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Effects of an allatostatin and a myosuppressin on midgut carbohydrate enzyme activity in the cockroach *Diploptera punctata*

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Abstract

Neuropeptides of the cockroach allatostatin (AST) family are known for their ability to inhibit the production of juvenile hormone by the corpora allata of cockroaches. Since their discovery, they have also been shown to modulate myotropic activity in a range of insect species as well as to act as neurotransmitters in Crustaceans and possibly in insects. The midgut of cockroaches contains numerous endocrine cells, some of which produce AST whereas others produce the FMRFamide-related peptide, leucomyosuppressin (LMS). We have determined if ASTs and LMS are also able to influence carbohydrate-metabolizing enzyme activity in the midgut of the cockroach, *Diploptera punctata*. Dippu-AST 7 stimulates activity of both invertase and α-amylase in a dose-dependent fashion in the lumen contents of ligatured midguts in vitro, but not in midgut tissue, whereas the AST analog AST(b)ϕ2, a cyclopropyl-ala, hydrocinnamic acid analog of Dippu-AST 6, has no effect. Leucomyosuppressin also stimulates enzyme activity in lumen contents only, although the EC_{50} is considerably greater than for Dippu-AST. Dippu-AST is also able to inhibit proctolin-induced contractions of midgut muscle, and this action had already been described for LMS [18]. Thus, in this organ, AST and LMS have at least two distinct physiological effects. © 1999 Elsevier Science Inc. All rights reserved.

Keywords: Allatostatin; Leucomyosuppressin; FMRFamide-related peptide (FaRP); Cockroach; *Diploptera;* Invertase; Amylase

1. Introduction

The insect midgut (mesenteron or ventriculus) has been described as the 'largest endocrine organ', containing a variety of putative endocrine cells along its length [8,36]. This statement is based on the number of apparent endocrine cells in the midgut, relative to the number of endocrine cells found in other tissues. The midgut is the defined site of production and release of several insect 'neuropeptides'. Despite its endodermal embryonic origin, the insect midgut contains numerous 'endocrine' cells that show both immunoreactivity and mRNA hybridization (by in situ hybridization) to several peptide families.

The insect midgut endocrine 'system' is considered to be analogous to the gastroenteropancreatic endocrine system of mammals [13,14,36]. The midgut endocrine cells probably originate in the midgut nidi and show a variable lifespan [15]. Peptidergic axons also extend along the length of the gut and originate from either the proctodeal or stomatogastric nervous system. Their presence, in concert with the endocrine cells, suggests that multiple modes of regulation of physiological processes by neuropeptides are possible. Examples of peptides present in the midgut include members of the allatostatin, tachykinin, and FMRFamide-related peptide (FaRP) families [4,29,45,49,50].

The allatostatins (ASTs) are a family of peptides originally isolated on the basis of their ability to inhibit the production of juvenile hormone in cockroaches [11,41,48]. They are characterized by a common pentapeptide C terminus (Y/F)XFG(L/I)amide and a variable N terminus of 1 to 13 residues. In addition to their ability to inhibit juvenile hormone production, the ASTs are able to inhibit both proctolin-induced and spontaneous contraction of muscle of the hindgut and the antennal pulsatile organ [25,26]. These

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peptides are present in putative endocrine cells, particularly in the anterior portion of the midgut of the cockroach, *Diploptera punctata,* as well as in nerves running much of the length of the gut [49]. The proctodeal nerves seem to terminate in the densest area of AST-IR (immunoreactive) endocrine cells and do not extend anterior beyond this region. Cells that show immunoreactivity to other peptide families, including some FaRPs, are abundant in the posterior region of the midgut whereas still other members of this family [e.g. leucomyosuppressin (LMS)], are concentrated in the anterior portion [19].

LMS was first isolated from the cockroach, *Leucophaea maderae*, based on its ability to inhibit hindgut muscle contractions [20] and has since been shown to inhibit proctolin-induced midgut contractions as well [18]. The myosuppressin subfamily comprises a group of decapeptides that share the common sequence XDVXHXFLRFamide, and that seem to be encoded on a gene separate from the other FaRPs [12,34,35,39]. The actions of myosuppressins on visceral muscle contractions of insects have been well documented (e.g. [20,27,30,38,47]). However, members of this family can also stimulate contractions of flight muscle [24] and cardiac muscle [46], as well as α-amylase secretion from weevil midgut and scallop digestive gland [31,32].

Studies on ASTs and LMS localization suggest that there is a differential spatial distribution, as well as a difference in function between LMS and other midgut FaRPs. The distribution of AST and LMS mRNA in the midgut of *D. punctata* [19,49] and the action of LMS on midgut contractions [18] have been described. We have determined whether these peptides also play a role in regulating enzyme secretion by this tissue. Here we describe the distribution of α-amylase and invertase activity in the cockroach midgut, and examine the ability of an AST, an AST analog and LMS to stimulate α-amylase and invertase activity in the midgut tissue and lumen.

