The Journal of Experimental Biology 215, 3394-3402

doi:10.1242/jeb.073189

RESEARCH ARTICLE

The neural and peptidergic control of gut contraction in *Locusta migratoria*: the effect of an FGLa/AST

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SUMMARY

The regulation of insect gut physiology is complex and involves the interactions of a number of mechanisms, including the neural regulation of gut contraction by altering neural input and the modulation of gut contractions by neuropeptides directly affecting the muscle. The FGLa-type allatostatins (FGLa/ASTs) are known brain/gut peptides with numerous physiological roles, including modulation of gut contraction and neural input. To further investigate the pleiotropic roles of FGLa/AST peptides in *Locusta migratoria*, we have examined the role of a locust FGLa/AST (Scg-AST-6) in the gut. Proctolin and Scg-AST-6 have opposing effects on gut contraction, where proctolin dose-dependently increases gut muscle tension, while Scg-AST-6 inhibits both muscle tension and spontaneous and neurogenic contractions in a dose-dependent manner. Results from neurophysiological recordings indicate that there may be a central pattern generator (CPG) within the ventricular ganglia regulated by descending inhibition, and the addition of Scg-AST-6 dose-dependently modulates this ventricular ganglion CPG. This work provides a comprehensive picture of how FGLa/ASTs may modulate and coordinate each region of the locust gut, and shows that FGLa/ASTs have both central effects, on the ventricular ganglion CPG, and peripheral effects on the gut muscle. Overall, this study shows how FGLa/ASTs contribute to the complex regulation and fine tuning of gut contraction.

Key words: allatostatin, neurophysiology, digestion, locust, proctolin, central pattern generator, ventricular ganglia.

Received 29 March 2012; Accepted 28 May 2012

INTRODUCTION

Movement of the food bolus posteriorly through the digestive tract is achieved by peristalsis, the coordinated contraction of the circular and longitudinal muscles that are present in each region of the gut (Davey, 1964; Miller, 1975). These peristaltic contractions are myogenic in nature, but can be modified by neural input. In the locust, foregut and hindgut myogenic contractions are modulated by input from the nervous system through the innervation from the stomatogastric nervous system (STNS) and last abdominal ganglion of the central nervous system (CNS), respectively (Albrecht, 1953; Donini et al., 2002; Möhl, 1972; Huddart and Oldfield, 1982). The frontal ganglion is involved in the control of movements of the anterior foregut or pharynx, and is the major source of innervations to the dilator muscles (Albrecht, 1953; Zilberstein and Ayali, 2002; Bräunig, 2008). Nerves arising from the paired ventricular (ingluvial) ganglia located bilaterally on the foregut directly supply the innervation to the posterior foregut, while the eleventh sternal nerve of the eighth abdominal ganglion innervates the hindgut (Robertson and Lange, 2010; Donini et al., 2002). The midgut receives innervation from both the STNS and the CNS, which results in an extensive nerve plexus over the entire midgut (Robertson and Lange, 2010; Donini et al., 2002; Albrecht, 1953).

In the locust, the ganglia of the STNS (frontal ganglion, hypocerebral ganglion and ventricular ganglia) control movement of the foregut (Fig. 1) (see Ayali and Lange, 2010). A central pattern generator (CPG) within the frontal ganglion controls motor patterns that result in coordinated peristaltic movements of the pharynx and muscles involved in swallowing (Ayali and Lange, 2010; Ayali et al., 2002; Zilberstein and Ayali, 2002). Cutting the nerves from the frontal ganglion to the gut decreases feeding activity and prevents the foregut from emptying its contents, suggesting the frontal ganglion plays an important role in feeding (Hill et al., 1966; Bignell, 1973). Recently, a CPG within the hypocerebral ganglion has been identified, which may interact with the frontal ganglion CPG to coordinate foregut contractions and crop emptying (Rand and Ayali, 2010). Neural activity within cells of the ventricular ganglion also directly control foregut contractions. For example, when the gastric nerves arising from the ventricular ganglia are severed from the foregut, muscle contractions are eliminated (Clarke and Grenville, 1960). Foregut contractions persist when only the ventricular ganglia are left attached to the gut and are abolished when the ganglia are completely removed from the gut (Lange and Chan, 2008).

The detection of several myoactive peptides within cell bodies of the STNS and CNS, and especially within the innervation to each of the gut regions suggests that peptides are involved in the neuromodulation of feeding and digestion, or in the control of gut motility (see Gäde et al., 1997; Robertson and Lange, 2010; Wei et al., 2000). For example, proctolin-like immunoreactivity is associated with cell bodies of the ganglia of the STNS and CNS, as well as within the nerves that innervate the gut (Clark et al., 2006a). The allatostatin (AST) family referred to as FGLa/ASTs (previously known as A-type or cockroach type ASTs ending in the amino acid sequence FGLamide) (see Coast and Schooley, 2011) are pleiotropic, and based on their distribution in the locust CNS and STNS and innervation to the gut it has been suggested that FGLa/ASTs also regulate digestive functions (Robertson and Lange, 2010).

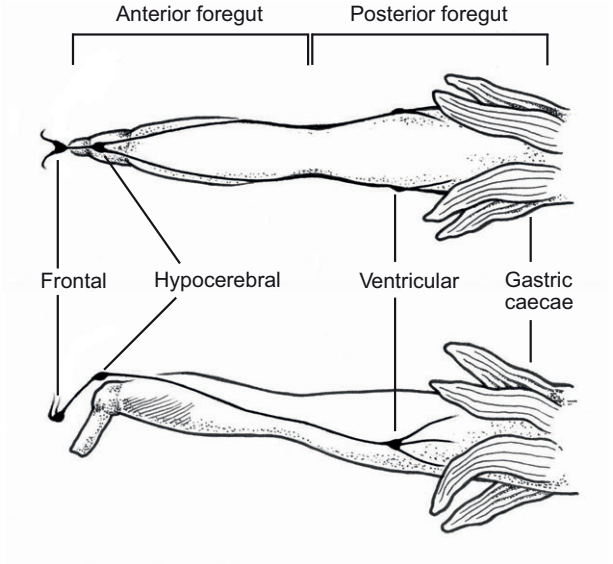

Fig. 1. Schematic representation of the locust stomatogastric nervous system (STNS). The STNS is situated on the dorsolateral surface of the gut. Two frontal connectives join the frontal ganglion to the brain. The hypocerebral ganglion is connected anteriorly to the frontal ganglion by the recurrent nerve and posteriorly to the paired ventricular ganglia by the oesophageal nerves. Each ventricular ganglion gives rise to three gastric nerves that arborize to form a network of processes over the posterior foregut and the gastric caecae. These ceacal processes then form a plexus over the anterior midgut, which can extend to the midgut–hindgut boundary.

