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Neuropeptides associated with the regulation of feeding in insects

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ABSTRACT

The stomatogastric nervous system plays a pivotal role in feeding behaviour. Central to this system is the frontal ganglion, which is responsible for foregut motor activity, and hence the passage of food through the gut. Many insect peptides, which exhibit myoactivity on the visceral muscles of the gut *in vitro*, have been detected in the stomatogastric nervous system by immunochemical or mass spectrometric techniques. This localisation of myoactive peptides, particularly in the frontal ganglion, implies roles for these peptides in the neural control and modulation of feeding in insects. Insect sulfakinins, tachykinins, allatotropin and proctolin have all been shown to stimulate the foregut muscles, whereas myosuppressins, myoinhibitory peptides and allatostatins all inhibited spontaneous contractions of the foregut in a variety of insects. Some of these peptides, when injected, inhibited feeding *in vivo*. Both the A-type and B-type allatostatins suppressed feeding activity when injected into the cockroach, *Blattella germanica* and the *Manduca sexta* C-type allatostatin and allatotropin inhibited feeding when injected into the larvae of two noctuid moths, *Lacanobia oleracea* and *Spodoptera frugiperda*, respectively. Injection of sulfakinins into the fly *Phormia regina*, the locust *Schistocera gregaria* and the cockroach *B. germanica* also suppressed feeding, whereas silencing the sulfakinin gene through the injection of double stranded RNA resulted in an increase in food consumption in the cricket *Gryllus bimaculatus*.

The regulation of feeding in insects is clearly very complex, and involves the interaction of a number of mechanisms, one of which is the release, either centrally or locally, of neuropeptides. However, the role of neuropeptides, their mechanisms of action, interactions with each other, and their release are still poorly understood. It is also unclear why insects possess such a number of different peptides, some with multiples copies or homologues, which stimulate or inhibit gut motility, and how their release, sometimes from the same neurone, is regulated. These neuropeptides may also act at sites other than visceral muscles, such as centrally through the brain or on gut stretch receptors.

1. Introduction

Initiation of feeding in insects, as with most animals, involves a complex combination of physical, chemical and nutritional factors. The insect mouthparts are regulated by the suboesophageal ganglion (SOG; Griss et al., 1991). The SOG is a composite of fused ganglion (mandibular, maxillary and labial) and is situated ventrally in the head capsule just below the brain and oesophagus. It is linked to the brain via a pair of circumoesophageal connectives and innervates the mandibles, maxillae, and labium, the hypopharynx, salivary glands and neck muscles (Gillott, 1980). Once feeding has been initiated in insects and food ingested, the alimentary canal and its associated glands triturate, lubricate, store, digest and absorb the food material and excrete and expel unwanted remains and compounds. The alimentary canal is basically made up of specialised regions, which may vary in their structures according to the feeding habits and life stage of an insect. The basic structure consists of the foregut, midgut and hindgut and associated glands (e.g. salivary glands). The foregut is generally differentiated into the pharynx, oesophagus, crop (for food storage) and the proventriculus (Gillott, 1980). The proventriculus serves as a valve to control food passage into the midgut, as a filter to separate solids and liquids, or as a grinder to break up solid material, depending on the insect and its food source. The midgut is predominantly involved with digestion (secretes enzymes into the gut lumen) and absorption of digested material, whilst the hindgut and associated Malpighian tubules comprise the excretory system, which ultimately determine haemolymph volume and composition by selective secretion and reabsorption of fluid and ions and metabolites. The functions of the salivary glands, the ducts of which enter the buccal cavity, vary in different insects (e.g. silk and venom glands), but typically produce a watery, enzyme rich fluid to lubricate the food and initiate digestion (Gillott, 1980).

For digestion and absorption to occur efficiently, food is moved along the alimentary by peristalsis. These contractions are myogenic, occurring within the muscles themselves rather than as a result of nervous stimulation. Myogenic centres have been located in

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the oesophagus, crop and proventriculus. The rate of food movement through the gut is not uniform, but varies according to the physiological state of the insect or the dietary components of the food, and is regulated by the proventricular and pyloric valves in the foregut and hindgut, respectively. Simpson and Bernays (1983) suggest that the regulation of feeding in insects is similar to that in mammals. Positive inputs from food and non-food stimuli to the central nervous system (chiefly the brain) are modified by deterrent stimuli and by feedbacks from peripheral systems such as gut stretch receptors, hormones and biogenic amines, and blood composition (Simpson and Bernays, 1983; Bowden and Dethier, 1986; Dacks et al., 2003). This review will concentrate on the neuropeptides associated with the stomatogastric nervous system (SNS) and their roles in the control of gut motility.

Food uptake and transport in the visceral organs (foregut and midgut) are regulated by the SNS that consists of a number of interconnected ganglia, which have links to the central nervous system (Penzlin, 1985; Hartenstein, 1997; Schoofs and Spieß, 2007). The precise structure of the SNS varies in different insects studied, but in general consists of the following features. Central to this system is the frontal ganglion (FG) that lies on the dorsal surface of the oesophagus, anterior to the brain to which it is linked via a pair of nerves, the frontal connectives. Nerves from the FG innervate the buccal region of the stomodaeum via the frontal nerve and a large recurrent nerve connects the FG to the hypocerebral ganglion (HCG), axons from which connect to the corpora cardiaca. The HCG is connected to a proventricular ganglion (PVG) by the oesophageal nerve (Fig. 1; Hartenstein, 1997). In cockroaches (*Periplaneta americana*), an ingluvial ganglion (IG) is also present, situated between the HCG and the PVG (see Penzlin, 1985). The FG and HCG are fused in *Manduca sexta*. From the HCG the oesophageal nerve is studded with neurones, and when this nerve reaches the proventriculus it bifurcates to form the enteric plexus (Hartenstein 1997).