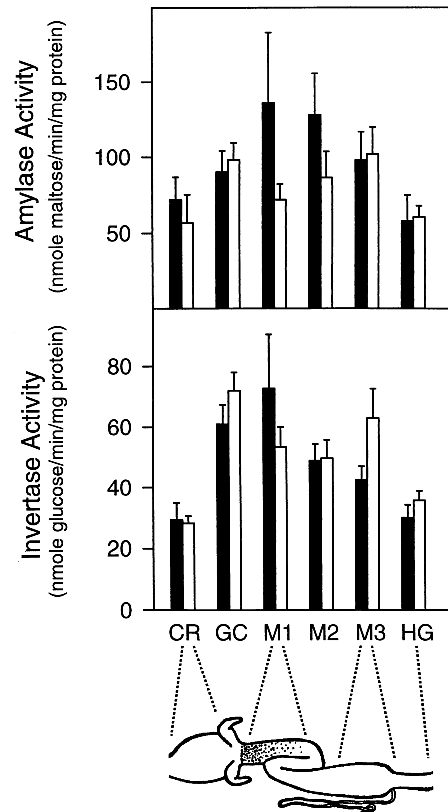

Fig. 1. The distribution of α-amylase (upper panel) and invertase (lower panel) activity in the alimentary canal of *D. punctata*, from fed (solid bars) and starved (clear bars) animals. Animals were starved for 18 h. The alimentary canal is drawn diagrammatically, and dashed lines separate the various regions assayed. Dots represent apparent endocrine cells expressing LMS and AST mRNA. CR, crop; GC, gastric caecae; M1–3, segments of the midgut in thirds, from anterior to posterior; HG, hindgut. Bars represent means $\pm$ SEM for 6 to 10 samples. There are no significant differences between enzyme activity in tissues of fed and unfed animals ($P < 0.5$), by using one-way ANOVA.

2. Materials and methods

2.1. Animals

The colony of *D. punctata* was maintained on lab chow and water at 27°C on a 12:12 h light:dark cycle as described previously [42]. All animals used were Day 3 adult males.

2.2. Enzyme distribution: tissue preparation

Insect midguts were dissected in low glucose saline (150 mM NaCl; 12 mM KCl; 10 mM $CaCl_2 \cdot 2H_2O$; 3 mM $MgCl_2 \cdot 6H_2O$; 4 mM HEPES; 0.01% D(+)-glucose; 2% FICOLL Type 400; pH 7.4) containing the protease inhibitors 1,10-Phenanthroline monohydrate, *N*-(α-Rhamnopyranosyloxyhydroxy-phosphinyl)-Leu-Trp (Phosphoramidon), and *N*-tosyl-L-phenylalanine chloromethyl ketone (TPCK) at 0.1 μM. The isolated midgut was defined as the region just posterior to the gastric caecae and just anterior to the Malpighian tubules (see Fig. 1).

For distribution studies, the crop, hindgut, and gastric caecae were separated from the midgut. The tissues were cut open to remove food particles, and the gut was divided into thirds. The tissue pieces were then sonicated or homogenized in fresh low glucose saline and centrifuged at 4000 $\times$ *g* for 5 min, at 4°C, to measure enzyme activity. Aliquots were taken from the supernatant to assay enzyme activity and to measure protein content, as described below.

2.3. Enzyme assay: α-amylase and invertase activity

The α-amylase and invertase assays were modified from previous protocols [21,37]. The assays were based on the ability of α-amylase to convert starch into maltose, and invertase to convert sucrose into glucose. Samples (40 μl) were incubated at 37°C for 20 min, with 40 μl saline, 40 μl buffer and 80 μl substrate. For α-amylase assays, the sub-

strate was 1% potato starch (amylum), and for invertase, the substrate was 1% sucrose. Standards comprising 0.1% D(+)-maltose monohydrate or D(+)-glucose, for the α-amylase and invertase assays, respectively, were incubated with saline, buffer, and substrate in the same manner. The buffer consisted of glacial acetic acid and sodium acetate, at pH 5.0 for α-amylase and pH 5.4 for invertase. After 20 min, the assay was terminated with 240 μl of a 1% 3–5-dinitrosalicylic acid solution containing 0.4N NaOH and 30% potassium/sodium tartrate, for 5 min at 100°C. The absorbency was measured at 540 nm.

Starch, D(+)-maltose monohydrate and 3–5-dinitrosalicylic acid were obtained from Fluka Biochemika, Caledon Laboratories Ltd. (Georgetown, ON, Canada). All other compounds were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.4. Enzyme release/activity studies

For the activity and release studies, midguts were ligatured and treated experimentally before enzyme assay of the tissues and the lumen contents. The midgut was separated from the crop, gastric caecae and hindgut, as one intact piece, and the food particles and peritrophic membrane were carefully removed with fine forceps. The midgut was then flushed out with a blunt syringe before ligature of both ends with fine silk thread. The tissues were suspended in 1ml of aerated low glucose saline at room temperature, with or without AST or LMS, for 60 min. The contents of the ligatured preparations were released into fresh saline by flushing out the guts, then processed as described above for assay of enzyme activity. The tissues were similarly processed.