Several insect peptides have been found to control insect gut contraction in many insect species, including the allatoregulatory hormones, myoinhibiting peptides (MIPs), proctolin and FMRFamide-related peptides (see Audsley and Weaver, 2009; Spit et al., 2012). The allatostatins and allatotropins have opposing effects on gut contraction. The FGLa/ASTs inhibit spontaneous and proctolin-induced gut contraction (Lange et al., 1993; Lange et al., 1995; Duve et al., 1995; Sarkar et al., 2003; Fusé et al., 1999). The MIPs (also known as B-type ASTs) were originally named because of their inhibition of hindgut and oviducal contractions in the locust (Schoofs et al., 1991) and members of this family inhibit gut contraction in other insect species, including cockroaches (Predel et al., 2001) (see Audsley and Weaver, 2009). While the FGLa/ASTs inhibit gut contraction, the allatotropins stimulate gut contraction in several insect species including moths (see Spit et al., 2012). Proctolin was isolated based on its excitation of hindgut muscle contraction in *Periplaneta americana* (Starratt and Brown, 1975). Since its discovery, proctolin has been found to have a stimulatory effect on the gut of several insect species including the locust midgut and foregut (Lange et al., 1988; Banner et al., 1987). Lastly, the FMRFamide-related peptides referred to as myosuppressins have an inhibitory effect on contraction of the gut regions, including the foregut and midgut of the locust (Banner and Osborne, 1989; Lange and Orchard, 1998).

The regulation of feeding in insects is complex, involving CPGs and sensory feedback, distension of the alimentary canal, nutrient effects and the modulation of activity by neuropeptides and hormones (Wei et al., 2000) (see Audsley and Weaver, 2009). One aim of the current study was to determine how one member of the FGLa/AST family (*Schistocerca gregaria* AST-6, Scg-AST-6) isolated from another locust, may modify the activity of the neurons in the ventricular ganglia that lead to the neurogenic contractions of the foregut. This particular FGLa/AST was chosen because it was isolated and sequenced from locust brain extracts (Veelaert et al., 1996) and the members of the FGLa/AST family show similarity in the C-terminal sequence and thus all have similar actions on visceral tissues, albeit with varying degrees of effectiveness (Lange et al., 1993). In addition, the modulatory role that neuropeptides play in the regulation of feeding and digestion is still poorly understood, and thus another aim of this study was to elucidate further the role of FGLa/ASTs (in particular Scg-AST-6) in gut physiology. This is important as it is the digestion and metabolism of nutrients from food that provides the energy for important physiological processes such as growth, flight and reproduction. As we have shown previously that proctolin and FGLa/ASTs act as releasing factors for adipokinetic hormone I and juvenile hormone, both of which are metabolically important peptides (Clark et al., 2006b; Clark et al., 2008), it is important to understand how proctolin and FGLa/ASTs may also affect gut physiology, and thus directly affect homeostasis and the physiology of the insect. Using a variety of techniques we show that the FGLa/AST peptide family influences foregut contractions in two ways; by modulating the CPG, which controls the timing and intensity of foregut contractions, and also in a complementary fashion by a direct action on the muscle to inhibit contraction.

MATERIALS AND METHODS

Animals

All experiments were conducted on mature 2–3 week old adult male *Locusta migratorioides* (Fairmaire and Reiche 1849). Locusts were housed in crowded conditions and were kept on a 12 h:12 h light:dark regime at 30°C at 50% humidity. The locusts were fed fresh wheat seedlings and bran, supplemented with carrots.

Chemicals

Scg-AST-6 (ARPYSFGL-NH_2) was custom synthesized by the Insect Biotech Canada Core Facility (Queen's University, Kingston, ON, Canada) and proctolin was obtained from Bachem (Torrance, CA, USA). Peptides were reconstituted in double-distilled water to yield a 10^{-3} mol l^{-1} stock solution, which was divided into 10 µl aliquots and frozen at –20°C until needed. Immediately prior to use, working dilutions of each peptide were made in physiological saline (150 mmol l^{-1} NaCl, 10 mmol l^{-1} KCl, 4 mmol l^{-1} $CaCl_2$, 2 mmol l^{-1} $MgCl_2$, 4 mmol l^{-1} $NaHCO_3$, 5 mmol l^{-1} Hepes pH 7.2, 90 mmol l^{-1} sucrose and 5 mmol l^{-1} trehalose).

Muscle contraction assays

The locust gut was dissected under physiological saline. To isolate the foregut, the gut was bisected at the cardiac valve (where the gastric caecae attach to the gut), and to isolate the hindgut the bisection was made just anterior to the pyloric valve at the point where the Malpighian tubules attach to the gut. Bisection at the cardiac valve and the pyloric valve also isolated the midgut. Once the appropriate region of the locust gut was dissected, one end was pinned securely to a Sylgard-coated dish using minuten pins and fine thread was tied tightly around the other end of the gut and then attached to a Grass FT 03 force transducer (Grass Medical Instruments, Quincy, MA, USA). The force transducer was connected to an amplifier and contractions were monitored on a flatbed chart recorder.

All preparations were maintained in either 400, 600 or 800 µl of saline (depending on the size of the preparation) and the peptide was added by removing half of the volume of saline and replacing

it with the same volume of saline containing peptide at twice the desired final concentration. Each preparation was washed extensively with saline between peptide applications. The effect of Scg-AST-6 on neurogenic contractions of the foregut was determined by leaving the ventricular ganglia attached to the foregut, but disconnected from the rest of the STNS.

Neurophysiology

The foregut was dissected out, as above, and bathed in 600 or 800 µl of saline in a Sylgard-coated dish. For extracellular nerve recordings using glass suction electrodes, the gut was dissected such that one ventricular ganglion and associated nerves was accessible. All nerve recordings were amplified and filtered (low band-pass filter 300 Hz and high band-pass filter 500 Hz) using an AM Systems model 1700 differential AC amplifier (Everett, WA, USA). In some preparations the muscle was attached to a force transducer to simultaneously monitor muscle contraction (see above). Extracellular nerve recordings and muscle contractions were displayed, stored and analysed using a Powerlab acquisition system (ADI Instruments, Colorado Springs, CO, USA) and LabChart 6 Pro. Analysis of nerve recordings was made for 2 min prior to and 2 min after application of peptide, and included measurement of the following variables: burst duration, interburst interval, cycle period, number of action potentials per burst and frequency of action potentials per burst.

