Available online at www.sciencedirect.com
SCIENCE DIRECT®
Regulatory Peptides 115 (2003) 171–177
REGULATORY PEPTIDES
www.elsevier.com/locate/regpep

Allatostatin gene expression in brain and midgut, and activity of synthetic allatostatins on feeding-related processes in the cockroach *Blattella germanica*

Ruth Aguilar, José L. Maestro, Lluïsa Vilaplana, Núria Pascual, Maria-Dolors Piulachs, Xavier Bellés*

Department of Physiology and Molecular Biodiversity, Institut de Biologia Molecular de Barcelona (CSIC), Jordi Girona 18, Barcelona 08034, Spain

Received 25 March 2003; received in revised form 3 June 2003; accepted 3 June 2003

Abstract

Allatostatins of the YXFGLamide group were discovered in cockroaches through their capacity to inhibit juvenile hormone biosynthesis. Here, we assess the occurrence of preproallatostatin (preproAST) mRNA in the brain and midgut of adult females of the cockroach *Blattella germanica*, and estimate brain and midgut preproAST mRNA levels during the first reproductive cycle. Reverse transcription polymerase chain reaction (RT-PCR) shows that brain preproAST mRNA levels increase slightly during the gonadotrophic cycle, and remain high during ootheca transport. In the midgut, preproAST mRNA levels decline around the middle of the gonadotrophic cycle. The pattern of allatostatin expression in gut tissues suggests that these peptides play roles related to feeding and nutrition. Our results have shown that synthetic allatostatins inhibit hindgut motility and activate midgut α-amylase secretion. In addition, injected allatostatins inhibit food consumption, which might be connected to the above activities.

Keywords: Allatostatin; *Blattella germanica*; German cockroach; Gene expression; Juvenile hormone; Food intake control

1. Introduction

Allatostatin peptides with a typical YXFGLamide C-terminal sequence were identified from brain extracts of the cockroach *Diploptera punctata* through their inhibitory activity on juvenile hormone biosynthesis by the corpora allata [1,2]. Again in cockroaches, it was later shown that these allatostatins elicit antimyotropic effects in the gut [3,4] and in the dorsal vessel [5], inhibit vitellogenin release by the fat body [6], and stimulate carbohydrate enzyme activity in the midgut [7]. Moreover, orthologous peptides have been reported in other species of Dictyoptera, and in Orthoptera, Lepidoptera, Diptera, and Phasmida, as well as in Crustacea Decapoda, although they inhibited juvenile hormone production only in cockroaches and crickets [8–13]. In addition to peptide identification, cDNAs or genes encoding for YXFGLamide allatostatins have been cloned and sequenced in Dictyoptera, Orthoptera, Lepidoptera, and Diptera [8,14–17]. These studies revealed that a single allatostatin gene encodes multiple similar YXFGLamide peptides that are posttranslationally cleaved by processing enzymes.

In the German cockroach *Blattella germanica*, four allatostatins have been identified from brain extracts [18], and nine more have been deduced from the gene that encodes the allatostatin prohormone [14]. Using an enzyme-linked immunosorbent assay (ELISA), allatostatin contents have been measured in the brain and midgut of *B. germanica* females, showing that peptide concentrations increase from adult emergence to the formation of the first ootheca, and remain high during the period of ootheca transport [19]. Moreover, immunocytochemical techniques have revealed allatostatin-like material in brain neurons, most of them unrelated to juvenile hormone regulation, in axons extended along the length of the gut and originating in neuronal bodies located in the brain and in the last abdominal ganglion, and in midgut endocrine cells [20]. This anatomical ubiquity suggests that allatostatins play a number of diverse physiological functions. In the present work, we have estimated the levels of preproallatostatin

* Corresponding author. Tel.: +34-93-4006124; fax: +34-93-2045904.
E-mail address: xbragr@ibmb.csic.es (X. Bellés).

0167-0115/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0167-0115(03)00165-4

(preproAST) mRNA in the brain and midgut of *B. germanica* adult females during the first reproductive cycle. In addition, the activities of synthetic allatostatins on myotropic midgut α-amylase release and food intake assays were tested. Results suggest that allatostatins, besides some other functions, are involved in the regulation of feeding-related processes.

2. Materials and methods

2.1. Insect rearing and tissue collection

Adult females of *B. germanica* (L.) (Dictyoptera, Blattellidae) were obtained from a colony fed dog chow and water and reared in the dark at 30 ± 1 °C and 60–70% relative humidity. Freshly moulted virgin females were isolated and used at the appropriate physiological ages within the first gonadotrophic cycle, which were assessed by measuring the basal oocyte length [21]. To study preproAST mRNA levels during the period of ootheca transport, mated specimens were used because they retain the ootheca throughout embryogenesis. Tissues were carefully dissected to ensure that they were anatomically homogeneous, and washed repeatedly in Ringer's saline (9 g/l NaCl, 0.2 g/l KCl, 0.2 g/l $NaHCO_3$, and 0.2 g/l $CaCl_2$) to remove all traces of haemolymph.