Midguts were incubated in AST (Dippu-AST 7) or LMS at concentrations ranging from 10^{-11} M to 10^{-5} M. The peptide solutions were made in low glucose saline with glacial acetic acid (0.005%) or 1 N HCl, and neutralized with 1 N NaOH. Controls consisted of low glucose saline with a similar acid composition, and enzyme buffers containing acid ranged in pH from 5.0 to 5.4.

Dippu-allatostatin 7 (APSGAQRLYGFGLamide) and LMS (pQDVDHVFLRFamide) were custom synthesized by Research Genetics (Huntsville, AL, USA). AST(b)ϕ2 was synthesized as previously described [33]. All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.5. Protein determination

Aliquots of supernatant from all processed samples were used to determine the total protein content. The Bio–Rad Protein Assay (Melville, NY, USA) [7], by using γ-globulin or bovine serum albumen as standard, was used. Data are expressed as 'nmole maltose(glucose) per min per mg of protein.'

3. Results

3.1. Distribution of α-amylase and invertase in the midgut

Two of the principal carbohydrate-metabolizing enzymes in the midgut of insects are α-amylase and invertase; these enzymes are responsible for the conversion of starch to maltose and sucrose to glucose, respectively. We initially determined if there was a differential distribution of these enzymes in the midgut of Day 3 adult males (Fig. 1). For the purposes of this study, the midgut was divided into three regions, anterior, middle and posterior. Enzyme content of the adjacent tissue, crop and gastric caecae (anterior) and hindgut (posterior) is also shown (Fig. 1). Midguts were taken from animals that were fed normally or were starved.

Fig. 1 shows that enzyme was detected in all tissues of the alimentary canal. The majority of invertase activity was associated with the midgut, particularly the caecae and anterior midgut, in which enzyme activity was significantly higher than other gut tissues ($P < 0.05$). Higher levels of α-amylase activity were also found in the anterior region of the midgut ($P < 0.05$) but individual measurements of α-amylase activity in this region showed high variability. Starvation of animals for 18 h resulted in a marked increase in variability of enzyme activity in the anterior and posterior regions of the midgut, although activities were not significantly different from fed controls ($P < 0.5$).

3.2. Time course of enzyme activity and release

Ligatured, isolated midguts were incubated in cockroach saline (see Ref. [18]) for 2 h to determine the change, over a 2-h interval, in tissue content of the enzymes and the release of the enzymes into the lumen, both in the absence of peptides (control) and in the presence of 1 μM Dippu-AST 7. Samples were taken at 30-min intervals.

Fig. 2 shows α-amylase activity in the contents of the midgut lumen and in the midgut tissue over this period. Control values showed some variation but in general, fluctuated around a basal level of about 200–300 nmol maltose/min/mg of protein. Enzyme activity in midgut tissue was lower and less variable (Fig. 2 inset). Addition of Dippu-AST 7 to the incubation medium resulted in a gradual increase in α-amylase activity in the lumen contents during the first 90 min of incubation and a decline to control levels by 120 min. The level of activity was significantly greater than control values, and α-amylase activity in lumen contents was about sevenfold higher than activity in the midgut tissue homogenates at 90 min.

Fig. 3 shows invertase activity in the contents of the lumen and the midgut tissue over a 120-min incubation period. Control (untreated) values of enzyme activity remained at about 50 nmole glucose/min/mg of protein in the lumen contents and between 30 to 40 nmole glucose/min/mg of protein in midgut tissue homogenates (inset). Treatment of ligatured midguts with Dippu-AST 7 resulted

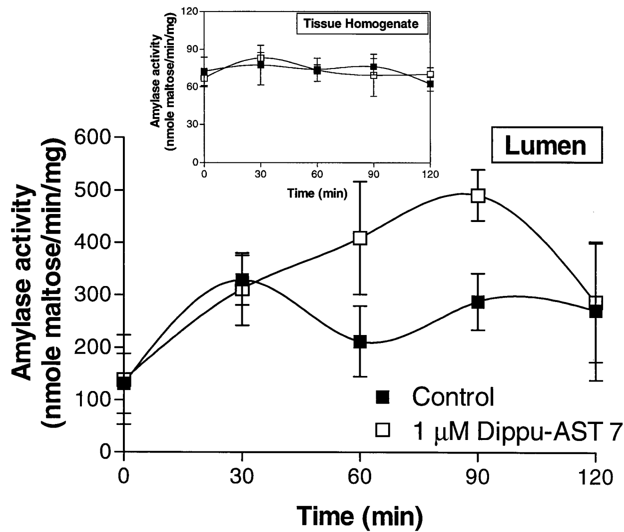

Fig. 2. Time course of change in α-amylase activity in midgut lumen contents (lumen) and tissue (tissue homogenate–inset) of *D. punctata* following treatment of ligatured midguts in vitro with 1 μM Dippu-allatostatin 7. Each point represents the mean of four measurements $\pm$ SEM. Significance was assessed by using two-way ANOVA. Treatment values are significantly different from control values at 90 min in the lumen ($P = 0.050$).

