Role of peptide hormones in insect gut physiology

Rania Abou El Asrar [a,b], Dorien Cools [a,b] and Jozef Vanden Broeck [a]

[a] Department of Biology, Research group of Molecular Developmental Physiology and Signal Transduction, Naamsestraat 59 box 2465, 3000 Leuven, Belgium

[b] equal contribution authorship
Rania Abou El Asrar (rania.abuelasrar@kuleuven.be)
Dorien Cools (dorien.cools@kuleuven.be)

Corresponding author:
Prof. Dr. Jozef Vanden Broeck (jozef.vandenbroeck@kuleuven.be)

Highlights

Gut physiology is regulated by a complex interplay between neuropeptides.

The gut is innervated by the central and stomatogastric nervous systems.

Biostable neuropeptide mimetics can be a promising approach for pest management

Abstract

Nutrient uptake and digestion are essential for optimal growth and development. In insects, these processes are regulated by the gut-brain axis, which is a neurohumoral communication system for maintaining gut homeostasis. The insect gut is a complex organ consisting of three distinct structures, denominated foregut, midgut and hindgut, each with their specific specializations. These specializations are tightly regulated by the interplay of several neuropeptides: a versatile group of signalling molecules involved in a multitude of processes including gut physiology. Neuropeptides take part in the regulation of gut processes ranging from digestive enzyme release to muscle activity and satiety. Some neuropeptide mimetics are a promising strategy for ecological pest management. This review focuses on a selection of neuropeptides that are well-known for their role in gut physiology, and neuropeptides for which their mode of action is yet to be unravelled.

Introduction

Physiological, developmental and behavioural events in insects are coordinated by an interplay of multiple signalling molecules. An important class of these are small proteins (generally about 5-80 amino acid residues) known as neuropeptides, which are released from neurons, neurosecretory cells (NSC) or (entero)endocrine cells. Thus, neuropeptides can behave as circulating (endocrine) or local hormones (paracrine), as neuromodulators, or as co-transmitters. Most neuropeptides are pleiotropic, since they often display multiple biological activities and generate regulatory effects in a variety of processes. Neuropeptides are derived from larger, inactive precursors that undergo post-translational cleavage and further modifications (carboxy terminus amidation, sulfation of tyrosyl groups, disulphide bridge formation, etc.) during transport from the endoplasmic reticulum-Golgi network to their release sites enclosed in secretory granules [1, 2]. Their action on target sites usually involves the activation of a class of membrane receptors known as G-protein coupled receptors (GPCRs). These initiate a cascade of intracellular events involving phospholipase C or adenylyl cyclase pathways, thus regulating the levels of second messengers, such as cyclic adenosine monophosphate (cAMP), diacylglycerol and inositol 1,4,5-trisphosphate [3].

Feeding behaviour is associated with three main physiological processes: (1) the perception and recognition of food, (2) endogenous nutritional and metabolic state, and (3) mechanical

and digestive activities within the alimentary canal (gut). The latter include food intake, gut peristalsis, digestive enzyme release, nutrient absorption and excretion. In this review, a selection of some of the important peptides **(Figure 1)** that are involved in regulating gut activities is discussed.

The enteric nervous system

The insect gut is a complex organ differentiated into three distinct structures, each with their corresponding specializations. The foregut consists of the pharynx and esophagus that open into the crop, and the proventriculus. Food stored in the crop undergoes initial digestion by salivary gland enzymes and enzymes regurgitated from the midgut. The midgut's main function is secretion of digestive enzymes and nutrient absorption. The hindgut consists of the pylorus, the ileum and the rectum; its general function is osmotic regulation of internal fluids, excretion and faeces formation [4]. The Enteric Nervous System consists (ENS) of interconnected ganglia and nerve plexuses that innervate the alimentary tract and regulate gut motility [5] **(Figure 2)**.

