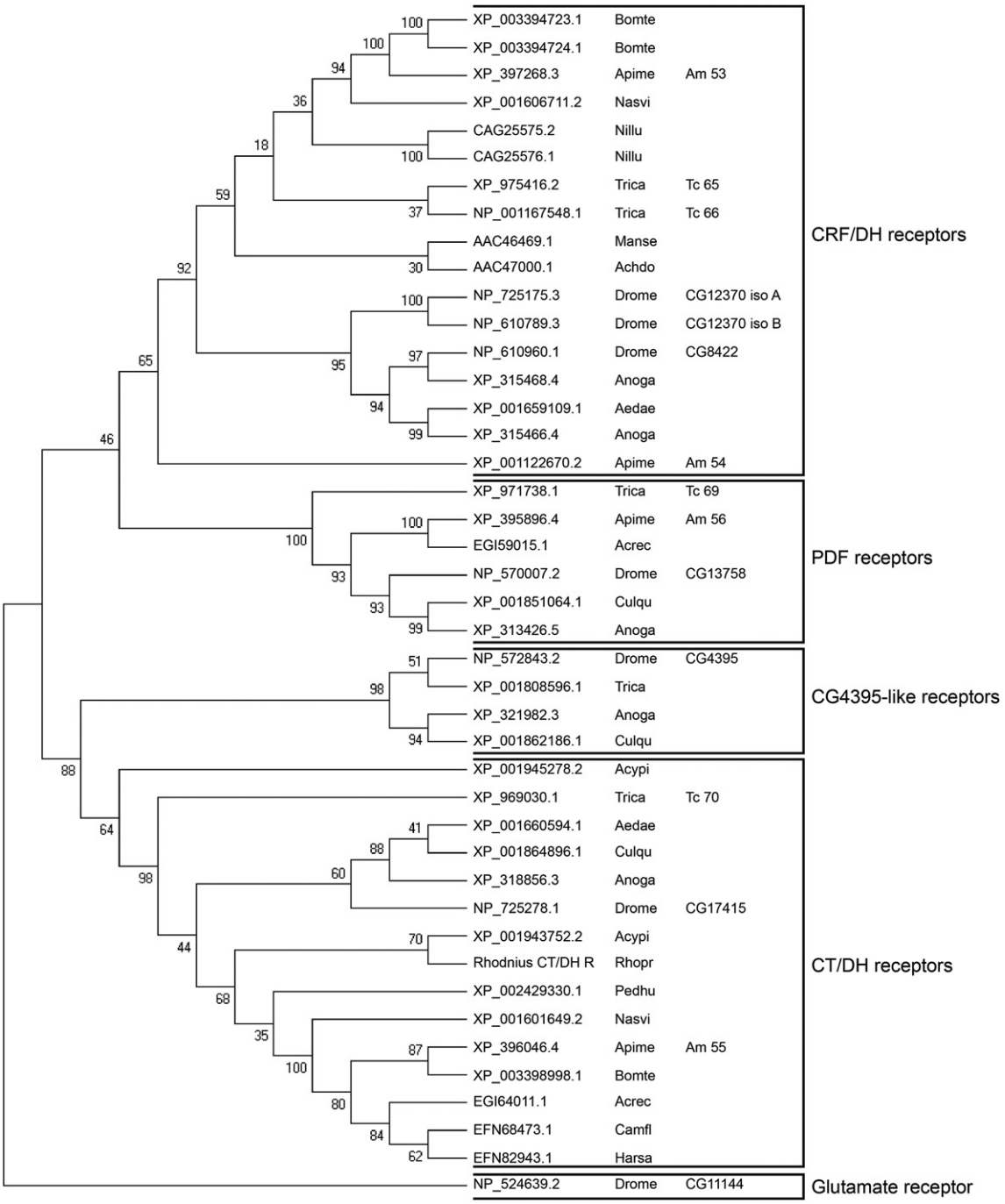

Fig. 4. Phylogenetic tree of insect GPCRs, belonging to family B. The maximum parsimonious tree was constructed using Close-Neighbor-Interchange (CNI) analysis and the bootstrap values obtained were based on 1000 replicates. The receptors are labeled using the associated GenBank accession number, five letter species code and, where available, gene id. Accession number has not been provided for *Rhodnius prolixus* CT/DH-R, however, it has been cloned and sequenced (Zandawala and Orchard, unpublished). The tree is rooted using the *D. melanogaster* metabotropic glutamate receptor (CG11144).