in an increase in enzyme activity by 60 min relative to control values and this elevation was also apparent at 90 min. No difference between control and treated values was noted in midgut homogenates over the incubation period. Invertase activity in lumen content was about threefold higher than in midgut homogenates at 90 min.

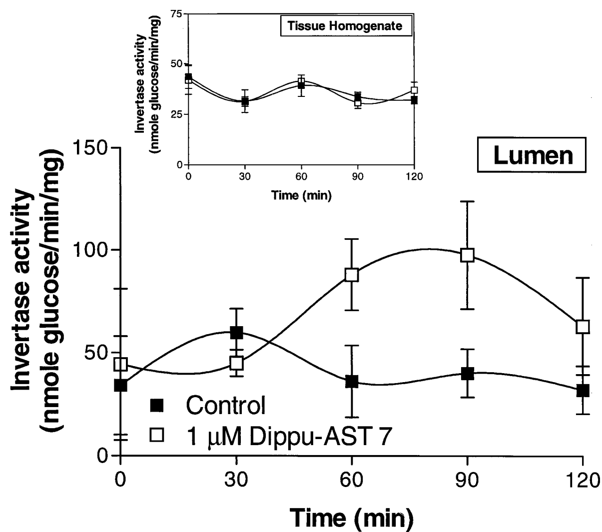

Fig. 3. Time course of change in invertase activities in midgut lumen contents (lumen) and tissue (tissue homogenate–inset) of *D. punctata* following treatment of ligatured midguts in vitro with 1 μM Dippu-AST 7. Each point represents the mean of four measurements $\pm$ SEM. Significance was assessed by using two-way ANOVA. Treatment values are significantly different from control values ($P = 0.045$) (60 + 90 min) in the lumen.

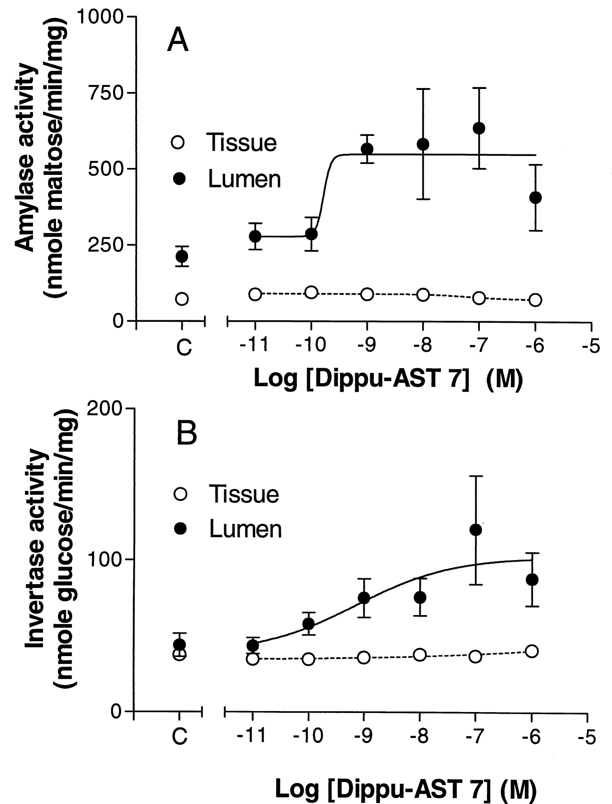

Fig. 4. Dose-response for α-amylase (A) and invertase (B) activities in midgut tissue and lumen contents of *D. punctata* as a result of treatment with Dippu-AST 7. The EC_{50} for α-amylase = 0.7 nM and for invertase = 56 nM. Control activity is represented by C. Each point represents the mean of 4–10 measurements $\pm$ SEM. For *midgut tissue*, there are no significant differences in α-amylase or invertase activities in the presence of Dippu-AST 7 relative to control values, by using ANOVA ($P > 0.5$). For *lumen contents,* activities are significantly higher than controls for α-amylase at 1, 10 and 100 nM ($P < 0.05$), and 1 and at 100 nM ($P < 0.01$) for invertase, as assessed by one-way ANOVA by using Newman–Keuls test for differences.