2.2. RNA isolation and cDNA preparation

Brains, midguts, and ovaries used for RNA extraction were immediately frozen in liquid nitrogen and stored at − 80 °C until use. Total RNA was isolated using the GenElute Mammalian Total RNA kit (Sigma, St. Louis, MO, USA). All RNA samples were treated with RQ1 DNase (Promega, Gaithersburg, MD, USA). cDNAs were prepared as described by Zhu et al. [22]. Aliquots of 0.5, 0.8, and 1 μg of total RNA extracted from brains, midguts, and ovaries, respectively, were retrotranscripted using SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA) and random hexamers (Promega) in a reaction volume of 20 μl. The reverse transcription products were then diluted to 40 μl with TE buffer, pH 8.0. Negative controls without the reverse transcriptase step were used to check for genomic contamination.

2.3. Northern blot analysis

A total of 15 μg of RNA from female brains was subjected to electrophoresis in 1.2% agarose gel containing formaldehyde, and was then transferred to Hybond-N^+ membranes (Amersham Biosciences, Buckinghamshire, UK). A 606-bp fragment of the allatostatin preprohormone cDNA (from positions 258 to 863; see Ref. [14]) was used as a probe. Probe labelling and detection of the corresponding preproAST mRNA band were carried out with the Gene Images random prime labelling module and detection system from Amersham, following the supplier's protocols.

2.4. Polymerase chain reaction (PCR) and Southern blot analysis

cDNA was used as PCR template. The primer pair (forward, 5′-GCGTCTTTATGATTTCGGACTTGGG-3′; and reverse, 5′-CCAAGACCAAAGGAAAACCTGTG-3′) were used to amplify a fragment of the allatostatin preprohormone cDNA, which includes 11 of 13 allatostatins encoded in the *B. germanica* preproAST gene. As a reference, cDNA corresponding to 16*S* rRNA, using forward 5′-TTACGCTGTTATCCCTTA-3′ and reverse 5′-CGCCTGTTTATCAAAAACAT-3′, was amplified in parallel for each cDNA sample. To obtain semiquantitative mRNA levels, a nonsaturating number of cycles in the PCR system were used. After PCR amplification, an aliquot of the total 50-μl reaction was fractionated in a 1.2% agarose gel and transferred onto a BioBond Nylon membrane (Sigma) for Southern blot analysis. Detection was carried out as for Northern blot.

2.5. Myotropic assay

Peptides were tested on the hindgut and foregut of *B. germanica* females in a standard organ bath, as previously described [23]. The activity was calculated as the difference of the mean of the force produced by the tissue 2 min after and 2 min before the treatment.

2.6. Enzyme release and α-amylase assay

The methodologies for enzyme release in vitro and α-amylase assay were modified from Ref. [7]. Basically, midguts from 5- to 6-day-old fed females were dissected out, ligated at both ends, and incubated in saline (154 mM NaCl, 2.7 mM KCl, 1.8 mM $CaCl_2$, 22 mM glucose, 5 mM Hepes, pH 6.8), with or without allatostatins. After the treatment, the contents of the incubated midguts were released into 100 μl of Ringer's saline. α-Amylase activity of midgut content was tested by measuring the capacity of midgut content to convert starch into maltose. The concentration of maltose was calculated in a colorimetric assay using 3,5-dinitrosalicylic acid as colour reagent and a maltose standard curve as reference. Absorbances were measured at $\lambda = 450$ nm.

2.7. Feeding bioassay

Feeding bioassay was performed as previously reported [23]. Briefly, freshly ecdysed adult females were starved for 48 h, injected with saline or with the corresponding allatostatin, and provided with carrot ad libitum. Animals were fed for 5 h, which guarantees that carotenoids were not yet

detected in the faeces, and then the whole gut was dissected out and extracted with methanol. Carotenoid concentration in the methanolic extracts was estimated by spectrophotometric measurement of the absorbance at 450 nm. The total weight of carrot ingested was estimated by interpolation on the standard curve constructed using methanolic extracts with increasing amounts of lyophilised carrot.

2.8. Synthetic allatostatins

B. germanica allatostatins BLAST-1 (LYDFGLa), BLAST-2 (DRLYSFGLa), BLAST-3 (AGSDGRLYSFGLa), and BLAST-4 (APSSAQRLYGFGLa) [18] were synthesised using standard Fmoc chemistry. The analogue of BLAST-2 with the C-terminus in free acid form (BLAST-2-COOH) was also synthesised following the same methodology.

3. Results

3.1. Northern blot analysis and tissue specificity of preproallatostatin gene expression

Northern blot analysis using total RNA isolated from brain tissues revealed a single 5.8-kb transcript corresponding to preproAST mRNA (Fig. 1A).