an equivalent dose of Drome-PDF. In contrast, Drome-CT/DH receptor is not sensitive to Drome-PDF (Johnson et al., 2005). This phenomenon is not unique as partial agonism by diverse ligands is a common feature among family B GPCRs (Hay et al., 2004). Furthermore, *de novo* predictions of Drome-PDF and Drome-CT/DH tertiary structures show that both these peptides form a helical region with the Drome-PDF helix being shorter than that of Drome-CT/DH (Maupetit et al., 2009). The relationship between a specific helix length and bioactivity has been examined previously using salmon CT where bioactivity was considerably reduced when the helix length was altered (Andreotti et al., 2006). Assuming similar structural requirements for the Drome-CT/DH receptor activation and assuming that the Drome-PDFR requires a ligand with a helical domain for activation, then this might explain the activation of Drome-PDFR by Drome-CT/DH and the absence of effect of Drome-PDF on Drome-CT/DH receptor. Clearly, additional work is needed to validate these speculations. CG8422, on the other hand, encodes a functional CRF/DH receptor (Drome-CRF/DH-R1) (Johnson et al., 2004). Hence despite the fact that the DH receptors isolated from the tobacco hornworm *M. sexta* (Reagan, 1994) and the house

cricket *Acheta domesticus* (Reagan, 1996) were originally classified broadly as members of the CT/secretin/CRF receptor family, these have now been determined to be receptors for CRF/DHs (Fig. 4) (Johnson et al., 2004). Likewise, the DH receptor cloned from the rice brown planthopper *Nilaparvata lugens* is a CRF/DH receptor (Price et al., 2004). Lastly, CG12370 (isoform A), which is paralogous to CG8422, has also been functionally characterized as a CRF/DH receptor (Drome-CRF/DH-R2) (Hector et al., 2009). Phylogenetic analysis groups this receptor along with other CRF/DH receptors (Fig. 4), which suggests possible gene duplication. Interestingly, FlyAtlas reports high expression of CG12370 transcript in the crop, midgut, MTs and hindgut but a lack of CG8422 transcript in these tissues (Chintapalli et al., 2007) which are associated with diuresis. The fact that Drome-CRF/DH-R1 is expressed in corazonin-expressing neurons, it is safe to assume that Drome-CRF/DH-R1 regulates processes centrally (e.g. corazonin secretion) whereas Drome-CRF/DH-R2 regulates peripheral physiological processes (e.g. diuresis).

7. Conclusion and future directions

Diuresis in insects is a process that is tightly regulated by various factors, one of which is the CT/DH. These factors work in concert to fine-tune the fluid secretion by MTs as well as the contractility of the insect's gut, which can also indirectly aid in stimulating fluid secretion (see Zandawala et al., 2012). They do so by reducing the unstirred layers around the MTs and by increasing the circulation of other diuretic hormones that may be present in the haemolymph. Like several other insect neuropeptides, CT/DHs are pleiotropic in nature. Hence in addition to their effect on peripheral tissues, they may also affect processes centrally including the regulation of corazonin transcript expression. Moreover, the presence of CT/DH in the CNS and gut of *D. melanogaster* and *O. fasciatus* suggests that this family of peptides might be a new class of brain-gut peptides. It is safe to conclude that CT/DHs are well conserved across insects, both structurally and functionally.

Acknowledgments

I would like to thank my advisor, Dr. Ian Orchard, for providing valuable input and support throughout the writing of this review. I am also thankful to Dr. Jean-Paul Paluzzi for his insightful comments on an earlier version of this manuscript. The unpublished work described here was supported by the Natural Sciences and Engineering Research Council of Canada.

References

Amodeo, P., Motta, A., Strazzullo, G., Castiglione Morelli, M.A., 1999. Conformational flexibility in calcitonin: the dynamic properties of human and salmon calcitonin in solution. J. Biomol. NMR 13, 161–174.

Andreotti, G., Mendez, B.L., Amodeo, P., Morelli, M.A., Nakamuta, H., Motta, A., 2006. Structural determinants of salmon calcitonin bioactivity: the role of the Leu-based amphipathic alpha-helix. J. Biol. Chem. 281, 24193–24203.

Chintapalli, V.R., Wang, J., Dow, J.A., 2007. Using FlyAtlas to identify better *Drosophila melanogaster* models of human disease. Nat. Genet. 39, 715–720.

Christie, A.E., 2008. Neuropeptide discovery in Ixodoidea: an *in silico* investigation using publicly accessible expressed sequence tags. Gen. Comp. Endocrinol. 157, 174–185.