Neuropeptides regulating gut physiology

Allatoregulatory peptides

Insects have three structurally unrelated allatostatins (ASTs) (A-, B- and C- type) and two allatotropins (ATs) (Table 1). The first allatoregulatory peptides were isolated based on their effect on juvenile hormone synthesis in the corpora allata [6-8]: allatostatin is inhibitory and allatotropin is stimulatory. Since then, a plethora of functions have been attributed to these peptides including heart rhythmicity [9], circulatory system regulation [10] and a role in sleep [11] and photic entrainment of the circadian clock [12] (reviewed by Verlinden et al.[13]). The role of these peptides in feeding is supported by several studies. In the cockroach *Blatella germanica*, expression of AST-A in the midgut declined around the middle of the gonadotrophic cycle at the same period when food consumption increases to maximum. [14]. Starvation of *Spodoptera littoralis* larvae decreased the number of AST-A expressing enteroendocrine cells in the midgut, which rose again after refeeding [15]. Immunolocalization studies detected the presence of allatoregulatory peptides in the stomatogastric nervous system (SNS) (Figure 2) of multiple insect species [16-19]. Immunoreactivity for AST was also present in the nerves extending to the anterior midgut, ileum and rectum of *Locusta migratoria* [19]. In Lepidopterans [20-22], it was demonstrated that AST and AT are co-localized in the frontal ganglion, recurrent nerve and branching extending to the crop, stomodeal valve and some parts of the midgut. In *Lacanobia oleracea*, dual localization of AST-A and AST-C was observed.

Since their discovery, these peptides have been shown to control movement of food by excitatory and inhibitory mechanisms on the gut. ASTs inhibit foregut [23], midgut [24], and hindgut [14, 25] peristalsis, which can possibly suppress feeding, as was demonstrated by *in vivo* studies. When administered into the moth *L. oleracea* and the aphid *Myzus persicae*,

Manduca sexta-AST (Manse-AST, Table 1) significantly reduced feeding, with a detrimental effect on weight gain and survival [26, 27]. A similar effect was seen with *B. germanica* AST-A [14]. In *Drosophila melanogaster*, activation of AST-A neurons inhibited starvation-induced feeding [28]. Interestingly, the opposite effect on feeding was not observed in Ast-A null mutant flies [11]. Controlled thermogeneic activation of AST expression indicated that the neurons in the posterior lateral protocerebrum and the enteroendocrine cells are sufficient to suppress feeding [11]. The effect of ASTs on feeding has led investigators to develop neuropeptide analogues for use as pest control agents [29, 30]

The function of ATs usually is opposite of ASTs. They are generally myostimulatory peptides [17]. In hematophagous insects, such as *Triatoma infestans*, an AT-like peptide released from the Malpighian tubules stimulates hindgut contractions to facilitate the mixing of urine and faeces during post-prandial diuresis [31, 32]. In agreement with the role of gut motility on feeding regulation, injection of Manse AT (Table 1) into *L. oleracea* or *S. littoralis* did not affect feeding or development [33]. Surprisingly, the reverse effect was observed in the Lepidopteran *Bombyx mori* [34]; AT inhibited spontaneous contractions in the pharynx, esophagus and ileum in both fed and starved larvae and prolonged latency to feeding. *In vivo* studies with *Spodoptera frugiperda* produced similar results; Manse-AT was associated with weight decrease and higher mortality whereas Manse-AST had no considerable effect on larval development [35]. The effect of allatoregulatory peptides on gut motility in this Lepidopteran insect was demonstrated in a later study [36]. *S. frugiperda* AST (AST-A) inhibited ileum contractions while Manse-AT stimulated them.

Allatoregulatory peptides may also control the release of digestive enzymes in response to food. AST-A increased the levels of amylase and protease activities in the midgut of *S. littoralis* [15], while in *S. frugiperda*, AST-A reduced the midgut levels of amylase and trypsin [36]. In the latter insect species, Manse-AT significantly increased the levels of amylase and trypsin.