3.3. Dose response of allatostatin effect

In view of the elevation in lumen contents of both α-amylase and invertase activities 60 and 90 min after treatment with 1 μM Dippu-AST 7, we incubated ligatured midguts for a 60-min interval in the presence of several AST concentrations. Fig. 4 reveals a dose-dependent elevation in both invertase and α-amylase activities with increasing concentrations of Dippu-AST 7. The EC_{50} values for these effects were 0.7 nM and 56 nM for α-amylase and invertase respectively (Fig. 4A and B). Thus, α-amylase activity in midgut lumen is almost 100-fold more sensitive to the peptide treatment than invertase activity. Enzyme activities in midgut tissue homogenates did not change with increasing AST concentrations and were not significantly different from control values. A decline in enzyme activities at the highest AST concentration was noted for both α-amylase and invertase.

Several AST analogs that are potent inhibitors of juve-

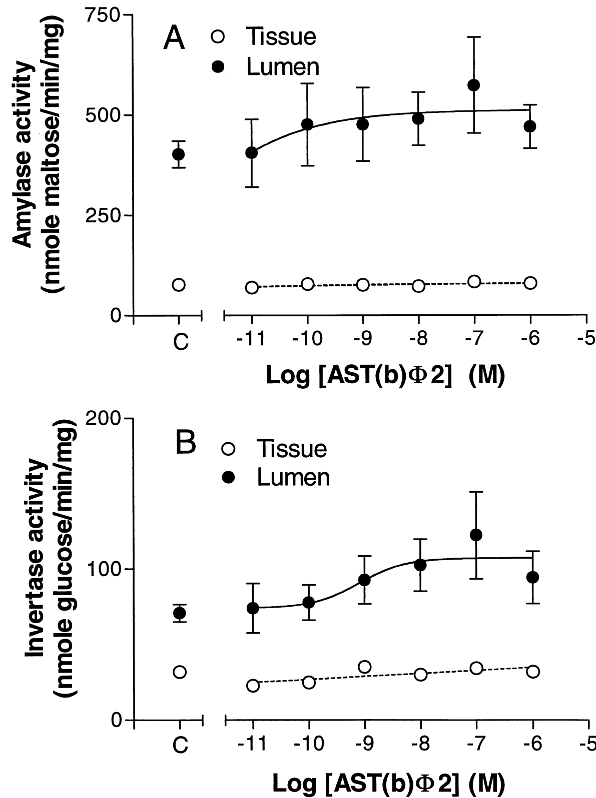

Fig. 5. Dose-response for α-amylase (A) and invertase (B) activity in midgut tissue and lumen contents of *D. punctata* as a result of treatment with AST analog AST(b)ϕ2. Control activity is represented by C. Each point represents the mean of 5 to 21 measurements $\pm$ standard error of the mean. In no instance is enzyme activity for analog treatment significantly different from control values ($P > 0.3$) as assessed by one-way ANOVA by using Newman–Keuls test for differences.

nile hormone biosynthesis have been developed recently [33]. Biologic activity of this analog can be attributed to persistence as a consequence of protease resistance and to the ability of these compounds to compete effectively at the level of the receptor(s). We have tested one of these peptidomimetics, (AST(b)ϕ2–a cyclopropyl-Ala, hydrocinnamic acid derivative of Dippu-AST 6) to determine whether it was also active in stimulating either α-amylase or invertase activity in the lumen contents or in the midgut tissue (see Ref. [33]). This analog was selected because of its almost complete resistance to degradation by midgut tissue [33]. Although it shows relatively low activity as an inhibitor of juvenile hormone production, this function may be unrelated to its ability to affect midgut enzyme activity, just as Dippu allatostatins show very different rank orders with respect to juvenile hormone production and modulation of muscle contraction [e.g. 26]. The use of this analog provides information on the specificity of the AST effect on the midgut enzyme-secreting cells and whether this compound effectively acts at the level of the midgut receptors for AST(s).

Fig. 5 shows dose-response curves for enzyme activity over a range of concentrations known to be active in inhibiting juvenile hormone biosynthesis [33]. All experiments were performed on ligatured midguts incubated for 60 min. The activity of α-amylase in both lumen contents and midgut homogenates following treatment with AST(b)ϕ2 are lower than those for comparable AST-treated midguts (Fig. 4), and in no instance are these values significantly different from the control values. Similarly, although mean invertase activities seemed somewhat elevated in lumen contents, in no case were the values significantly different from control values. In midgut tissue homogenates, no effect on either α-amylase or invertase activity was observed.

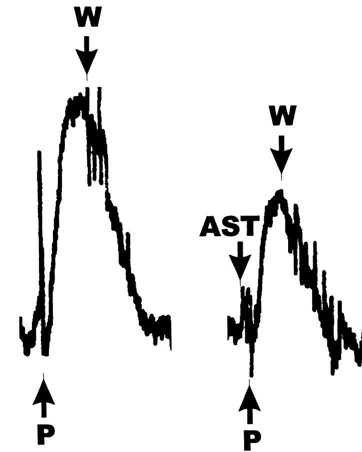

Fig. 6. Examples of sustained tonic contractions of the anterior portion of cockroach midgut after application of proctolin (P), or Dippu-AST 7 (AST) and proctolin (P). Washes are denoted by W, and arrows denote the point of application of test substances.