As early as 1863 functional studies had implicated the SNS in the control of feeding and gut motility (Faivre, 1863). It is now well established that the SNS is generally involved with the regulation of foregut motor activity associated with feeding, with the FG responsible for generating contractions (peristaltic waves) of the foregut (Schoofs and Spieß, 2007). A number of studies demonstrated that severing the link between the frontal ganglion and the gut, either by removal of the ganglion itself, or by severing the recurrent nerve, results in the accumulation of food in the foregut. This has been demonstrated in *M. sexta*, where removal of the FG from larvae resulted in slower growth (Bell, 1986), and reduced ingestion of food (Griss et al., 1991). In a variety of insects (*M. sexta*, *P. americana*, *Schistocerca gregaria* and *Heliothis zea*) removal of the frontal ganglion or severance of the recurrent nerve results in the accumulation of food in the foregut (Highnam et al., 1966; Hill et al., 1966; Bignell, 1973; Bushman and Nelson, 1990), and in both *M. sexta* larvae and adult locusts the FG has been shown to control foregut contractions (Miles and Booker, 1994, 1998; Ayali et al., 2002; Ayali, 2004; Ayali and Zilberstein, 2004). Miles and Booker (1994) also demonstrated that cutting the frontal nerve stops all activity to the buccal region and cutting the recurrent nerve stops all muscle activity in the pharyngeal and oesophageal regions of the foregut.

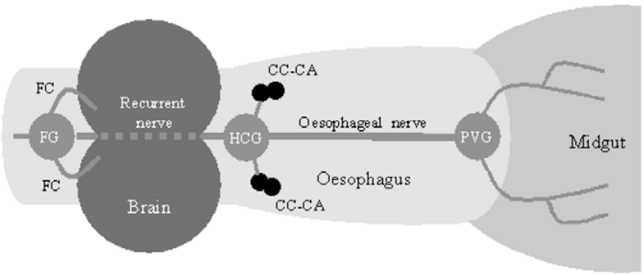

Fig. 1. Generalised structure of the stomatogastric nervous system of a larval insect showing ganglia and interconnecting nerves. Abbreviations: CC-CA, corpus cardiacum – corpus allatum; FG, frontal ganglion; FC, frontal connectives; HCG, hypocerebral ganglion; PVG, proventricular ganglion.

Many insect peptides exhibit myoactivity on the visceral muscles, especially the gut, and, because one of the first consequences of food intake is to stretch the foregut followed by contraction and relaxation of the gut muscles, it is hypothesised that the control of gut motility is involved in the regulation of feeding (Wei et al., 2000). These peptides include proctolin, the insect kinins (e.g. tachykinins), FMRFamide-related peptides, and the allatoregulatory peptides (reviewed by Gäde et al., 1997). All of these groups of peptides appear to be widely distributed, both in the central and peripheral nervous systems, and their specific localisation in neurones, nerve axons and tissues gives an indication of their putative roles.

2. Neuropeptides associated with the stomatogastric nervous system

The presence of (myoactive) neuropeptides in the SNS of insects suggests roles for these peptides in the neural control and modulation of feeding in insects. Using immunocytochemical techniques a variety of peptides have been detected in the frontal ganglion and/or other ganglia and nerves of the SNS. *In vitro* and *in vivo* bioassays have been used to demonstrate a role for these myoactive peptides in the regulation of feeding, predominantly in phytophagous insects.

2.1. FMRFamide-like peptides

The neuropeptide FMRFamide was initially characterised from the clam, *Macrocallista nimbosa* (Prince and Greenburg, 1977). There is now a large family of FMRFamide related peptides (FaRPs), with at least three subfamilies in insects (Orchard et al., 2001). These are the (extended) FMRFamides, the FLRFamides and the HMRFamides (reviewed by Gäde et al., 1997; Orchard et al., 2001). The FaRPs have all been shown to have strong inhibitory activity on visceral muscles such as the locust heart (Cuthbert and Evans, 1989) and oviduct (Lange et al., 1991), cockroach gut (Aguilar et al., 2004; Predel et al., 2001) and foregut of the blood sucking bug *Rhodnius prolixus* (Tsang and Orchard, 1991) and locust (Banner and Osbourne, 1989) suggesting they have a role as neurotransmitters or neuromodulators (Tsang and Orchard, 1991).

2.1.1. FMRFamides

Insect FMRFamides have only been identified in dipterans (reviewed by Orchard et al., 2001), but using antisera to FMRFamide, immunoreactivity has been detected in many different insect orders. However, antisera developed against FMRFamide most likely recognises the epitope formed by the C-terminal RFamide (Tsang and Orchard, 1991; Orchard et al., 2001), and hence will cross-react with a broad spectrum of FaRPs.

In Dictyoptera, RFamide-like immunoreactivity was detected in the ingluvial nerves of *Nauphoeta cinerea* and *Blabera craniifer* (Žitňan et al., 1993). Žitňan et al. (1989) reported the presence of FMRFamide-like immunoreactivity in the ganglia and nerves of the SNS of the waxmoth *Galleria mellonella* (Lepidoptera) and suggested that the close association of neurosecretory fibres with muscles of the gut indicates an effect on muscle contraction. Immunoreactivity to FaRPs has also been detected in the FG of *M. sexta* (Copenhaver and Taghert, 1989) and may be co-localised with *M. sexta* allatotropin in this tissue (Taylor et al., 1996). In the visceral tissues of *R. prolixus*, FMRFamide-like immunoreactiv-