Kinins

Kinins are pleiotropic factors acting at multiple levels of food intake and processing, diuresis and release of digestive enzymes. They are characterized by the FxxWG C-terminal sequence (Table 1). Kinins were first isolated from the Madeira cockroach *Leucophaea maderae*, as myotropic peptides acting on the hindgut [37]. Later, they were identified in other insects, either expressing a single kinin peptide or multiple kinin isoforms, encoded by a single precursor [38-40]. Kinins are expressed in the CNS and throughout the alimentary canal where they stimulate muscle contractions. Immunohistochemical and radioimmunoassay (RIA) techniques demonstrated that, in the blood-feeding bug *Rhodnius prolixus*, kinins are co-expressed with corticotropin-releasing factor-related diuretic hormones (CRF/DH) in the posterior lateral neurosecretory cells of the mesothoracic ganglionic mass and in neurohemal areas on abdominal nerves, suggesting the possibility of co-release of the peptides into the haemolymph [41].

In search of eco-friendly insecticides for pest control, kinins have been considered possible candidates. Kinin analogues cause detrimental effects when delivered to various insects; inhibition of larval weight gain was observed for the corn earworm, *Helicoverpa zea* [42, 43],

and higher diuretic activity was measured in the housefly *Musca domestica* [42], both resulting in high mortality rates. More recently, tests with analogues in the blood-gorging *R. prolixus* demonstrated that the presence of biostable kinin analogues in the haemolymph resulted in smaller blood meals and slower diuresis rate, leading to reduction in urine excretion and an inability to moult [44, 45]. This anti-feedant activity together with a reduction in honeydew production was also observed for the pea aphid *Acyrthosiphon pisum* [46].

CRF-related diuretic hormone

Corticotropin-releasing factor (CRF)-related diuretic hormones (DH) (example of structure is presented in Table 1) are named after their diuretic function (as reviewed by Gäde [47]). A correlation between CRF/DH release and feeding behaviour was first demonstrated in *M. sexta*, where high doses of CRF/DH-injections in larvae resulted in weight loss and decreased food consumption [48]. Likewise, it was shown that CRF/DH titers in the hemolymph of starved *L. migratoria* gradually rose after feeding, initiating post-feeding diuresis [49, 50]. Stretch receptors in the foregut may detect the presence of food, inducing a signal to release diuretic hormones. *L. migratoria* nymphs showed increased latency to the first meal upon injection of CRF/DH analogues as well as a decrease in meal duration suggesting that CRF-related diuretic hormones play a role in satiety [51]. These findings were later confirmed in *Schistocerca gregaria* by knockdown of CRF/DH through RNA interference, which resulted in increased food uptake. A rescue of this RNAi-induced knockdown by administering CRF/DH resulted in reduced food consumption [52]. Recently, immunohistochemical assays in the CNS of the blood-gorging insect *R. prolixus* demonstrated diminished CRF-like staining in NSC following feeding and partial restocking in the period after the meal [53].These data were supported by temporal qPCR analysis of the CNS. Elevated haemolymph CRF/DH titres prior to feeding resulted in lower blood meal consumption, while seemingly having no effect on the rate of postprandial diuresis [53]. *Rhopr*-CRF/DH-receptor transcripts are expressed in the CNS and throughout the gut, mainly the foregut, suggesting that CRF/DH maybe involved in muscle contraction in the gut [54]. Myotropic activity was already proposed for the cricket *Acheta domesticus*, where CRF/DH increases the frequency and amplitude of contractions of the foregut [55]. Muscle contractions in the hindgut might be related to expulsion of urine produced by the Malpighian tubules [56].

Proctolin

Proctolin is a potent myotropic with activity on visceral, skeletal and cardiac muscles. It is involved in a variety of processes including egg-laying [57] sexual behaviour [58], hemolymph circulation [59]and heart rhythmicity [9]. It was originally isolated and sequenced (Table 1) based on its myotropic activity on the hindgut of *Periplanta americana* [60, 61]. Proctolin might be unique to invertebrates [62-64], with no homologs in vertebrates. Genomic search for proctolin precursor showed that it is absent in many insect species [65, 66] and there is no compelling evidence for presence of proctolin signalling in Lepidopterans [67-70]. An analogue of proctolin (Table 1) was also isolated from the brain of the Colorado potato beetle [71, 72].