Statistics

The data are reported as means ± s.e.m. A one-tailed paired *t*-test was used to assess the difference between groups. Significance for all statistical tests was $P<0.05$.

RESULTS

Muscle contraction assays

Proctolin

Proctolin was stimulatory on the foregut and hindgut, causing dose-dependent increases in basal tension that were reversible with washing (Fig. 2A; Fig. 3A). The thresholds for contraction occurred at 10^{-10} mol l^{-1} for the foregut and between 10^{-11} and 10^{-10} mol l^{-1} for the hindgut (Fig. 2B; Fig. 3B). The maximum increase in basal tension for both of these gut regions occurred at 10^{-6} mol l^{-1} proctolin (Fig. 2B; Fig. 3B).

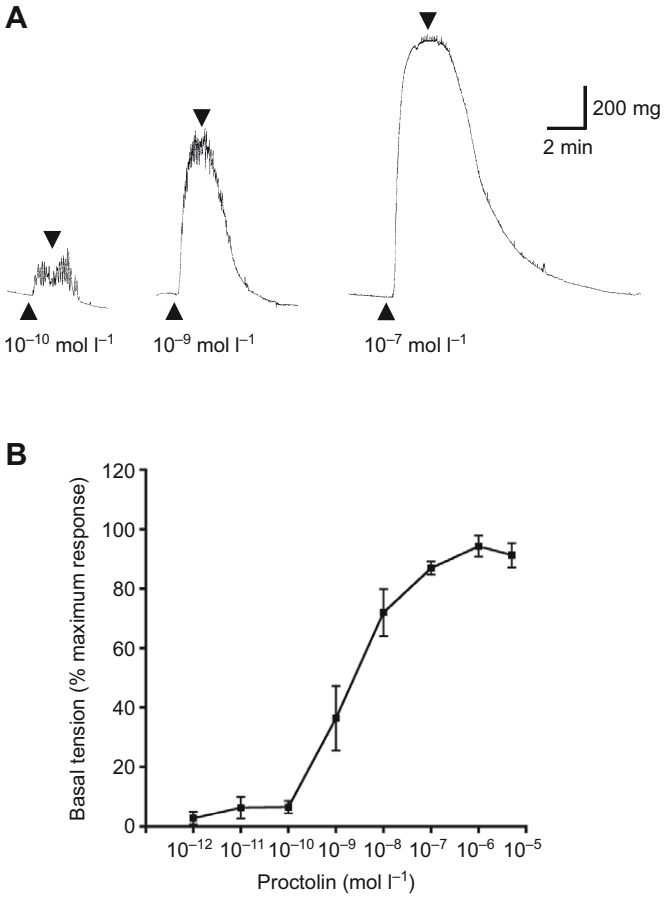

Fig. 2. Proctolin stimulates locust foregut contraction. (A) Sample trace showing the effect of increasing concentrations of proctolin on basal tension. The phasic contractions (vertical deflections) superimposed on the basal tension change are also induced by proctolin. Upward pointing arrowheads indicate application of proctolin and downward pointing arrowheads indicate the beginning of the saline wash. (B) Dose–response curve illustrating the dose-dependent increase in basal tonus of the foregut upon application of proctolin (values are means ± s.e.m. of 4–6 preparations).

Scg-AST-6

Our studies examined the effect of Scg-AST-6 on contraction of the locust gut. Scg-AST-6 inhibited contractions of all regions of the locust gut (Figs 4–7).

Foregut

The inhibitory effect of Scg-AST-6 was determined in two ways: directly on a proctolin-induced contraction when the ventricular ganglia were removed from the foregut, and on the neurogenic contractions produced by the ventricular ganglia that innervate the foregut muscle when the ganglia were left attached. To assess the inhibitory effect of Scg-AST-6 on proctolin-induced contractions of the foregut, a standard dose of 10^{-9} mol l^{-1} proctolin was chosen, which produced a contraction that was approximately 40% maximal (see Fig. 2B). Scg-AST-6 dose-dependently inhibited the proctolin-induced contraction of the foregut (Fig. 4). Maximum inhibition of the 10^{-9} mol l^{-1} proctolin-induced contraction occurred at 10^{-6} mol l^{-1} Scg-AST-6, where Scg-AST-6 inhibited the proctolin-induced contraction by approximately 55% (Fig. 4B).

When the ventricular ganglia and associated nerves were left attached to the foregut, the foregut muscle revealed a rhythmic pattern of neurogenic contractions (Fig. 5A) that were not seen when the ganglia were removed (see Fig. 4A). Scg-AST-6 reduced the frequency and amplitude of the neurogenic contractions of the foregut in a dose-dependent manner (Fig. 5). The inhibitory effect on frequency and amplitude was reversible with washing and the rhythmic pattern of contractions returned to saline levels after 4 min. Maximum inhibition of contraction frequency occurred at 10^{-6} mol l^{-1} (Fig. 5B), where the neurogenic contractions of the foregut were completely abolished in 80% of the preparations (4 out of 5 preparations).

Midgut

The isolated midgut possesses spontaneous contractions (Fig. 6A). Scg-AST-6 caused a dose-dependent reduction of midgut basal tension that was reversible upon washing (Fig. 6B). Maximum relaxation of the midgut was measured at 5×10^{-6} mol l^{-1} Scg-AST-6. Scg-AST-6 also led to an inhibition of the frequency of spontaneous contractions (Fig. 6A).