The possible occurrence of preproAST mRNA in brains, midguts, and ovaries from 1-day-old (previtellogenic) and 5-day-old (vitellogenic) females was studied using reverse transcription polymerase chain reaction (RT-PCR). A 606-bp product, corresponding to the expected fragment that includes 11 of 13 allatostatins, was amplified from brains

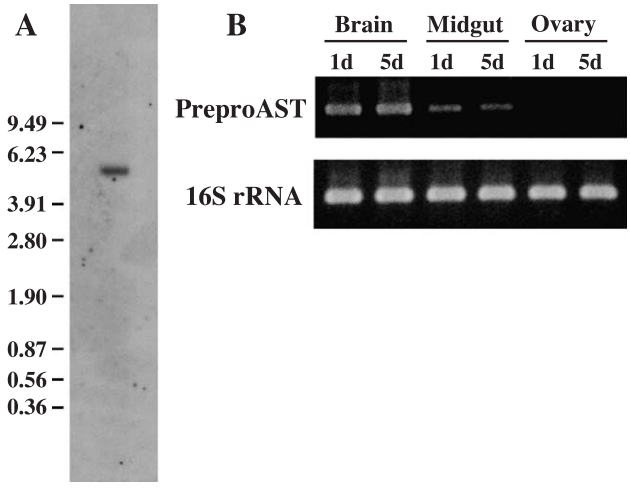

Fig. 1. (A) Northern blot analysis of RNA (15 μg) isolated from *B. germanica* brain tissues and hybridised with a cDNA probe for preproallatostatin. Sizes of molecular weight markers are indicated on the left of the figure. (B) RT-PCR amplification of preproallatostatin mRNA and 16*S* rRNA in brain, midgut, and ovary tissues of 1- and 5-day-old *B. germanica* adult females (3d and 5d, respectively). A total of 10 μl of each PCR product was separated on a 1% agarose gel.

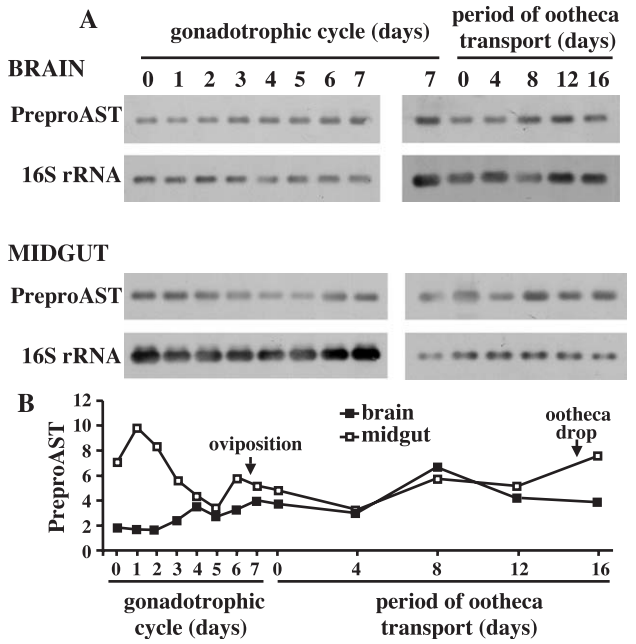

Fig. 2. Preproallatostatin (PreproAST) mRNA and 16*S* rRNA levels in brain and midgut of *B. germanica* females. (A) Southern blot showing preproAST RT-PCR levels on each day of the first gonadotrophic cycle and on selected days during the period of ootheca transport. The Southern blots are representative of two to three replicates. (B) Densitometry of the bands shown in (A). Absorbance is expressed as arbitrary units; the results have been corrected with respect to rRNA values, and those from the gonadotrophic cycle have been normalised with those of the period of ootheca transport using day 7 as a reference, which is present in both gels.

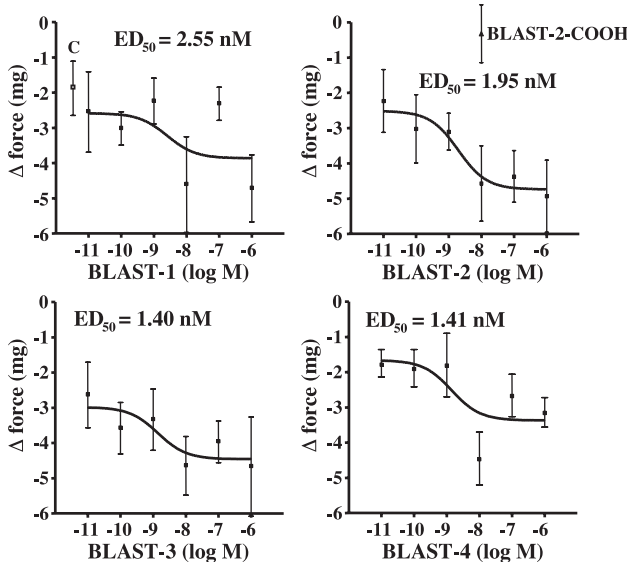

Fig. 3. Inhibitory effect of BLAST-1, BLAST-2, BLAST-3, BLAST-4, or BLAST-2-COOH on hindgut motility in *B. germanica* females. Results (mean ± S.E.M.; $n = 5–8$) are expressed as the difference of the mean of the force produced by the tissue during the 2 min after and before the treatment. ED_{50} for each allatostatin is also shown. "C" indicates values for water control experiments. The activity of BLAST-2-COOH at 10^{-8} M is significantly lower than that of BLAST-2 at the same concentration (Student's *t* test; $P < 0.05$).