In *L. migratoria*, proctolin is present in all ganglia of the SNS, including the frontal ganglion, which supports its role in regulation of feeding and gut motility [73]. Proctolin immunoreactivity also reveals its presence in the thoracico-abdominal ganglion of the adult blow fly *Calliphora erythrocephala* [74] and the 8th abdominal ganglion of *L. migratoria* [73], from which neurons extend to innervate the hindgut musculature. No proctolin immunoreactivity was observed in enteroendocrine (EE) cells of *L. migratoria*.

Much of the research on proctolin has focused on its effect on muscle contraction. In addition to the cockroach hindgut [61], proctolin stimulated contraction in the foregut of *S. gregaria* [75, 76] and *L. migratoria*, the midgut of *L. migratoria* [77] and *R. prolixus* [78], the pyloric sphincter in *D. melanogaster* [24], and the hindgut of *Leucophaea maderae* [79] and *Nicrophorus vespilloides* [55]. Therefore, it is regarded as a major neuromuscular modulator in the gut of insects. Injection of proctolin into *B. germanica* adults was not associated with food intake inhibition [80], correlating with its effect on foregut motility.

RYamides

RYamides are a group of neuropeptides very recently discovered and characterized in insects [65]. They are characterized by a C-terminal FFxxxRY-amide C-terminal sequence (Table 1). Their function, therefore, has yet to be elucidated, but expression of the RYamide receptors in the hindgut of *D. melanogaster* adult flies suggests a role of insect RYamides in digestion or water reabsorption [81]. In *B. mori*, RYamides are expressed in several neurons in the brain and terminal abdominal ganglion as well as in the EE cells of the anterior midgut of larvae, pupae and adults [82]. This expression pattern reinforces the previous suggestion of RYamides playing a role in the regulation of feeding and digestion. RYamide orthologs are found in most other insects with sequenced genomes, except for four sequenced ant genomes [83]. The ortholog for *D. melanogaster*, however, does not contain an exon encoding the signal peptide, suggesting that the gene is degenerating in this species [84]. RYamides have been described in other invertebrates, where they exert similar functions in feeding behaviour [85, 86].

Trissin

Another recently discovered peptide is trissin, first identified and characterized (Table 1) as a ligand of the *D. melanogaster* GPCR CG34381 [87]. Trissin-related neuropeptides and receptors of other insects have been predicted through sequence comparison with sequenced insect genomes and transcriptomes, among these are the orders of Homoptera, Neuroptera, Lepidoptera, Hymenoptera and Diptera [87, 88]. FlyAtlas [89]shows that trissin and its receptor are predominantly expressed in the brain and thoracico-abdominal ganglion in *D. melanog*aster. Through in situ hybridization, trissin expression was examined in *B. mori*, concluding that this neuropeptide's expression is restricted to only two pairs of small protocerebral interneurons and four to five large neurons in the frontal ganglion, which is a part of the SNS, innervating the alimentary canal [88]. In these neurons, trissin is co-expressed with several excitatory and inhibitory factors of gut musculature, such as AT, AST-A and myoinhibitory peptides [88].

Conclusions

Food intake stimulates the release of multiple neuropeptides from the central- or stomatogastric nervous systems and some are also released from the enteroendocrine cells in the gut. Our understanding of the neuropeptide regulation of feeding and digestion is still poor and fragmentary. Why have insects evolved to depend on several neuropeptides that perform similar functions? Gut motility is regulated by myostimulants (proctolin, allatotropin and kinins) and myoinhibitants (allatostatin, myosupressin and myoinhibitory peptides). It is also not well understood how the differential release and action of peptides, in some cases localized within the same neurons, is regulated.

Another interesting question concerns the interchangeable nature of some peptides. Insects have three allatostatins families and in many cases more than one family is present in the same species. Other peptides seem to be absent in some species (RYamides in the ant *Acromyrmex echinatior*) or in entire insect orders (proctolin in Lepidoptera).

The elucidation of the role of neuropeptides is further complicated by the observation of the species-dependent effects and the action of some peptides in a multitude of processes in gut physiology. Omics techniques and recent advances in technology are driving our understanding of these complex processes, while also aiding in the characterization of new insect neuropeptides that add to the complexity of gut physiology.