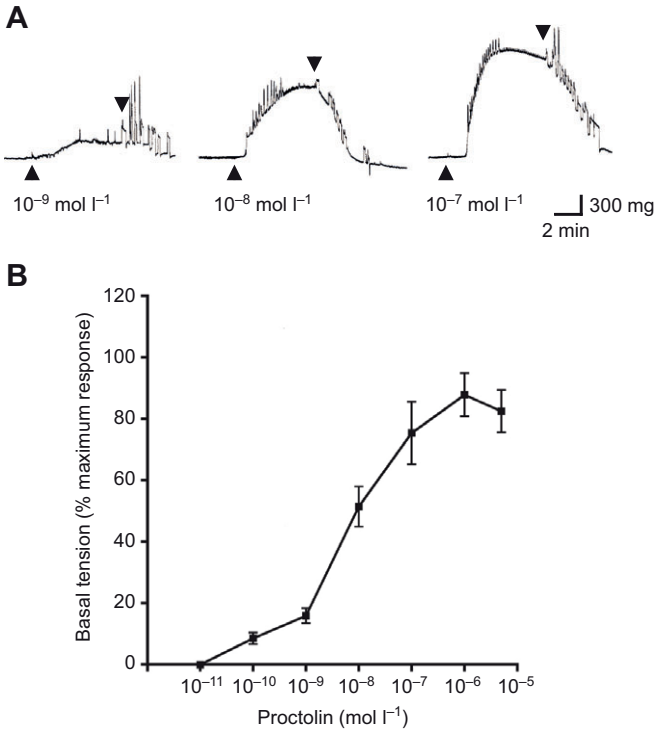

Fig. 3. Proctolin stimulates locust hindgut contraction. (A) Sample trace illustrating the dose-dependent increase in basal tension of the hindgut. Proctolin also causes phasic contractions, seen as vertical deflections extending from the tension curve. Upward pointing arrowheads indicate when proctolin was applied and downward pointing arrowheads indicate when the saline wash began. (B) Dose–response curve illustrating the dose-dependent increase in hindgut basal tension. Points represent means ± s.e.m. of 4–7 preparations.

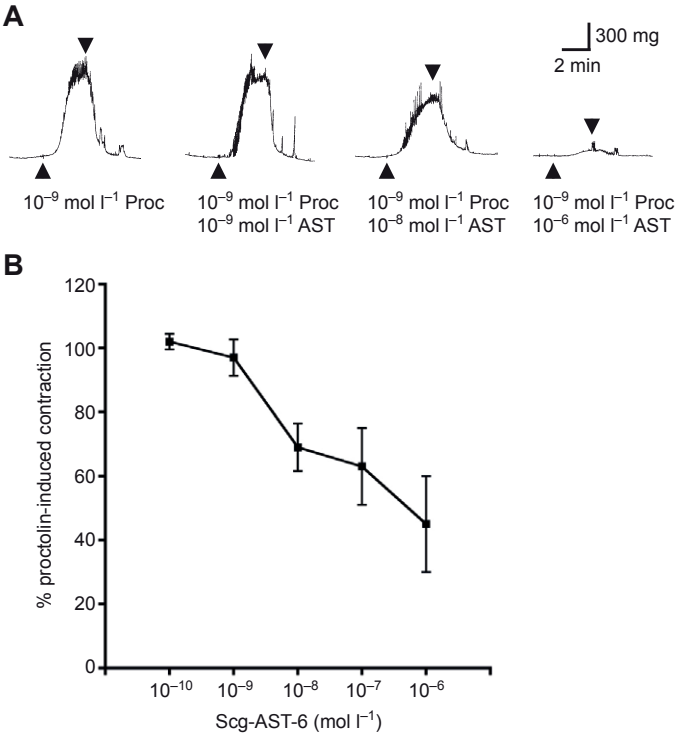

Fig. 4. Scg-AST-6 inhibits proctolin-induced contractions of the foregut. (A) Sample trace showing that Scg-AST-6 (AST) dose-dependently inhibits a standard contraction produced by 10^{-9} mol l^{-1} proctolin (Proc). Arrowheads pointing upwards indicate when peptide(s) was applied and arrowheads pointing downwards indicate when the saline wash commenced. (B) Dose–response curve showing the dose-dependent decrease in proctolin-induced contraction of the foregut. Values are plotted as the mean ± s.e.m. of 3–5 preparations.

Hindgut

The locust hindgut was not spontaneously active; thus, the inhibitory effect of Scg-AST-6 was assessed on a contraction induced by 10^{-8} mol l^{-1} proctolin (Fig. 7). Scg-AST-6 caused a dose-dependent inhibition of the proctolin-induced contraction, which was reversible with washing (Fig. 7A). Scg-AST-6 was not capable of fully inhibiting the 10^{-8} mol l^{-1} proctolin-induced contraction of the hindgut. Scg-AST-6 caused maximum inhibition at 10^{-7} mol l^{-1}, resulting in an approximately 60% decrease in the proctolin-induced contraction (Fig. 7B). Scg-AST-6 appeared to increase the time it took to reach maximum proctolin-induced contraction, with 10^{-7} mol l^{-1} Scg-AST-6 causing a significant delay (Fig. 7C).

Neurophysiology

When the ventricular ganglia were left connected to the rest of the STNS and brain there were no apparent bursts of action potentials recorded from the gastric nerves that innervate the foregut muscle (Fig. 8A). Upon isolation of the ventricular ganglia by transecting the oesophageal nerves (which connect the ventricular ganglia to the hypocerebral ganglion; see Fig. 1), bursts of action potentials were seen in the gastric nerves of the ventricular ganglia. These bursts contained a variety of sizes of action potentials (Fig. 8B). The onset and pattern of bursting activity was variable, which may be related to the degree of fullness of the foregut. For guts that were empty or partially full, either the bursting motor patterns were not exhibited or the bursting was intermittent, whereas guts that were full exhibited bursts that were more coordinated. Thus, all experiments were conducted on preparations where the foregut was full of fresh food.

When simultaneous neurophysiological recordings and foregut muscle contraction assays were performed it was seen that the bursts of action potentials were coordinated with foregut contractions (Fig. 9). In saline, the cycle period was fairly constant and coordinated 1:1 with foregut contractions, which were all of similar force (Fig. 9A). At a dose of 10^{-8} mol l^{-1} Scg-AST-6, the burst duration and interburst interval significantly decreased, leading to a shorter and more irregular cycle period (Fig. 9B; Fig. 10A). The contraction frequency also increased concurrent with the decrease in cycle frequency but the magnitude of the contractions was more variable (Fig. 9B). After washing, the bursting pattern maintained a shorter cycle period and a greater frequency of foregut contractions (Fig. 9C). After the addition of 10^{-6} mol l^{-1} Scg-AST-6 the foregut muscle underwent an initial period of irregularity, with bursts of varying length and frequency of action potentials coordinated with irregular contractions. This was followed by a period where bursting was abolished and where no contractions of the foregut were seen. Over time, the bursting pattern gradually reappeared, as did foregut contractions (Fig. 9D). On examining preparations where bursting persisted at 10^{-6} mol l^{-1} Scg-AST-6 (20% of the preparations), trends in the data suggest that the burst duration decreased slightly compared with saline, while the interburst interval and cycle period increased (Fig. 10B). In addition, Scg-AST-6 caused a dose-