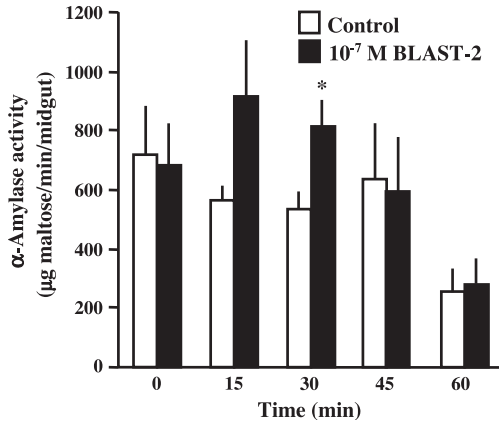

Fig. 4. α-Amylase activity in the contents of midguts of *B. germanica* females incubated at different times with 10^{-7} M BLAST-2. Results are expressed as the mean ± S.E.M. ($n = 3–29$). The asterisk indicates a significant difference between treated and control (Student's *t* test; $P < 0.05$).

and midguts, although it was more abundant in the amplification from brain cDNA (Fig. 1B). No amplification was obtained when using cDNA from ovaries (Fig. 1B).

3.2. Preproallatostatin mRNA levels in brain and midgut tissues

PreproAST mRNA levels were estimated using semi-quantitative RT-PCR in brain and midgut tissues during the female reproductive cycle. Brain levels of preproAST mRNA slightly increased from days 1 to 7 (when oviposition occurs) of the first gonadotrophic cycle and remained high, although with some fluctuations, during the period of ootheca transport (Fig. 2). This period finishes between days 14 and 16 after the formation of the ootheca, when it is dropped.

In the midgut, preproAST mRNA levels were high after adult emergence, declined around the middle of the gonadotrophic cycle, and recovered thereafter (Fig. 2).

3.3. Activity of allatostatins on gut motility

The peculiar pattern of preproAST mRNA in midgut tissues suggested that allatostatins may have a role related to feeding and nutrition. To begin with, we studied the activities of allatostatins BLAST-1, BLAST-2, BLAST-3, and BLAST-4, and the free acid analogue of BLAST-2 (BLAST-2-COOH) upon foregut and hindgut motility. None of the peptides showed any effect on foregut motility (results not shown). Conversely, each of the four allatostatins showed a moderate inhibitory effect on both frequency (not shown) and amplitude (Fig. 3) of hindgut contractions, with an ED_{50} within the nanomolar range and a maximum activity (between 4 and 5 mg of decreasing force) at 10^{-8} M. The analogue BLAST-2-COOH was inactive (Fig. 3).

3.4. Activity of allatostatins on α-amylase secretion by the midgut

In a preliminary series of experiments, we studied the release of α-amylase in vitro as a function of the incubation time. For this purpose, isolated midguts were incubated for 15, 30, 45, and 60 min in the absence (control) or presence of 10^{-7} M BLAST-2; then, lumen content was assayed for α-amylase activity. Enzymatic activity in controls remained around 600 µg maltose/min/midgut during the first 45 min and dropped to around half this value at 60 min (Fig. 4). In treated midguts, the pattern was different, showing the highest values of enzyme release at 15 and 30 min, the latter being significantly higher than the respective control (Fig. 4). Therefore, the comparative study of all allatostatins was carried out through incubations of 30 min.

The comparison of the dose–response results for the four allatostatins revealed that BLAST-1 was the most active, eliciting an 84% increase of α-amylase secretion at 10^{-10} M, although no significant effect was seen at higher or lower doses. BLAST-2 showed a significant 53% increase of enzyme secretion only at 10^{-7} M, and BLAST-3, BLAST-