One fact that is clear is that a multitude of questions still need to be answered before we can begin to understand the full complexity of the intestinal processes in insects. However, since these neuropeptides are clearly one of the key factors in gut physiology, targeting their GPCRs to disrupt specific function(s) would be an interesting strategy for development of biorational pesticides. An interesting example are biostable kinin analogues, which have already been proposed as candidates for pest control.

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Declaration of interests

The authors declare no conflict of interest.

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Table 1: The structure of neuropeptides with the insect species from which they were isolated. The consensus sequence is indicated in bold.

Peptide group	Sequence	Insect species	References
Allatostatin-A	SPSGMQRL**Y**G**F**G**L**-amide	*Periplaneta americana*	[90]
Allatostatin-B	G**W**QDLNGG**W**-amide	*Gryllus bimaculatus*	[91]
Allatostatin-C	pEVRFRQCYFNPISCF	*Manduca sexta*	[92]
Allatotropin	GFKNVEMMTARGF-amide	*Manduca sexta, Spodoptera frugiperda*	[6], [93]
Allatotropin-2	RVRGNPISCF	*Spodoptera frugiperda*	[94]
Kinins	RPS**F**NS**WG**-amide	*Periplaneta americana*	[38]
CRF/DH	RMPSLSIDLPMSVLRQKLSLEKERKVHALRAAA NRNFLNDI-amide	*Manduca sexta*	[95]
Proctolin	R**YLPT**	*Periplaneta americana*	[60]
Ala^{1}-proctolin	A**YLPT**	*Leptinotarsa decemlineata*	[72]
RYamide	SEDRSSGNSLKESS**FF**SPG**RY**-amide	*Nasonia vitripennis*	[65]
Trissin	IK**C**DT**CG**K**EC**ASA**C**GTKH**FR**T**CC**F**N**YL	*Drosophila melanogaster*	[87]

Figure 1: A generalized schematic overview of the insect gastrointestinal tract, with the neuropeptides acting on each gut section.