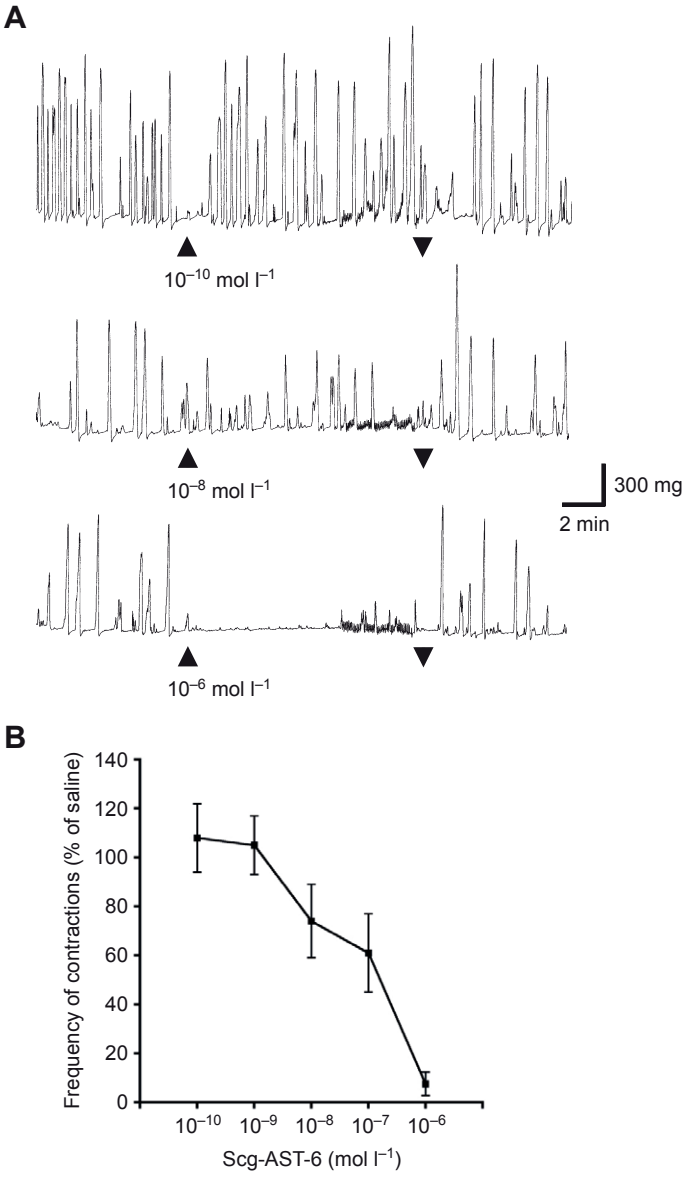

Fig. 5. Scg-AST-6 inhibits the frequency of neurogenic contractions of the foregut. (A) Sample trace showing the effect of increasing the concentration of Scg-AST-6. Upward pointing arrowheads indicate when Scg-AST-6 was applied, while downward pointing arrowheads indicate when the saline wash commenced. (B) Dose–response curve illustrating the dose-dependent decrease in the frequency of foregut neurogenic contractions (plotted as a percentage of the number of contractions in saline). Values are shown as the mean ± s.e.m. of 4–6 preparations.

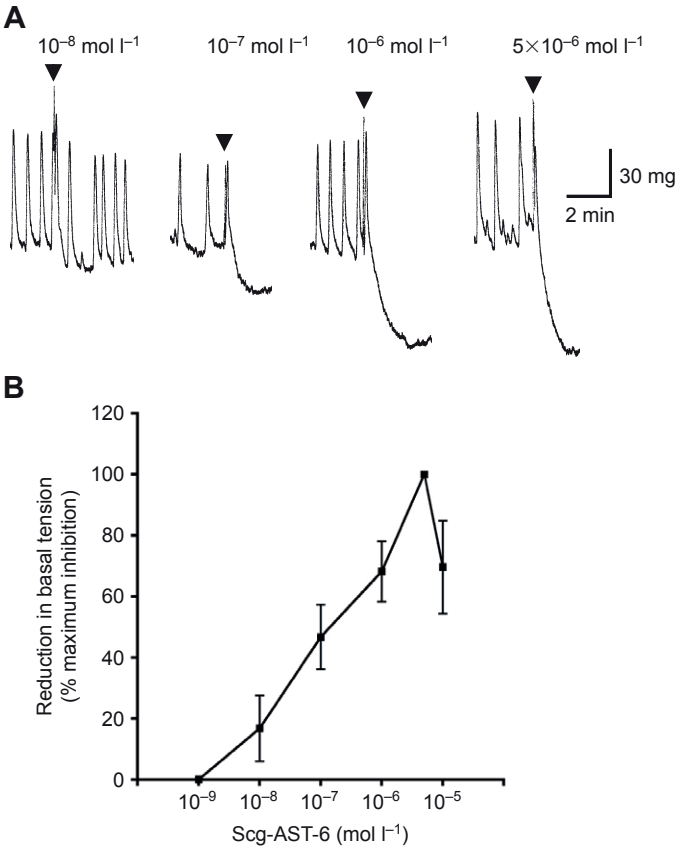

Fig. 6. Scg-AST-6 decreases midgut basal tension. (A) Sample trace illustrating the inhibitory effect of Scg-AST-6 on muscle tone. Vertical deflections indicate spontaneous contractions, which were also inhibited in a dose-dependent manner by Scg-AST-6. (B) Dose–response curve showing the dose-dependent decrease in midgut muscle tension (plotted as a percentage of the maximum inhibition). Points are means ± s.e.m. of 3–5 preparations.

dependent decrease in the number of action potentials per burst (Fig. 11A) and a dose-dependent decrease in the frequency of action potentials within a burst (Fig. 11B).