3.4. Effect of allatostatin on myotropic activity in midgut

Members of the AST family modulate the activity of muscle of the hindgut of *D. punctata* [25,26]. In particular, ASTs attenuate the effect of proctolin-induced contractions in hindgut muscle, in a dose-dependent fashion. This effect shows a high degree of specificity with respect to the AST employed and a rank order for the AST attenuation has been determined [26], with Dippu-AST 9, 8 and 7 ranking as the most potent of the inhibitors of proctolin-induced muscle contraction.

We have used Dippu-AST 7, because of its likely occurrence in midgut endocrine cells and its effect on enzyme activity in the midgut (Fig. 4), to determine if the peptide is able to modulate contractions of the muscle of the midgut. As has been demonstrated in hindgut [26], Dippu-AST 7 at a concentration of 500 nM is capable of inhibiting significantly the proctolin-induced contractions of *D. punctata* midgut muscle (see Fig. 6) (54.2% $\pm$ 14.2% inhibition, n = 4). In these experiments, Dippu-AST 7 was added to the midgut preparation just before proctolin addition (100 nM). Fig. 6 clearly shows that in the presence of Dippu-AST 7, contractions in the presence of proctolin were dramatically

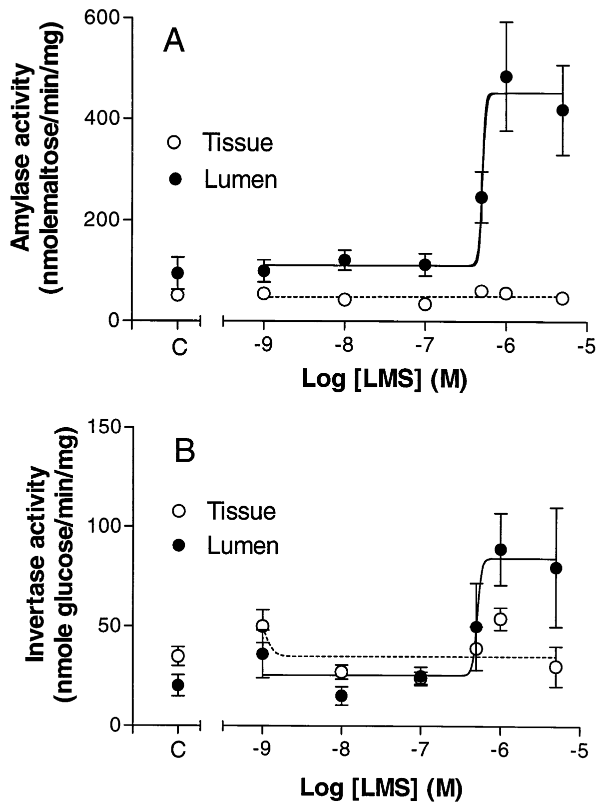

Fig. 7. Dose-response for α-amylase (A) and invertase (B) activity in midgut tissue and lumen contents as a result of treatment with leucomyosuppressin (LMS). The EC_{50} for α-amylase = 510 nM and for invertase = 510 nM. Control activity is represented by C. Each point represents the mean of 5 or more measurements ± SEM. For *midgut tissue*, there are no significant differences in α-amylase or invertase activities in the presence of LMS relative to control values, by using one-way ANOVA ($P > 0.5$). For *lumen contents,* activities are significantly higher than controls for α-amylase at 500 nM and 5 μM ($P < 0.001$), and at 500 nM ($P < 0.05$) and 1 and 5 μM ($P < 0.001$) for invertase, as assessed by one-way ANOVA by using Newman–Keuls test for differences.

attenuated. This peptide thus seems to be pleiotropic in its effects, affecting both digestive enzyme activity and muscle contraction.

3.5. Dose-response of leucomyosuppressin effect

LMS, another peptide identified in the midgut of cockroaches by both immunoassay and in situ hybridization [19], has been reported to exert similar pleiotropic effects, both in terms of inhibition of proctolin-induced contractions (in *D. punctata*) [18] and in stimulation of α-amylase secretion in weevil midgut [31]. To determine the effect of LMS in *D. punctata* on enzyme secretion, we have assayed enzyme release from ligatured midgut. We incubated ligatured midguts for 60 min, alone or in the presence of a range of concentrations of LMS, as described for the AST experiments above. LMS stimulated a dose-dependent increase in both α-amylase and invertase activity in the lumen contents of the midguts (Fig. 7A and B, respectively). The threshold response for α-amylase activity was observed at a concentration of about 100 nM LMS, with a half-maximal response at approximately 500 nM, and a maximal response at 1 μM LMS.