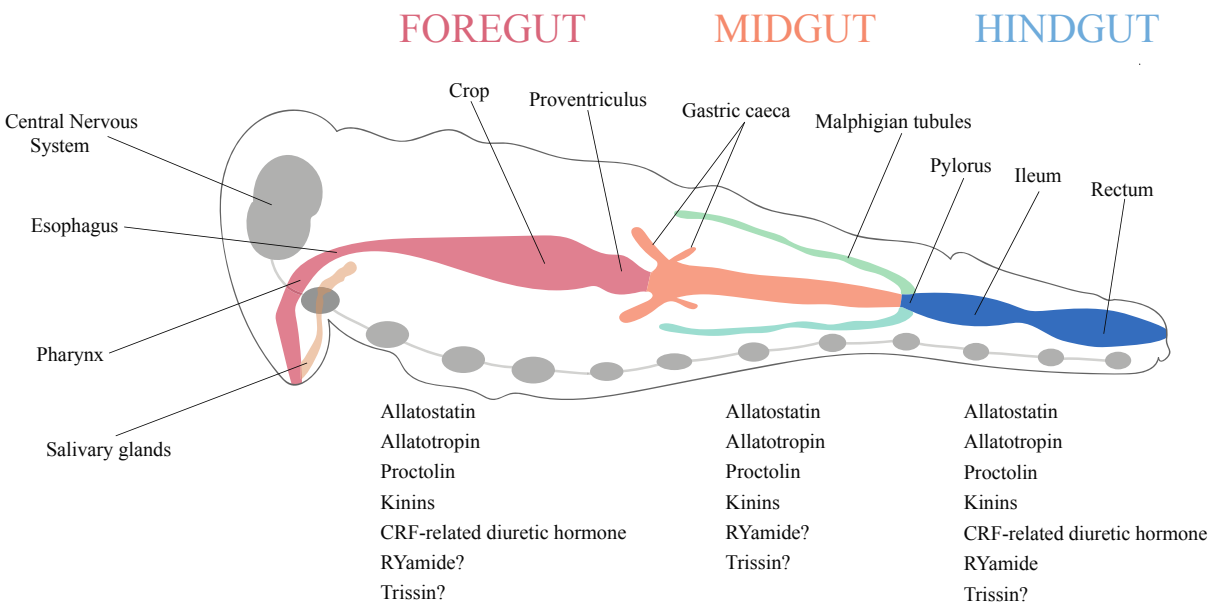
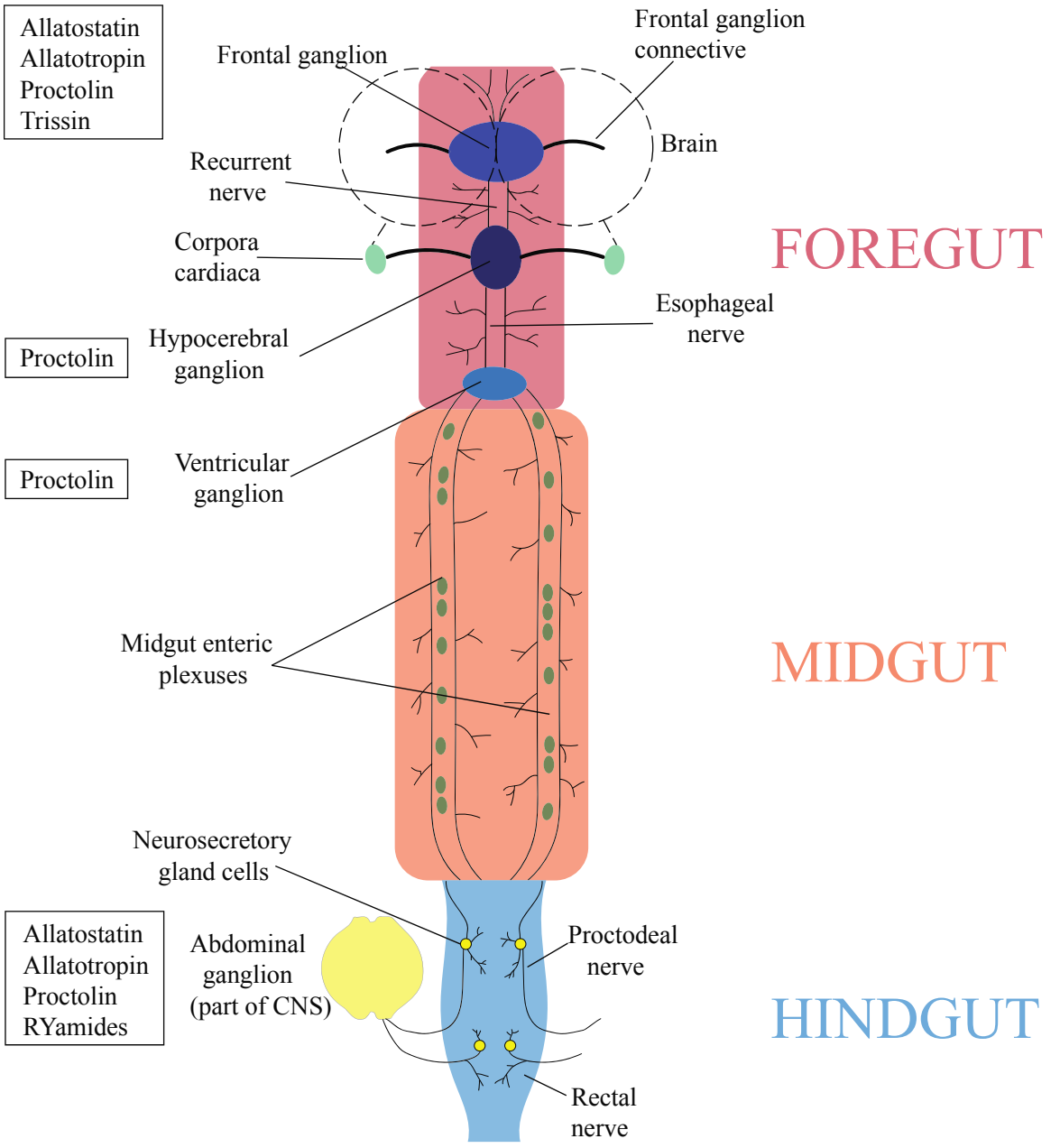