DISCUSSION

The distribution of FGLa/AST-like immunoreactivity in *L. migratoria* suggests these peptides have diverse physiological functions (Clark et al., 2008). Recently, FGLa/AST innervation to the locust gut was described, indicating the source of FGLa/AST-like peptides associated with the foregut to be cell bodies within the brain, and the source of those associated with the hindgut to be cell bodies within the eighth abdominal ganglion (Robertson and Lange, 2010). These cells may release FGLa/AST-like peptides locally as neurotransmitters or neuromodulators onto the gut tissue or into the haemolymph to act as neurohormones. The results from this study show that proctolin can stimulate muscle contraction of the foregut and hindgut of the locust and that Scg-AST-6 can act directly on the foregut and hindgut muscle to inhibit proctolin-induced contractions. Scg-AST-6 can also decrease tonus of midgut tissue. This myoinhibitory property of FGLa/ASTs is well documented in a number of insect species. For example, FGLa/ASTs inhibit peristaltic contractions of the foregut in *Calliphora vomitoria*, *S. gregaria* and *Leucophaea maderae* (Duve and Thorpe, 1994; Zilberstein et al., 2004; Duve et al., 1995), proctolin-induced contractions of the midgut in *Diploptera punctata* (Fusé et al., 1999), and spontaneous and proctolin-induced contractions of the hindgut in *D. punctata* and *S. gregaria* (Lange et al., 1993; Lange et al., 1995; Veelaert et al., 1996). Other myoinhibitory effects of FGLa/ASTs include inhibition of spontaneous oviducal contractions in *S. gregaria* (Veelaert et al., 1996) and modulation of the cardiac rhythm in *Blattella germanica* (Vilaplana et al., 1999). However, we now also report that FGLa/ASTs indirectly inhibit foregut contractions *via* altering activity from a CPG located in the ventricular ganglia and thereby inhibiting neurogenic contractions.

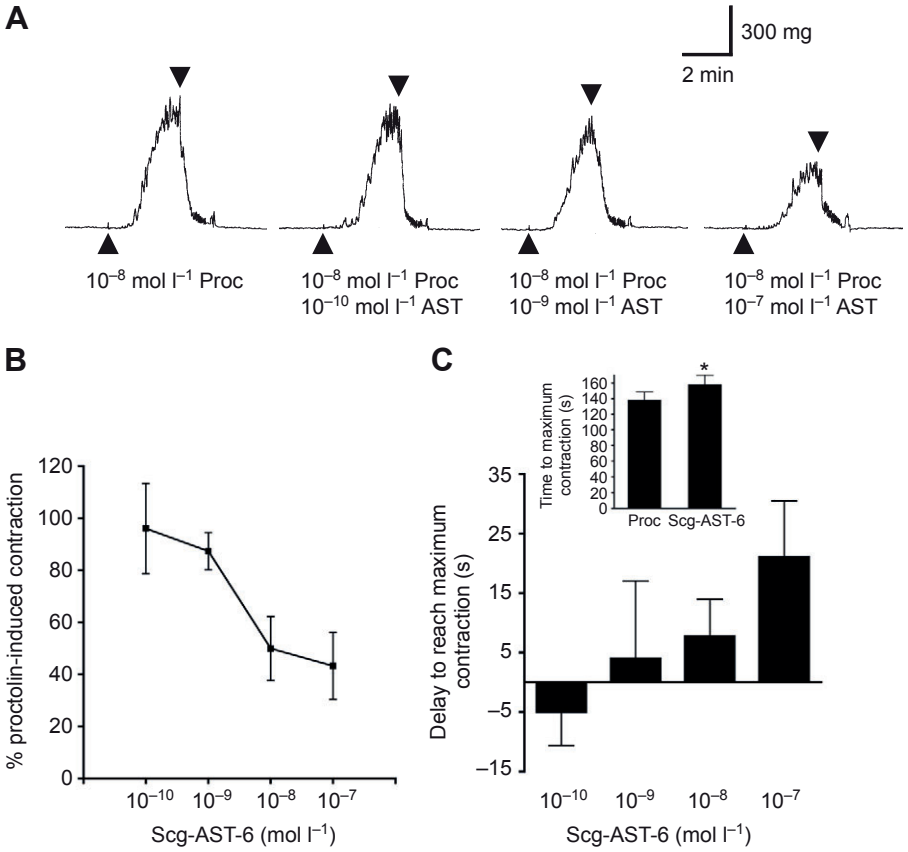

Fig. 7. Scg-AST-6 inhibits proctolin-induced contraction of the hindgut. (A) Sample trace showing the inhibition of the effects of a standard 10^{-8} mol l^{-1} dose of proctolin (Proc) by increasing doses of Scg-AST-6 (AST). Arrowheads pointing upwards indicate when peptide was applied, while arrowheads pointing downwards indicate when peptide was washed off with saline. (B) Dose–response curve illustrating the dose-dependent decrease in the percentage of proctolin-induced contraction. Points represent the mean ± s.e.m. of 4–7 preparations. (C) Scg-AST-6 dose-dependently delays the time to reach maximum proctolin-induced contraction compared with a standard contraction produced by 10^{-8} mol l^{-1} proctolin. Bars represent the mean ± s.e.m. of 4–7 preparations. This delay to reach maximum contraction is statistically significant for 10^{-7} mol l^{-1} Scg-AST-6 (inset, *P<0.05; one-tailed t-test, P<0.05).

A motor pattern controlling the contractions of the foregut is generated by the isolated ventricular ganglia, as shown by the coordination of the bursts of action potentials recorded from the nerves arising from the ventricular ganglion with contraction of the foregut. This confirms previous work (Lange and Chan, 2008; Clarke and Grenville, 1960) predicting that a CPG within the ventricular ganglia directs foregut contraction. This CPG is probably under descending inhibitory control, as transection of the oesophageal nerves activates the CPG, leading to a rhythmic motor pattern. In the intact locust, a distinct food passage rhythmic motor pattern associated with the start of feeding has been recorded from the frontal ganglion nerves (see Ayali and Lange, 2010). This motor pattern, which is also under descending inhibitory control, coordinates with peristaltic contractions of the foregut and increases in cycle frequency as the foregut and crop distend, and ceases when the gut is fully stretched and full (Zilberstein and Ayali, 2002). A similar observation was made in the current study, where bursts of action potentials from the ventricular ganglia gastric nerves were not observed or were intermittent when the foregut was empty or minimally distended, but were observed when the foregut was full of food. Neuromodulation of foregut rhythm from the frontal ganglion by peptides or amines has been documented (Zilberstein and Ayali, 2002; Zilberstein et al., 2004). Neurophysiological studies have previously suggested that FGLa/ASTs modulate the foregut rhythm *in vitro* and *in vivo* in the desert locust (Zilberstein et al., 2004). The effect of FGLa/AST on the characteristics of the