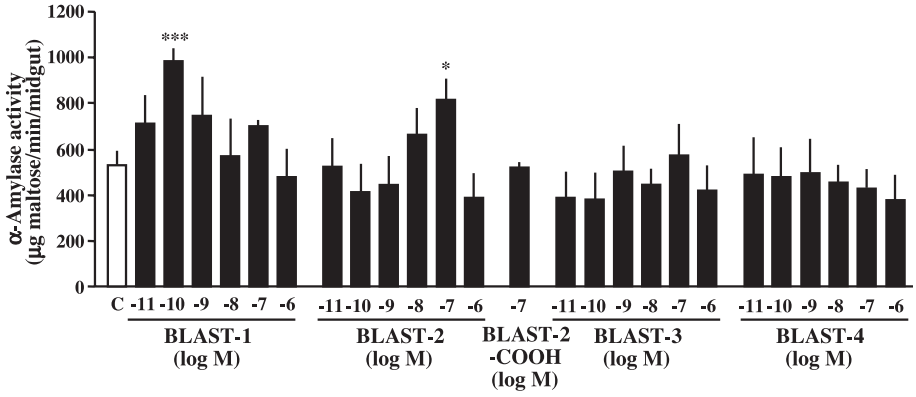

Fig. 5. α-Amylase activity in the contents of midguts of *B. germanica* females incubated 30 min with BLAST-1, BLAST-2, BLAST-3, BLAST-4, or BLAST-2-COOH. Results are expressed as the mean ± S.E.M. ($n = 4–29$). Asterisks indicate significant differences (Student's *t* test) with respect to control (C, white column) (*$P < 0.05$; ***$P < 0.001$).

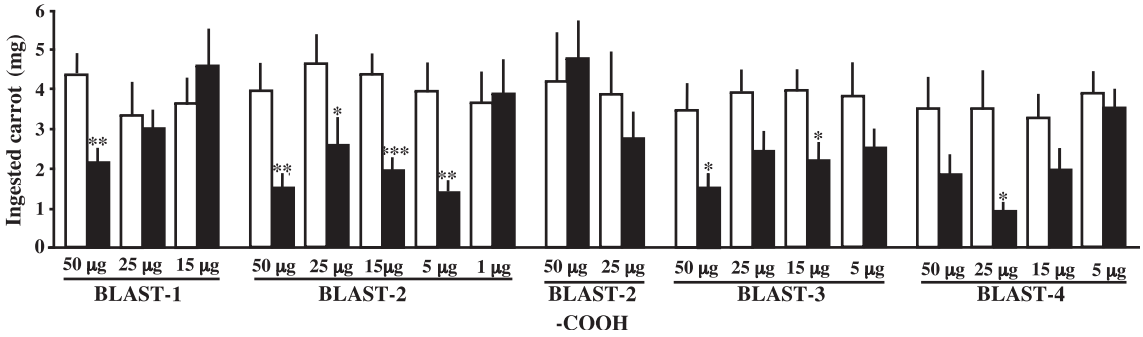

Fig. 6. Food intake inhibition induced by BLAST-1, BLAST-2, BLAST-3, BLAST-4, or BLAST-2-COOH in *B. germanica* following the carrot assay. Results (treated: black columns; control: white columns) are expressed as mean ± S.E.M. ($n = 12–24$). Asterisks indicate significant differences (Student's t test) with respect to control (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

4, and BLAST-2-COOH were inactive at all the concentrations tested (Fig. 5).

3.5. Activity of allatostatins as food intake inhibitors

Allatostatins BLAST-1, BLAST-2, BLAST-3, BLAST-4, and BLAST-2-COOH were tested as food intake inhibitors using the carrot assay and peptide doses from 1 to 50 µg per specimen. BLAST-2 was the most active peptide, resulting in ca. 60% inhibition of food intake at doses between 5 and 50 µg (Fig. 6). BLAST-1 was the less active, inducing ca. 50% inhibition at a dose of 50 µg (Fig. 6). BLAST-3 and BLAST-4 showed intermediate activities, whereas the analogue BLAST-2-COOH was inactive (Fig. 6).

4. Discussion

Northern blot analysis of brain RNA from *B. germanica* revealed a single preproAST mRNA transcript of approximately 5.8 kb (Fig. 1A). Using similar procedures and the same tissue, Ding et al. [24] revealed single transcripts of preproAST mRNA of 5.0 and 9.2 kb in the cockroaches *D. punctata* and *Periplaneta americana*, respectively. In the desert locust *Schistocerca gregaria*, Vanden Broeck et al. [25] detected a preproAST transcript of 2.4 kb in both central nervous system and midgut tissues. In Lepidoptera, Northern blot analysis in the gut and central nervous system tissues revealed a single preproAST transcript of 1.6 kb in *Helicoverpa armigera* [15] and of 2.1 kb in *Bombyx mori* [26]. These results indicate that there is a considerable variability in length of the preproAST mRNA, at least comparing Lepidoptera, Orthoptera, and Dictyoptera species. In *B. germanica*, the coding region for preproAST protein comprises 1128 nucleotides [14] that, compared with the Northern blot estimated length for the whole mRNA (Fig. 1A), indicates the occurrence of long untranslated regions in the mRNA.

Our results show the occurrence of preproAST mRNA in brain and midgut tissues, but not in the ovaries. In addition, semiquantitative nonsaturated RT-PCR suggests that the relative amount of preproAST mRNA in the brain is higher than in midgut in the two ages tested, 1- and 5-day-old females (Fig. 1B). The occurrence of preproAST mRNA in the brain and midgut is consistent with previous studies in a number of cockroach species. In situ hybridisation studies revealed the presence of preproAST mRNA in cell bodies of brain neurones of *D. punctata* [27] and *P. americana* [24], and in endocrine cells of *D. punctata* midgut [28,29]. In *B. germanica*, Maestro et al. [20] reported the occurrence of allatostatin immunoreactive material in several areas of the brain, ventral nerve cord, and stomatogastric nervous system. Furthermore, numerous immunoreactive endocrine cells were observed to be evenly distributed over the whole midgut epithelium. Concerning the tissue specificity, Garside et al. [30] reported low but significant levels of DippuAST mRNA in ovaries of *D. punctata*, although they did not find any cell body being immunoreactive against allatostatins, either in oviducts or in ovaries.