Figure 2: A generalized schematic overview of the insect enteric nervous system (ENS) and the site of production (in cases where this is known) of each neuropeptide within the ENS. The frontal ganglion, hypocerebral ganglion and ventricular ganglion are components of the SNS. The abdominal ganglion lies in the ventral nerve cord and is part of the central nervous system (CNS). The frontal ganglion connects to the brain by the frontal ganglion connective, while the recurrent nerve links it to the hypocerebral ganglion. The hypocerebral ganglion is in turn connected to the corpora cardiaca and the ventricular ganglion via the esophageal nerve. These ganglia also give rise to diffuse nerve plexuses that innervate the foregut musculature. From the ventricular ganglion, a branching nerve plexus (the midgut enteric plexus) extends along the superficial musculature of the midgut. The hindgut is innervated by branches of the proctodeal and rectal nerves that extend from the terminal abdominal ganglion; branches of the proctodeal nerve also extend onto the posterior midgut.



**Duan Sahbaz, B., et al., Ligand binding pocket of a novel Allatostatin receptor type C of stick insect, Carausius morosus. Sci Rep, 2017. 7: p. 41266.

This paper reports on the structural study of allatostatins C with its receptor in *Carausius morosus* to identify the allatostatin C binding pocket. The study uses a combination of molecular docking onto the 3D structure of the receptor and *in silico* analysis to predict the binding motif in the allatostatin receptor as well as atomic force microscopy and site-directed mutagenesis to measure the physical interaction between allatostatin and different mutated versions of the receptor. This can have important implications for design of neuropeptide analogues for pest control.

*Huang, S.S., et al., Structure-Based Discovery of Nonpeptide Allatostatin Analogues for Pest Control. J Agric Food Chem, 2018. 66(14): p. 3644-3650.

This paper reports on the use of molecular docking of FGL-amide allatostatin (AST-A) in the *Diploptera punctata* allatostatin receptor and structure-activity relationship to design non-peptide allatostatin analogues, of which two had a significant larvicidal effect, when administered orally. These compounds can be considered as potential insecticidal agents.

** Chen, J., et al., Allatostatin A Signalling in Drosophila Regulates Feeding and Sleep and Is Modulated by PDF. PLoS Genet, 2016. 12(9): p. e1006346.

Using transgenic flies with a temperature controlled thermogenic activator for allatostatin-A expression, the group of Wegener demonstrate the effect of AST-A neuron/cell expression on feeding and the subset of neurons/cells essential for suppression of feeding.

*Mollayeva, S., I. Orchard, and A.B. Lange, The involvement of Rhopr-CRF/DH in feeding and reproduction in the blood-gorging insect Rhodnius prolixus. Gen Comp Endocrinol, 2018. 258: p. 79-90.

This paper reported on CRF/DH in *Rhodnius prolixus*, a blood gorging insect and vector for human Chagas disease. The study highlights targets to alter both feeding, diuresis and reproduction for this disease vector.

** Holtof M, Lenaerts C, Cullen D, Vanden Broeck J: Extracellular nutrient digestion and absorption in the insect gut. Cell Tissue Res 2019, 377:397–414

This extensive review discusses the anatomy of the insect gut with a focus on the insect midgut and how macronutrients (proteins, carbohydrates and lipids) are digested and absorbed in the highly specialized compartments of the insect gut.

* Veenstra JA, Khammassi H. 2017. Rudimentary expression of RYamide in Drosophila melanogaster relative to other Drosophila species points to a functional decline of this neuropeptide gene. Insect Biochem Mol Biol 83:68-79

This paper reported on the *D. melanogaster* RYamide gene. They suggest that the RYamide *Drome*-gene is evolving into a pseudogene, since an exon encoding a signal peptide is absent, in contrast to other *Drosphila* and insect species. When expression of the neuropeptide was studied in several *Drosophila* species using specific antisera, it was found that the Drome-RYamide is only expressed in two neurons, in contrast to other *Drosophila* species, where it is expressed in numerous neurons and enteroendocrine cells.