Christie, A.E., Durkin, C.S., Hartline, N., Ohno, P., Lenz, P.H., 2010a. Bioinformatic analyses of the publicly accessible crustacean expressed sequence tags (ESTs) reveal numerous novel neuropeptide-encoding precursor proteins, including ones from members of several little studied taxa. Gen. Comp. Endocrinol. 167, 164–178.

Christie, A.E., Stevens, J.S., Bowers, M.R., Chapline, M.C., Jensen, D.A., Schegg, K.M., Goldwaser, J., Kwiatkowski, M.A., Pleasant Jr., T.K., Shoenfeld, L., Tempest, L.K., Williams, C.R., Wiwatpanit, T., Smith, C.M., Beale, K.M., Towle, D.W., Schooley, D.A., Dickinson, P.S., 2010b. Identification of a calcitonin-like diuretic hormone that functions as an intrinsic modulator of the American lobster, *Homarus americanus*, cardiac neuromuscular system. J. Exp. Biol. 213, 118–127.

Christie, A.E., Nolan, D.H., Garcia, Z.A., McCoole, M.D., Harmon, S.M., Congdon-Jones, B., Ohno, P., Hartline, N., Congdon, C.B., Baer, K.N., Lenz, P.H., 2011. Bioinformatic prediction of arthropod/nematode-like peptides in non-arthropod, non-nematode members of the Ecdysozoa. Gen. Comp. Endocrinol. 170, 480–486.

Coast, G.M., 2006. Chapter 24. Insect diuretic and antidiuretic hormones. In: Abba, J.K. (Ed.), Handbook of Biologically Active Peptides. Academic Press, Burlington, pp. 157–162.

Coast, G.M., Webster, S.G., Schegg, K.M., Tobe, S.S., Schooley, D.A., 2001. The *Drosophila melanogaster* homologue of an insect calcitonin-like diuretic peptide stimulates V-ATPase activity in fruit fly Malpighian tubules. J. Exp. Biol. 204, 1795–1804.

Coast, G.M., Garside, C.S., Webster, S.G., Schegg, K.M., Schooley, D.A., 2005. Mosquito natriuretic peptide identified as a calcitonin-like diuretic hormone in *Anopheles gambiae* (Giles). J. Exp. Biol. 208, 3281–3291.

Coast, G.M., Nachman, R.J., Lopez, J., 2011. The control of Malpighian tubule secretion in a predacious hemipteran insect, the spined soldier bug *Podisus maculiventris* (Heteroptera, Pentatomidae). Peptides 32, 493–499.

Donini, A., O'Donnell, M.J., Orchard, I., 2008. Differential actions of diuretic factors on the Malpighian tubules of *Rhodnius prolixus*. J. Exp. Biol. 211, 42–48.

El-Salhy, M., Falkmer, S., Kramer, K.J., Speirs, R.D., 1983. Immunohistochemical investigations of neuropeptides in the brain, corpora cardiaca, and corpora allata of an adult lepidopteran insect, *Manduca sexta* (L). Cell Tissue Res. 232, 295–317.

Evans, B.N., Rosenblatt, M.I., Mnayer, L.O., Oliver, K.R., Dickerson, I.M., 2000. CGRP-RCP, a novel protein required for signal transduction at calcitonin gene-related peptide and adrenomedullin receptors. J. Biol. Chem. 275, 31438–31443.

Furuya, K., Milchak, R.J., Schegg, K.M., Zhang, J., Tobe, S.S., Coast, G.M., Schooley, D.A., 2000. Cockroach diuretic hormones: characterization of a calcitonin-like peptide in insects. Proc. Natl. Acad. Sci. U.S.A. 97, 6469–6474.

Gard, A.L., Lenz, P.H., Shaw, J.R., Christie, A.E., 2009. Identification of putative peptide paracrines/hormones in the water flea *Daphnia pulex* (Crustacea; Branchiopoda; Cladocera) using transcriptomics and immunohistochemistry. Gen. Comp. Endocrinol. 160, 271–287.

Gruber, C.W., Muttenthaler, M., 2012. Discovery of defense- and neuropeptides in social ants by genome-mining. PLoS ONE 7, e32559.

Hansen, B.L., Hansen, G.N., Scharrer, B., 1982. Immunoreactive material resembling vertebrate neuropeptides in the corpus cardiacum and corpus allatum of the insect *Leucophaea maderae*. Cell Tissue Res. 225, 319–329.