In our study, brain preproAST mRNA levels showed a modest increase through the first gonadotrophic cycle of the female, and remained high during ootheca transport (Fig. 2). This pattern differs from that reported by Vilaplana et al. [19] for allatostatin brain contents measured by ELISA, which shows a 12-fold increase from days 2 to 5 of the first gonadotrophic cycle, remaining approximately stable afterwards. The differences between peptide and mRNA levels, especially at the beginning of the gonadotrophic cycle, suggest that mechanisms other than transcriptional regulation contribute to the modulation of allatostatin concentration. These mechanisms may include regulation of translation, processing of the precursor protein, and peptide catabolism.

The pattern of preproAST mRNA in brain tissues observed in our study contrasts with that of juvenile hormone biosynthesis, which is cyclic and shows a sharp peak on day 6 [21,31,32]. Although allatostatins were isolated through their inhibitory activity on juvenile hormone production by the corpora allata [1,2], immunocytochemical studies have revealed that allatostatin material in brain cells that project to corpora allata accounts only for a small percentage of the total amount observed in other areas

of the brain (for *B. germanica*, see Ref. [20]). These observations may explain why the pattern of juvenile hormone biosynthesis is not correlated with that of allatostatin production. Moreover, the ubiquitous occurrence of allatostatin material in distinct cells of the insect brain, including interneurons, suggests that these peptides may play an additional, more general neuromodulatory role (e.g., see Ref. [9]). Indeed, a clear neuromodulatory role of allatostatins on acetylcholine and glutamate actions on the stomatogastric neuromuscular junction in the crab *Cancer borealis* has been described [33].

PreproAST mRNA levels in midgut show a decrease around the middle of the gonadotrophic cycle. Interestingly, the mRNA pattern is approximately inverse to that of food consumption [32], which shows maximal values between days 3 and 5, but contrasts with that of peptide concentration measured by ELISA, which steadily increase from days 0 to 7 [19]. Again, differences between mRNA and peptide patterns are observed, and the occurrence of posttranscriptional regulatory mechanisms is suggested. In addition, in the case of midgut, the differences in the pattern of allatostatin peptide [19] and mRNA may be explained because of the different peptide and mRNA contributions of axons from cell bodies outside the midgut and intrinsic midgut endocrine cells.

The pattern of allatostatin expression in gut tissues led us to test the effect of allatostatins in different feeding-related functions. Firstly, the activity of allatostatins upon gut musculature contraction was tested. Allatostatins showed inhibitory activity on hindgut contractions, on both frequency and amplitude, but no activity was detected on foregut assays. Contrarily to the inhibitory activity on juvenile hormone production, which has been proven only in cockroaches and crickets, the inhibitory activity of allatostatins on gut contractions has been reported in many insect species of different orders, and even in crustaceans [9,11,34]. In cockroaches, antimyotropic activity induced by allatostatins has been reported on the foregut of *Leucophaea maderae* [3] and the hindgut of *D. punctata* [4]. In the later species, ED_{50} for most of the 13 tested allatostatins on the inhibitory activity of proctolin-induced contractions of hindgut was between 2 and 5×10^{-7} M [4].

Besides antimyotropic activity, allatostatins showed differential responses on the activation of α-amylase secretion in the midgut of *B. germanica*, with BLAST-1 being the most active, followed by BLAST-2, whereas BLAST-3 and BLAST-4 had no effect. The absence of activity at high peptide concentrations may be due to a more rapid action of allatostatin at these concentrations, such that at the end of the 30-min incubation, the enzyme has been already secreted and degraded. The results of the time course assay, which give lower enzymatic activities at longer incubation times, are also in agreement with such an explanation. In *D. punctata*, the allatostatin Dippu-AST 7 (APSGAQRLYGFGLa) stimulates α-amylase activity in the midgut lumen, with an ED_{50} of 0.7 nM [7]. In this species, a decrease in allatostatin stimulation of α-amylase activity has also been reported at longer times and higher concentrations [7]. The effects of allatostatins on the digestive system would seem to reflect a physiological role for these peptides, given that the allatostatin receptor has been detected in the midgut of different insects, including a cockroach species [26,35].

Finally, an inhibitory effect of allatostatins on food intake has been also demonstrated, with allatostatin BLAST-2 being the most active, showing ca. 60% inhibition at a dose of 5 μg. In *B. germanica*, inhibitory activity on food intake has been also reported for sulfakinin [EQFDDY(SO_3H)GHMRFa], a totally unrelated peptide, which inhibited food intake when injected at doses of 1 μg (50% inhibition) and 10 μg (60% inhibition) [23].