The effects of LMS on invertase activity were more variable. Although a 5 μM concentration of LMS elicited a significant increase above control levels ($P < 0.05$), this value was not significantly higher than the 1 nM response ($P < 0.1$) and only concentrations greater than 1 μM LMS elicited a response significantly greater than both the control and the 1 nM response ($P < 0.001$). Variability in invertase activity was also noted in tissue samples, although values were not significantly different from controls ($P > 0.5$).

The EC_{50} values for the elevation in enzyme activities following LMS treatment were 510 nM for both α-amylase and invertase (Fig. 7A and B). This is almost 1000-fold greater than the EC_{50} for AST in the case of amylase, and tenfold in the case of invertase. It is also striking that α-amylase and invertase activity in midgut lumen show identical sensitivities to LMS treatment, whereas in the case of Dippu-AST 7, the sensitivities differ by 100-fold (see above).

4. Discussion

4.1. Enzyme distribution

In most insect species, the midgut is structurally and functionally differentiated along its length [5] and is often separated into regions of water absorption, enzymatic activity and nutrient absorption. In general, the anterior midgut is usually higher in carbohydrase activity, whereas the posterior region shows higher protease activity [43]. This suggests that enzymes with different pH optima are likely to be distributed differentially in the midgut.

Amylase and invertase activities are detected in all tissues of the alimentary canal of *D. punctata* although the majority seems to be in the anterior portion of the midgut. This is in agreement with studies in other insect species, in which the highest α-amylase and invertase activity is associated primarily with the gastric caecae or the anterior region of the midgut [10,23,40]. However, few studies have described enzyme activity in the crop or hindgut, as demonstrated here, and this suggests that there may be species-specific differences in enzyme activity or distribution. α-Amylase and invertase activities in *D. punctata* midguts do seem to fluctuate under different feeding regimes. Although there are no significant changes in enzyme activity along the length of the midgut, noticeably higher variability in measurements of α-amylase activity were observed in the anterior region following starvation of cockroaches for 18 h ($P < 0.5$). This variability suggests that in some cases, the levels of enzyme activity are fluctuating. Decreases in enzyme activity in midguts of scale insects after a 24 h period

of starvation have been described [21]. It remains to be determined if longer periods of starvation will result in a corresponding reduction in enzyme activity.

Other studies have described sex- and species-specific differences in enzyme activity associated with differences in time after feeding [23]. For example, α-amylase and invertase activity are generally highest in the anterior region of the midgut of recently fed animals, and are often higher in females than in males [1,10,23,40]. It will be instructive to compare the distribution of enzyme activity in the alimentary canal of *D. punctata* after various periods of feeding, and between males and females. We have not detected histochemical differences in LMS mRNA expression in midguts of males or females, although this has not been quantified experimentally (M.F., unpublished observations).

4.2. Regulation of enzyme activity

Enzyme secretion in insect midgut has been suggested to be regulated by factors from the brain median neurosecretory cells (MNSCs), the salivary glands, and endocrine cells of the midgut (see below). For example, although α-amylase activity in the midgut of *P. americana* seems to originate from the saliva of the salivary glands [1,3], midgut invertase secretion is regulated by hormonal factors from the salivary glands [2].

The MNSCs regulate midgut α-amylase activity in *Tenebrio molitor* [22], invertase activity in *Dysdercus cingulatus* [28], and protease activity in *Calliphora erythrocephala* [44] and *D. cingulatus* [28]. However, whether the MNSCs are only indirectly involved, by stimulating food consumption, and thereby triggering digestive enzyme secretion, is unclear.

Hormones from the midgut itself seem to regulate enzyme secretion [17,40]. The midgut contains endocrine cells of both the open and closed variety, some of which may monitor the nutrient content and stretch of the gut to trigger the release of digestive enzymes [17]. Numerous 'neuropeptides', including both ASTs and LMS, have been detected in putative endocrine cells of the midgut (e.g. [9,19,45,49]). Some of these peptides likely act in a paracrine fashion on neighboring enzyme-containing cells. Changes in peptide content in such cells are correlated to the states of digestion of the animal. For example, FMRFamide-like immunoreactivity decreases in putative endocrine cells of the midgut of recently fed adult female mosquitoes [9]. Although peptides are also associated with nerves traversing the length of the midgut, morphologic evidence argues against the nervous control of enzyme secretion [10].