Harbison, S.T., Chang, S., Kamdar, K.P., Mackay, T.F., 2005. Quantitative genomics of starvation stress resistance in *Drosophila*. Genome Biol. 6, R36.

Hatje, K., Keller, O., Hammesfahr, B., Pillmann, H., Waack, S., Kollmar, M., 2011. Cross-species protein sequence and gene structure prediction with fine-tuned Webscipio 2.0 and Scipio. BMC Res. Notes 4, 265.

Hauser, F., Cazzamali, G., Williamson, M., Blenau, W., Grimmelikhuijzen, C.J., 2006. A review of neurohormone GPCRs present in the fruitfly *Drosophila melanogaster* and the honey bee *Apis mellifera*. Prog. Neurobiol. 80, 1–19.

Hauser, F., Cazzamali, G., Williamson, M., Park, Y., Li, B., Tanaka, Y., Predel, R., Neupert, S., Schachtner, J., Verleyen, P., Grimmelikhuijzen, C.J., 2008. A genome-wide inventory of neurohormone GPCRs in the red flour beetle *Tribolium castaneum*. Front. Neuroendocrinol. 29, 142–165.

Hauser, F., Neupert, S., Williamson, M., Predel, R., Tanaka, Y., Grimmelikhuijzen, C.J., 2010. Genomics and peptidomics of neuropeptides and protein hormones present in the parasitic wasp *Nasonia vitripennis*. J. Proteome Res. 9, 5296–5310.

Hay, D.L., Conner, A.C., Howitt, S.G., Smith, D.M., Poyner, D.R., 2004. The pharmacology of adrenomedullin receptors and their relationship to CGRP receptors. J. Mol. Neurosci. 22, 105–113.

Hector, C.E., Bretz, C.A., Zhao, Y., Johnson, E.C., 2009. Functional differences between two CRF-related diuretic hormone receptors in *Drosophila*. J. Exp. Biol. 212, 3142–3147.

Hewes, R.S., Taghert, P.H., 2001. Neuropeptides and neuropeptide receptors in the *Drosophila melanogaster* genome. Genome Res. 11, 1126–1142.

Hill, C.A., Fox, A.N., Pitts, R.J., Kent, L.B., Tan, P.L., Chrystal, M.A., Cravchik, A., Collins, F.H., Robertson, H.M., Zwiebel, L.J., 2002. G protein-coupled receptors in *Anopheles gambiae*. Science 298, 176–178.

Holtzhausen, W.D., Nicolson, S.W., 2007. Beetle diuretic peptides: the response of mealworm (*Tenebrio molitor*) Malpighian tubules to synthetic peptides, and cross-reactivity studies with a dung beetle (*Onthophagus gazella*). J. Insect Physiol. 53, 361–369.

Hyun, S., Lee, Y., Hong, S.T., Bang, S., Paik, D., Kang, J., Shin, J., Lee, J., Jeon, K., Hwang, S., Bae, E., Kim, J., 2005. *Drosophila* GPCR Han is a receptor for the circadian clock neuropeptide PDF. Neuron 48, 267–278.

Johnson, E.C., Bohn, L.M., Taghert, P.H., 2004. *Drosophila* CG8422 encodes a functional diuretic hormone receptor. J. Exp. Biol. 207, 743–748.

Johnson, E.C., Shafer, O.T., Trigg, J.S., Park, J., Schooley, D.A., Dow, J.A., Taghert, P.H., 2005. A novel diuretic hormone receptor in *Drosophila*: evidence for conservation of CGRP signaling. J. Exp. Biol. 208, 1239–1246.

Kaskani, C., Poulos, C.P., Goldsworthy, G.J., 2012. The effects of linear and cyclic analogs of Locmi-DH, Dippu-DH(46) and Dippu-DH(31) on appetitive behavior in *Locusta migratoria*. Peptides 34, 258–261.

Kim, Y.J., Zitnan, D., Cho, K.H., Schooley, D.A., Mizoguchi, A., Adams, M.E., 2006. Central peptidergic ensembles associated with organization of an innate behavior. Proc. Natl. Acad. Sci. U.S.A. 103, 14211–14216.

LaJeunesse, D.R., Johnson, B., Presnell, J.S., Catignas, K.K., Zapotoczny, G., 2010. Peristalsis in the junction region of the *Drosophila* larval midgut is modulated by DH31 expressing enteroendocrine cells. BMC Physiol. 10, 14.

Lear, B.C., Merrill, C.E., Lin, J.M., Schroeder, A., Zhang, L., Allada, R., 2005. A G protein-coupled receptor, *groom-of-PDF*, is required for PDF neuron action in circadian behavior. Neuron 48, 221–227.