Taking all the data into account, it can be hypothesised that the various effects of allatostatins herein described may be closely connected each other. A plausible integrative hypothesis for allatostatin activity upon feeding-related processes could be as follows: A direct inhibitory effect on gut contraction would stop food passage, thus indirectly promoting the inhibition of further food intake. In turn, the accumulation of food in different parts of the gut would allow a longer exposure to an increased amount of digestive enzymes, thus optimising the digestive process. Whether the results obtained and the above hypothesis have physiological or simply pharmacological sense remains to be assessed. However, the occurrence of allatostatins in gut tissues, together with the relatively low doses of peptides used, at least for some of the allatostatins tested, suggests that allatostatins may be effectively involved in regulating digestive and feeding processes.

Acknowledgements

Thanks are due to David Martín for critically reading the manuscript. Financial support from the Ministry of Science and Technology, Spain (projects PTR95.0595.OP and AGL2002-01169) and the Generalitat de Catalunya (2001 SGR 003245) is also gratefully acknowledged.

References

[1] Pratt GE, Farnsworth DE, Siegel NR, Fok KF, Feyereisen R. Identification of an allatostatin from adult *Diploptera punctata*. Biochem Biophys Res Commun 1989;163:1243–7.
[2] Woodhead AP, Stay B, Seidel SL, Khan MA, Tobe SS. Primary structure of four allatostatins: neuropeptide inhibitors of juvenile hormone synthesis. Proc Natl Acad Sci U S A 1989;86:5977–6001.
[3] Duve H, Wren P, Thorpe A. Innervation of the foregut of the cockroach *Leucophaea maderae* and inhibition of spontaneous contractile activity by callatostatin neuropeptides. Physiol Entomol 1995;20:33–44.
[4] Lange AB, Bendena WG, Tobe SS. The effect of the thirteen Dip-Allatostatins on myogenic and induced contractions of the cockroach (*Diploptera punctata*) hindgut. J Insect Physiol 1995;41:581–8.
[5] Vilaplana L, Maestro JL, Piulachs MD, Bellés X. Modulation of