Our experiments have shown that both Dippu-AST 7 and LMS can increase enzyme activity in lumen contents of midgut of *D. punctata* in a dose-dependent fashion. This suggests that these peptides enhance the release of at least these two enzymes from the midgut cells into the lumen. Alternatively or in addition, the peptides could regulate the activation of these enzymes, for example by modifying enzyme structure through phosphorylation. LMS, as well as the sulfakinins [30], often considered members of the FaRP family, have also been shown to stimulate α-amylase secretion in the weevil midgut [31]. The differential expression of both AST and LMS mRNA in apparent endocrine cells in the anterior region of *D. punctata* midgut [19,49], and the presence of immunoreactive nerves along the midgut [18, 49], suggest that both peptides act throughout the midgut in several capacities, including regulation of enzyme secretion. However, both AST and LMS mRNA have also been detected in MNSCs of the brain of *D. punctata* [11,12], and immunoreactivity has been shown in the corpora cardiaca [19,41]. Hence, regulation of midgut enzyme activities by peptides originating in cells of the brain or in other CNS regions cannot be ruled out in these studies.

There is large variability in measurements of invertase activity, relative to those of α-amylase activity, in the midguts in response to peptide treatment. This suggests that there are multiple responses to the peptides in the midgut, with very different thresholds. A similar differential response has been demonstrated for ASTs; for example, this peptide family shows very different EC_{50} values and rank order in terms of ability to inhibit juvenile hormone biosynthesis and to modulate hindgut proctolin-induced muscle contraction [25,26,41].

There are no doubt numerous other peptides present within endocrine cells of midgut of cockroaches, as earlier work suggests [8,9,14,15]. Endocrine cells containing both AST and LMS mRNA are differentially distributed along the midgut [19,49] and the peptides may therefore regulate digestion in the cockroach midgut, in different regions, perhaps in response to different feeding cues, or stages of digestion.

It remains uncertain whether AST and LMS stimulate production of the enzymes, enhance release of the enzymes from the midgut epithelium, or cause activation of inactive forms (e.g. by directly modifying enzyme structure through phosphorylation). Histochemistry, immunochemistry, and in situ hybridization may provide partial answers to these questions, so that the relationship between the enzyme-containing cells and the endocrine cells can be assessed. At least two types of endocrine cells have been reported in cockroach midgut [16], 'open' and 'closed' types (see Refs. [16,17]) and both AST- and LMS-immunoreactive cells in the midgut seem to be primarily of the open type, traversing the entire midgut wall, from lumen to basal lamina [19,49]. Such cells have been hypothesized to act as 'primary sensors' that detect the nutrient content of the midgut lumen [17]. In response to appropriate signals, these cells could release their peptides in a paracrine fashion (both basally and laterally), to effect release from neighboring enzyme-containing cells. Whether there are specific associations between either AST- or LMS-containing cells and specific epithelial enzyme-secreting cells (e.g. amylase or invertase)

is unknown. Similarly, we do not know if epithelial cells contain or secrete more than one enzyme type. Our results, however, indicate that both of these enzymes are affected by the peptides, suggesting that either both enzymes are contained within a single cell type or that the peptides exert their effects on several types of epithelial cells. In either case, the presence of receptors for both AST and LMS on midgut cells is implied and recent work has demonstrated that specific AST receptors are present in midgut tissue of *D. punctata* [6].

It should be noted that the midgut is not a homogeneous tissue–there are at least three tissue types in midgut, principally muscle and epithelial tissue with lesser amounts of endocrine (neural) tissue. The two principal tissues both seem to be targets for the ASTs and LMS: midgut muscle responds directly to AST and LMS treatment through an attenuation of proctolin-induced contractions (Fig. 6 and Ref. [18]), and in the epithelium, there is an enhancement of release or activation of amylase and invertase. At present, it is unknown if there are two distinct receptor subtypes associated with these tissues but recent data suggest the presence of only a single receptor subtype for ASTs in midgut tissue [6]. Characterization and isolation of receptors from these two tissues will provide the answer to this question. It will also be important to assess the abilities of peptides such as the AST family (i.e. rank order) to increase enzyme activities on the one hand and modulate myotropic activity on the other.

The absence of a significant response of midgut enzyme activity to treatment with the AST analog AST(b)ϕ2 (Fig. 5) suggests that this compound acts exclusively on the corpora allata, to inhibit the production of juvenile hormone [33]. Although this compound is moderately active as an inhibitor of juvenile hormone production ($IC_{50} = 1.5\ \mu M$) [33], it is much less active than other peptide analogs, suggesting that its interaction with the receptor in the corpora allata is suboptimal. At present, it is unknown if this compound shows activity with respect to modulation of myotropic activity. However, the absence of a significant effect on midgut enzyme activities indicates that there are likely differences between the receptors in the corpora allata and in the midgut epithelium. In addition, it suggests that because of its low potency with respect to juvenile hormone production, its concentration may have been suboptimal for an effect on enzyme activities.

Peptides, including ASTs and myosuppressins, may function in the cockroach midgut to both stimulate release of digestive enzymes and to alter rate of movement of food through the gut. These two actions could ultimately result in an improved efficiency of digestion and an increased efficiency of uptake of nutrients from the midgut. Thus, the pleiotropic effects of these peptides may be coordinated, to ultimately improve the absorption of nutrients from the midgut.

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