Li, B., Predel, R., Neupert, S., Hauser, F., Tanaka, Y., Cazzamali, G., Williamson, M., Arakane, Y., Verleyen, P., Schoofs, L., Schachtner, J., Grimmelikhuijzen, C.J., Park, Y., 2008. Genomics, transcriptomics, and peptidomics of neuropeptides and protein hormones in the red flour beetle *Tribolium castaneum*. Genome Res. 18, 113–122.

Li, Y., Hoxha, V., Lama, C., Dinh, B.H., Vo, C.N., Dauwalder, B., 2011. The hector G-protein coupled receptor is required in a subset of fruitless neurons for male courtship behavior. PLoS ONE 6, e28269.

Lovejoy, D.A., Al Chawaf, A., Cadinouche, M.Z., 2006. Teneurin C-terminal associated peptides: an enigmatic family of neuropeptides with structural similarity to the corticotropin-releasing factor and calcitonin families of peptides. Gen. Comp. Endocrinol. 148, 299–305.

Ma, W., Chabot, J.G., Powell, K.J., Jhamandas, K., Dickerson, I.M., Quirion, R., 2003. Localization and modulation of calcitonin gene-related peptide-receptor component protein-immunoreactive cells in the rat central and peripheral nervous systems. Neuroscience 120, 677–694.

Maupetit, J., Derreumaux, P., Tuffery, P., 2009. PEP-FOLD: an online resource for de novo peptide structure prediction. Nucleic Acids Res. 37, W498–W503.

McLatchie, L.M., Fraser, N.J., Main, M.J., Wise, A., Brown, J., Thompson, N., Solari, R., Lee, M.G., Foord, S.M., 1998. RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. Nature 393, 333–339.

Mertens, I., Vandingenen, A., Johnson, E.C., Shafer, O.T., Li, W., Trigg, J.S., De Loof, A., Schoofs, L., Taghert, P.H., 2005. PDF receptor signaling in *Drosophila* contributes to both circadian and geotactic behaviors. Neuron 48, 213–219.

Nicolson, S.W., 1976. The hormonal control of diuresis in the cabbage white butterfly *Pieris brassicae*. J. Exp. Biol. 65, 565–575.

Ons, S., Sterkel, M., Diambra, L., Urlaub, H., Rivera-Pomar, R., 2011. Neuropeptide precursor gene discovery in the Chagas disease vector *Rhodnius prolixus*. Insect Mol. Biol. 20, 29–44.

Orchard, I., 2009. Peptides and serotonin control feeding-related events in *Rhodnius prolixus*. Front. Biosci. 1, 250–262.

Orchard, I., Lange, A.B., Cook, H., Ramirez, J.M., 1989. A subpopulation of dorsal unpaired median neurons in the blood-feeding insect *Rhodnius prolixus* displays serotonin-like immunoreactivity. J. Comp. Neurol. 289, 118–128.

Park, D., Veenstra, J.A., Park, J.H., Taghert, P.H., 2008. Mapping peptidergic cells in Drosophila: where DIMM fits in. PLoS ONE 3, e1896.

Petzel, D.H., Hagedorn, H.H., Beyenbach, K.W., 1985. Preliminary isolation of mosquito natriuretic factor. Am. J. Physiol. 249, R379–R386.

Petzel, D.H., Hagedorn, H.H., Beyenbach, K.W., 1986. Peptide nature of two mosquito natriuretic factors. Am. J. Physiol. 250, R328–R332.

Petzel, D.H., Berg, M.M., Beyenbach, K.W., 1987. Hormone-controlled cAMP-mediated fluid secretion in yellow-fever mosquito. Am. J. Physiol. 253, R701–R711.

Price, D.R., Du, J., Dinsmore, A., Gatehouse, J.A., 2004. Molecular cloning and immunolocalization of a diuretic hormone receptor in rice brown planthopper (*Nilaparvata lugens*). Insect Mol. Biol. 13, 469–480.

Reagan, J.D., 1994. Expression cloning of an insect diuretic hormone receptor. A member of the calcitonin/secretin receptor family. J. Biol. Chem. 269, 9–12.

Reagan, J.D., 1996. Molecular cloning and function expression of a diuretic hormone receptor from the house cricket, *Acheta domesticus*. Insect Biochem. Mol. Biol. 26, 1–6.