cardiac rhythm by allatostatins in the cockroach *Blattella germanica* (L.) (Dictyoptera Blattellidae). J Insect Physiol 1999;45:1057–64.
[6] Martín D, Piulachs MD, Bellés X. Inhibition of vitellogenin production by allatostatin in the German cockroach. Mol Cell Endocrinol 1996;121:191–6.
[7] Fusé M, Zhang JR, Partridge E, Nachman RJ, Orchard I, Bendena WG, et al. Effects of an allatostatin and a myosuppressin on midgut carbohydrate enzyme activity in the cockroach *Diploptera punctata*. Peptides 1999;20:1285–93.
[8] Bendena WG, Garside CS, Yu CG, Tobe SS. Allatostatins: diversity in structure and function of an insect neuropeptide family. Ann NY Acad Sci 1997;814:53–66.
[9] Duve H, Johnsen AH, Maestro JL, Scott AG, East PD, Thorpe A. Identification of the dipteran Leu-callatostatin peptide family: the pattern of precursor processing revealed by isolation studies in *Calliphora vomitoria*. Regul Pept 1996;67:11–9.
[10] Duve H, Johnsen AH, Maestro JL, Scott AG, Jaros PP, Thorpe A. Isolation and identification of multiple neuropeptides of the allatostatin superfamily in the shore crab *Carcinus maenas*. Eur J Biochem 1997;250:727–34.
[11] Dircksen H, Skiebe P, Abel B, Agricola H, Buchner K, Muren JE, et al. Structure distribution and biological activity of novel members of the allatostatin family in the crayfish *Orconectes limosus*. Peptides 1999;20:695–712.
[12] Lorenz MW, Kellner R, Hoffmann KH, Gäde G. Identification of multiple peptides homologous to cockroach and cricket allatostatins in the stick insect *Carausius morosus*. Insect Biochem Mol Biol 2000;30:711–8.
[13] Duve H, Johnsen AH, Scott AG, Thorpe A. Allatostatins of the tiger prawn, *Penaeus monodon* (Crustacea: Penaeidea). Peptides 2002; 23:1039–51.
[14] Bellés X, Graham LA, Bendena WG, Ding Q, Edwards JP, Weaver RJ, et al. The molecular evolution of the allatostatin precursor in cockroaches. Peptides 1999;20:11–22.
[15] Davey M, Duve H, Thorpe A, East P. Characterisation of the helicostatin peptide precursor gene from *Helicoverpa armigera* (Lepidoptera: Noctuidae). Insect Biochem Mol Biol 1999;29:1119–27.
[16] Lenz C, Williamson M, Grimmelikhuijzen CJP. Molecular cloning and genomic organization of an allatostatin preprohormone from *Drosophila melanogaster*. Biochem Biophys Res Commun 2000;273: 1126–31.
[17] Meyering-Vos M, Wu X, Huang J, Jindra M, Hoffmann KH, Sehnal F. The allatostatin gene of the cricket *Gryllus bimaculatus* (Ensifera Gryllidae). Mol Cell Endocrinol 2001;184:103–14.
[18] Bellés X, Maestro JL, Piulachs MD, Johnsen AH, Duve H, Thorpe A. Allatostatic neuropeptides from the cockroach *Blattella germanica* (L.) (Dictyoptera Blattellidae). Identification immunolocalization and activity. Regul Pept 1994;53:237–47.
[19] Vilaplana L, Maestro JL, Piulachs MD, Bellés X. Determination of allatostatin content in relation to the gonadotrophic cycle in the female of *Blattella germanica* (L.) (Dictyoptera Blattellidae). Physiol Entomol 1999;24:213–9.
[20] Maestro JL, Bellés X, Piulachs MD, Thorpe A, Duve H. Localization of allatostatin-immunoreactive material in the central nervous system stomatogastric nervous system and gut of the cockroach *Blattella germanica*. Arch Insect Biochem Physiol 1998;37:269–82.
[21] Bellés X, Casas J, Messeguer A, Piulachs MD. In vitro biosynthesis of JH III by the corpora allata of adult females of *Blattella germanica* (L.). Insect Biochem 1987;17:1007–10.
[22] Zhu J, Miura K, Chen L, Raikhel AS. AHR38 a homolog of NGFI-B inhibits formation of the functional ecdysteroid receptor in the mosquito *Aedes aegypti*. EMBO J 2000;19:253–62.
[23] Maestro JL, Aguilar R, Pascual N, Valero ML, Piulachs MD, Andreu D, et al. Screening of antifeedant activity in brain extracts led to the identification of sulfakinin as a satiety promoter in the German cockroach. Are arthropod sulfakinins homologous to vertebrate gastrins–cholecystokinins? Eur J Biochem 2001;268:5824–30.
[24] Ding Q, Donly BC, Tobe SS, Bendena WG. Comparison of the allatostatin neuropeptide precursors in the distantly related cockroaches *Periplaneta americana* and *Diploptera punctata*. Eur J Biochem 1995;234:737–46.
[25] Vanden Broeck J, Veelaert D, Bendena WG, Tobe SS, De Loof A. Molecular cloning of the precursor cDNA for schistostatins locust allatostatin-like peptides with myoinhibiting properties. Mol Cell Endocrinol 1996;122:191–8.
[26] Secher T, Lenz C, Cazzamali G, Sorensen G, Williamson M, Hansen GN, et al. Molecular cloning of a functional allatostatin gut/brain receptor and an allatostatin preprohormone from the silkworm *Bombyx mori*. J Biol Chem 2001;276:47052–60.
[27] Donly BC, Ding Q, Tobe SS, Bendena WG. Molecular cloning of the gene for the allatostatin family of neuropeptides from the cockroach *Diploptera punctata*. Proc Natl Acad Sci U S A 1993;90:8807–11.
[28] Reichwald K, Unnithan GC, Davis NT, Agricola H, Feyereisen R. Expression of the allatostatin gene in endocrine cells of the cockroach midgut. Proc Natl Acad Sci U S A 1994;91:11894–8.
[29] Yu CG, Stay B, Ding Q, Bendena WG, Tobe SS. Immunochemical identification and expression of allatostatins in the gut of *Diploptera punctata*. J Insect Physiol 1995;41:1035–43.
[30] Garside CS, Koladich PM, Bendena WG, Tobe SS. Expression of allatostatin in the oviducts of the cockroach *Diploptera punctata*. Insect Biochem Mol Biol 2002;32:1089–99.
[31] Maestro JL, Danés MD, Piulachs MD, Cassier P, Bellés X. Juvenile hormone inhibition in corpora allata from ovariectomized *Blattella germanica*. Physiol Entomol 1994;19:342–8.
[32] Osorio S, Piulachs MD, Bellés X. Feeding and activation of corpora allata in the cockroach *Blattella germanica* (L.) (Dictyoptera Blattellidae). J Insect Physiol 1998;44:31–8.
[33] Jorge-Rivera JC, Marder E. Allatostatin decreases stomatogastric neuromuscular transmission in the crab *Cancer borealis*. J Exp Biol 2000;200:2937–46.
[34] Duve H, Johnsen AH, Maestro JL, Scott AG, Crook N, Winstanley D, et al. Identification, tissue localisation and physiological effect in vitro of a neuroendocrine peptide identical to a dipteran Leu-callatostatin in the codling moth *Cydia pomonella* (Tortricidae: Lepidoptera). Cell Tissue Res 1997;289:73–83.
[35] Bowser PR, Tobe SS. Partial characterization of a putative allatostatin receptor in the midgut of the cockroach *Diploptera punctata*. Gen Comp Endocrinol 2000;119:1–10.