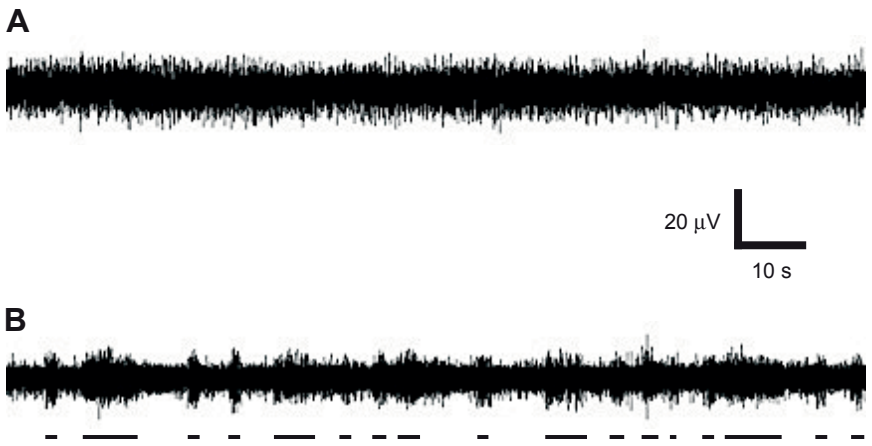

Fig. 8. Extracellular recordings from a gastric nerve of the ventricular ganglion that innervates the foregut of *Locusta migratoria*. (A) Extracellular recordings with an intact STNS. Note that there is no apparent bursting activity. (B) Extracellular recordings after the transection of the oesophageal nerves to isolate the ventricular ganglia from the STNS. Note the bursting activity. Lines below the trace indicate the duration of each burst. This is a representative trace of the bursting motor pattern seen (N=20).

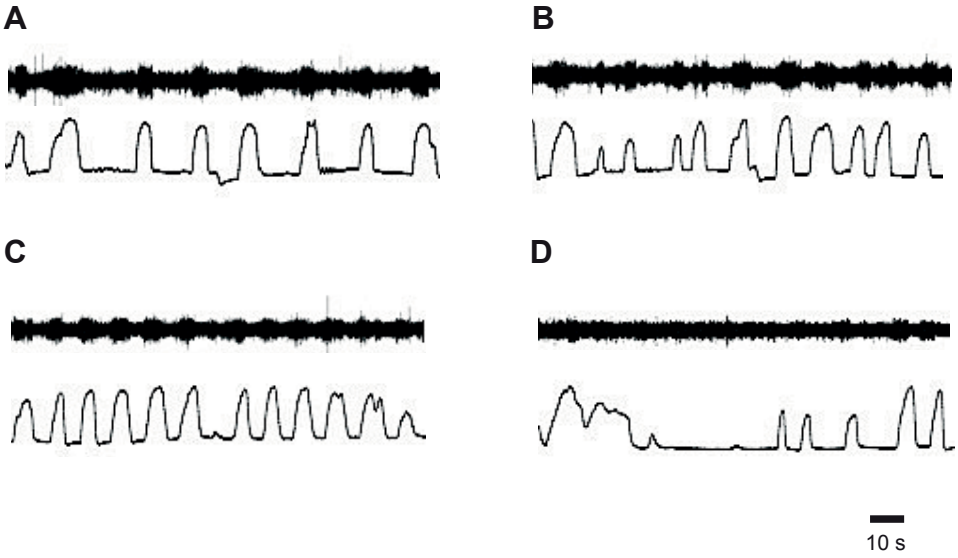

Fig. 9. Coordination of bursting motor patterns with foregut contraction. (A) Extracellular recordings (top trace) and foregut contractions (bottom trace) recorded in saline are coordinated. (B) Recordings after the addition of 10^{-8} mol l^{-1} Scg-AST-6. (C) Recordings after a saline wash. Note that cycle period decreases. (D) Recordings made after the addition of 10^{-6} mol l^{-1} Scg-AST-6. Note the irregular activity and absence of a repeating pattern. These are representative traces (N=10).

rhythm from the frontal ganglion were complex, with low doses of FGLa/AST having an excitatory effect on the motor pattern while 10^{-6} mol l^{-1} Scg-AST-6 caused complete inhibition of both nerve and muscle activity (Zilberstein et al., 2004). In the current study, neuromodulation of the foregut motor pattern arising from the ventricular ganglion by Scg-AST-6 indicates a similar control to that shown by Zilberstein and colleagues (Zilberstein et al., 2004). A low dose of Scg-AST-6 (10^{-8} mol l^{-1}) caused a decrease in cycle period due to a significant decrease in the interval between bursts, thereby leading to an increase in the frequency of contractions, whereas at 10^{-6} mol l^{-1} the neural and muscle activity were completely abolished or greatly reduced. In preparations where neural and muscle activity were still detected at 10^{-6} mol l^{-1} Scg-AST-6, the rhythm was altered such that there was a decrease in burst duration and frequency of action potentials within the burst. These changes led to less forceful contractions of the foregut muscle. Thus, FGLa/ASTs control foregut movement at two levels; first, by influencing the motor patterns from the ventricular ganglia, and second, by direct inhibitory action on the foregut muscles. Interestingly, there may be an added complexity in that the frequency of foregut contractions increased after washing out the allatostatin. Thus, there may be a 'post-inhibitory rebound' effect whereby the CPG becomes more robust following the removal of the peptide. Perhaps this is a true physiological effect allowing for even greater fine tuning depending upon the context of events.

To aid in the passage of food from one region to another or to increase the mixing of the food within a specific region, the motility of the various regions of the gut must be altered. The FGLa/ASTs can inhibit contractions, while proctolin can stimulate contractions. Thus, proctolin may be released to increase the motility of the muscle. This is reasonable given proctolin was originally discovered based on its myotropic effect on cockroach hindgut (Brown and Starratt, 1975), and has since been implicated in the regulation of feeding (see Audsley and Weaver, 2009). When peristaltic contractions need to be reduced or abolished completely, FGLa/ASTs may be released to decrease the contractile activity of the gut region to allow for more efficient absorption of nutrients and absorption of water by increasing the time the food bolus remains in the midgut or hindgut. FGLa/ASTs may also act on the cardiac and pyloric sphincters to relax them, allowing the passage of food into the next gut region more easily.