Roller, L., Yamanaka, N., Watanabe, K., Daubnerova, I., Zitnan, D., Kataoka, H., Tanaka, Y., 2008. The unique evolution of neuropeptide genes in the silkworm *Bombyx mori*. Insect Biochem. Mol. Biol. 38, 1147–1157.

Schooley, D.A., Horodyski, F.M., Coast, G.M., 2005. 3.10 – Hormones controlling homeostasis in insects. In: Lawrence, I.G., Kostas, I., Sarjeet, S.G. (Eds.), Comprehensive Molecular Insect Science. Elsevier, Amsterdam, pp. 493–550.

Schooley, D.A., Horodyski, F.M., Coast, G.M., 2012. 9-Hormones controlling homeostasis in insects. In: Lawrence, I.G. (Ed.), Insect Endocrinology. Academic Press, San Diego, pp. 366–429.

Shafer, O.T., Kim, D.J., Dunbar-Yaffe, R., Nikolaev, V.O., Lohse, M.J., Taghert, P.H., 2008. Widespread receptivity to neuropeptide PDF throughout the neuronal circadian clock network of *Drosophila* revealed by real-time cyclic AMP imaging. Neuron 58, 223–237.

Soderberg, J.A., Birse, R.T., Nassel, D.R., 2011. Insulin production and signaling in renal tubules of *Drosophila* is under control of tachykinin-related peptide and regulates stress resistance. PLoS ONE 6, e19866.

Stroop, S.D., Nakamuta, H., Kuestner, R.E., Moore, E.E., Epand, R.M., 1996. Determinants for calcitonin analog interaction with the calcitonin receptor N-terminus and transmembrane-loop regions. Endocrinology 137, 4752–4756.

Te Brugge, V.A., Orchard, I., 2008. Distribution and activity of a Dippu DH_{31}-like peptide in the large milkweed bug *Oncopeltus fasciatus*. Peptides 29, 206–213.

Te Brugge, V.A., Schooley, D.A., Orchard, I., 2002. The biological activity of diuretic factors in *Rhodnius prolixus*. Peptides 23, 671–681.

Te Brugge, V.A., Lombardi, V.C., Schooley, D.A., Orchard, I., 2005. Presence and activity of a Dippu-DH_{31}-like peptide in the blood-feeding bug, *Rhodnius prolixus*. Peptides 26, 29–42.

Te Brugge, V.A., Schooley, D.A., Orchard, I., 2008. Amino acid sequence and biological activity of a calcitonin-like diuretic hormone (DH31) from *Rhodnius prolixus*. J. Exp. Biol. 211, 382–390.

Te Brugge, V., Ianowski, J.P., Orchard, I., 2009. Biological activity of diuretic factors on the anterior midgut of the blood-feeding bug, *Rhodnius prolixus*. Gen. Comp. Endocrinol. 162, 105–112.

Te Brugge, V., Paluzzi, J.P., Schooley, D.A., Orchard, I., 2011. Identification of the elusive peptidergic diuretic hormone in the blood-feeding bug *Rhodnius prolixus*: a CRF-related peptide. J. Exp. Biol. 214, 371–381.

Tobe, S.S., Zhang, J.R., Schooley, D.A., Coast, G.M., 2005. A study of signal transduction for the two diuretic peptides of *Diploptera punctata*. Peptides 26, 89–98.

Veenstra, J.A., 2009. Peptidergic paracrine and endocrine cells in the midgut of the fruit fly maggot. Cell Tissue Res. 336, 309–323.

Veenstra, J.A., Romberg-Privee, H.M., Schooneveld, H., Polak, J.M., 1985. Immunocytochemical localization of peptidergic neurons and neurosecretory cells in the neuro-endocrine system of the Colorado potato beetle with antisera to vertebrate regulatory peptides. Histochemistry 82, 9–18.

Zandawala, M., Paluzzi, J.P., Orchard, I., 2011. Isolation and characterization of the cDNA encoding DH(31) in the kissing bug, *Rhodnius prolixus*. Mol. Cell. Endocrinol. 331, 79–88.

Zandawala, M., Lytvyn, Y., Taiakina, D., Orchard, I., 2012. Cloning of the cDNA, localization, and physiological effects of FGLamide-related allatostatins in the blood-gorging bug, *Rhodnius prolixus*. Insect Biochem. Mol. Biol. 42, 10–21.

Zhao, Y., Bretz, C.A., Hawksworth, S.A., Hirsh, J., Johnson, E.C., 2010. Corazonin neurons function in sexually dimorphic circuitry that shape behavioral responses to stress in Drosophila. PLoS ONE 5, e9141.