We also show here that Scg-AST-6 inhibits midgut muscle contraction by lowering basal tonus. Previous studies have indicated a role of FGLa/ASTs in midgut physiology. For example, FGLa/AST content of the cockroach midgut changes with nutritional status. In starved and dehydrated cockroaches there is an initial

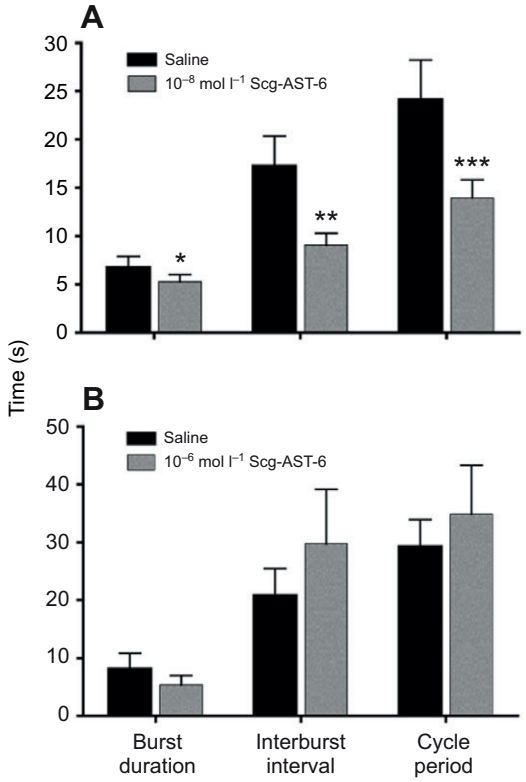

Fig. 10. The effect of Scg-AST-6 on neurophysiological pattern characteristics. (A) Scg-AST-6 at 10^{-8} mol l^{-1} significantly decreased burst duration, interburst interval and cycle period relative to saline (*P=0.03, **P=0.01; one-tailed paired t-test, P<0.05). Bars represent means + s.e.m. of 7 preparations. (B) There was no significant effect of 10^{-6} mol l^{-1} Scg-AST-6 on burst duration, interburst interval and cycle period relative to saline for preparations where bursting persisted (one-tailed paired t-test, P<0.05). Bars represent means + s.e.m. of 10 preparations.

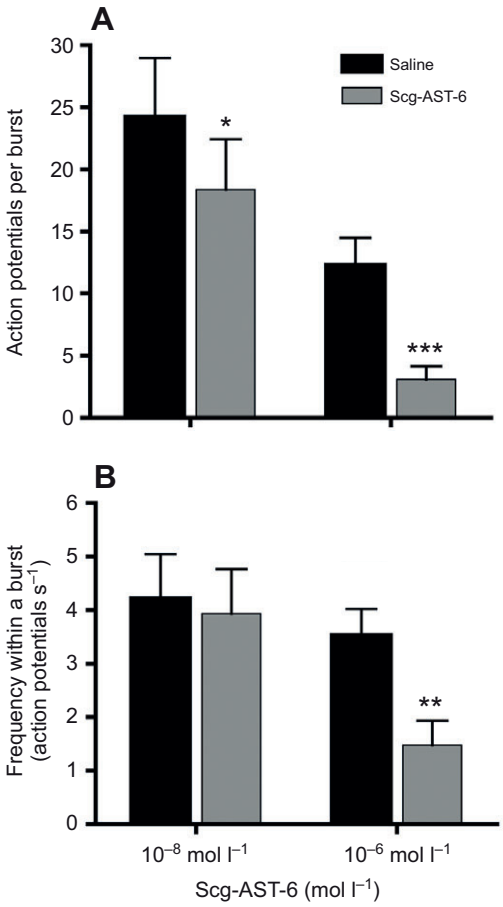

Fig. 11. Dose-dependent effect of Scg-AST-6 on (A) the number of action potentials per burst and (B) the frequency of action potentials within a burst. Bars represent the mean + s.e.m. of 7 preparations for 10^{-8} mol l^{-1} and 10 preparations for 10^{-6} mol l^{-1} Scg-AST-6. *P<0.05, **P=0.002, ***P=0.007; one-tailed paired t-test, P<0.05.

increase in midgut FGLa/AST content, which decreases as the duration of the nutritional stress increases (Yu et al., 1995). During periods of water and food deprivation, FGLa/ASTs may be released into the haemolymph to modulate local midgut contraction, thus decreasing metabolic activity and increasing the time the food bolus remains in this region, allowing increased time for chemical digestion and the absorption of nutrients. This is in line with the suggestion that these peptides function as neurotransmitters/neuromodulators at the locust gut, based on the presence of networks of FGLa/AST-like immunoreactive processes and varicosities on the surface of the foregut, midgut and hindgut (Robertson and Lange, 2010). In addition, FGLa/AST-like peptides have been detected in the haemolymph and are released from the cockroach midgut into the haemolymph during feeding, acting as endocrine hormones (Woodhead et al., 1993; Yu et al., 1995).

As FGLa/ASTs modulate hindgut muscle contractility, the action of FGLa/ASTs may also be related to excretion. It is possible that FGLa/ASTs can affect both muscle contractility and reabsorption because FGLa/ASTs have been shown to alter ion transport in other insect species (Onken et al., 2004). Future work will examine the role that FGLa/ASTs play in locust gut ion transport to further elucidate the physiological roles that this peptide family plays in digestion.

Here, we have shown that the FGLa/ASTs are important in the regulation of gut muscle activity, by modulating not only the neural input but also, directly, the gut muscle tissue. FGLa/ASTs modulate each region of the gut, coordinating the functioning of each region. It would appear that a physiological function in the gut is a common role for FGLa/ASTs in insects (see Audsley and Weaver, 2009; Lange et al., 1995; Duve and Thorpe, 1994) (see also current study). The ancestral role for the FGLa/ASTs may be as brain/gut peptides, as a myoinhibitory role for visceral muscle contraction is so widespread for the FGLa/ASTs, and because the FGLa/ASTs do not function as true allatostatins in most species of insects (see Gäde, 2002). FGLa/ASTs and proctolin are pleiotropic and involved in several physiological processes in animals, so it is not surprising that the control of the contractile activity of insect gut musculature is modulated by these peptides. By having opposing effects on gut contraction, proctolin and FGLa/ASTs can regulate gut motility to increase the efficiency of nutrient absorption and the passage of food along the alimentary canal.

ACKNOWLEDGEMENTS

The authors wish to thank Ian Orchard for his guidance and for reviewing the manuscript. We would also like to thank Zach McLaughlin for drawing the schematic diagram of the stomatogastric nervous system.

FUNDING

This work was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) grant to A.B.L. and an NSERC Alexander Graham Bell Canada Graduate Scholarship to L.A.R.

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