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Leucomyosuppressin modulates cardiac rhythm in the cockroach *Blattella germanica*

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ABSTRACT

Several lines of evidence point to leucomyosuppressin (LMS) and myosuppressin-related peptides as inhibitory modulators of heartbeat frequency in arthropods. Previous studies in *Blattella germanica* demonstrated that heartbeat frequency decreases after ootheca formation, and remains low during the period of ootheca transport. Subsequent work in this cockroach resulted in the characterization of LMS and the cloning and sequencing of its precursor. The present paper describes the activity of LMS on modulation of heartbeat in *B. germanica*. Assays using semi-isolated heart preparations revealed that LMS reduces heartbeat frequency in a dose dependent manner, at physiological concentrations. Additional experiments showed that LMS inhibits heartbeat rates *in vivo*. Finally, injection of dsRNA for LMS elicited a decrease in LMS mRNA to virtually undetectable levels and heartbeat frequency increased significantly in females carrying oothecae. These data suggest that LMS contributes to the modulation of cardiac rhythm in *B. germanica* during the reproductive cycle.

1. Introduction

Reproduction in cockroaches is characterized by the formation of an egg-case or ootheca, which protects the oviposited eggs. A number of species drop the ootheca immediately after its formation, whereas others, which are evolutionarily more modified, transport it until the emergence of the young (Roth, 1970). The German cockroach, *Blattella germanica*, is a good example of this mode of reproduction. It transports the ootheca attached to the genital atrium for a period of ca. 20 days, during which the female produces lower rates of juvenile hormone (Gadot et al., 1989; Maestro et al., 1994), consumes less food (Lee and Wu, 1994; Osorio et al., 1998) and shows a decreased locomotory activity (Lee and Wu, 1994). Moreover, heartbeat frequency decreases after ootheca formation, and remains low during the period of ootheca transport (Vilaplana et al., 1999). Regulatory peptides have been postulated as modulators of cardiac rhythm, and proposed candidates for such a modulation have been YXFGLamide allatostatins, with an inhibitory role, and corazonin with a stimulatory one (Vilaplana et al., 1999).

However, a number of lines of evidence point to myosuppressin peptides as repressor modulators of heartbeat frequency in insects. Myosuppressins form a subfamily of FMRFamide-related peptides (Orchard et al., 2001), and the first member to be identified was leucomyosuppressin (LMS), with the structure pQDVDHVFLRFamide, which was isolated from nervous tissues of the cockroach *Leucophaea maderae* by monitoring its ability to decrease spontaneous hindgut contractions (Holman et al., 1986). Subsequent heterologous assays showed that LMS inhibits the amplitude and frequency of contractions in semi-isolated heart preparations of the desert locust *Schistocerca gregaria* (Cuthbert and Evans, 1989), whereas the endogenous LMS-like peptide of this locust (PDVDHVFLRFamide), also inhibited the spontaneous contractions of the heart (Robb et al., 1989). In tenebrionid beetles, cockroach LMS significantly decreased the heartbeat frequency in *Tenebrio molitor* (Wasielewski and Skonieczna, 2008), whereas the same peptide, isolated from the neuroendocrine system of *Zophobas atratus*, had a similar effect in this species (Marciniak et al., 2011). In crustaceans, a neuropeptide with the structure pQDLDHVFLRFamide, thus belonging to the myosuppressin subfamily, has been isolated from a number of species (Ma et al., 2008; Stemmler et al., 2007). Subsequently, the cDNA corresponding to the peptide precursor has been cloned and sequenced in the American lobster *Homarus americanus* (Stevens et al., 2009), where the synthetic peptide decreases heartbeat frequency *in vivo* and in semi-isolated heart preparations (Stevens et al., 2009).

In the cockroach *B. germanica*, we have isolated and chemically characterized LMS (Aguilar et al., 2004), and we have also cloned and sequenced the cDNA corresponding to the LMS precursor (Vilaplana et al., 2004). However, we have not studied the activity of LMS on modulation of heartbeat in this cockroach. The present contribution aims to establish a possible role of LMS on cardiac rhythm modulation, using RNAi to silence the expression of the peptide precursor.

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2. Materials and methods

2.1. Insects

Adult females of *B. germanica* were obtained from a colony reared with dog chow and water, in the dark at 30 ± 1 °C and 60–70% relative humidity. Freshly molted adult females were isolated and used at the appropriate ages. Virgin females were used in the experiments, which were carried out during the first 7 days of the gonadotrophic cycle. To study the period of ootheca transport, mated females were used instead, since they retain the ootheca throughout the entire period of embryogenesis.

2.2. Synthetic peptides

Synthetic version of LMS (pQDVDHVFLRFamide), and the analog of ManducaFLRFamide with the C-terminus in acid form (pQDVVHSFLRF-OH), were synthesized in the Proteomics Facility of the Universitat Pompeu Fabra (UPF), using standard Fmoc chemistry, purified to near-homogeneity (>95%) by HPLC and characterized by amino acid analysis and MALDI-TOF mass spectrometry.

2.3. Semi-isolated heart preparations

For semi-isolated heart preparations, we used 5-day-old *B. germanica* females as previously described (Vilaplana et al., 1999). Briefly, the specimens were pinned ventral side up on a paraffin wax plate and the dorsum with the heart attached was dissected free. The heart preparation was covered with 500 μL of Ringer saline and oxygenated with an air flow above the liquid surface. After 20 min, 150 μL of saline was removed, and the same volume containing the desired concentration of peptide, was added. Heartbeat frequency was recorded every minute for 5 min and then every 5 min for 15 min.

2.4. Recording heartbeat rate in vivo

In the experiments of peptide treatments, 1 μL of a MilliQ water peptide solution was injected into the abdomen of CO_2-anaesthetised 5-day-old adult *B. germanica* females. Peptide solution was calculated to obtain the desired peptide concentration (10^{-6}, 10^{-7} or 10^{-8} M) in the haemolymph, assuming that a 5-day-old adult *B. germanica* female has a haemolymph volume of ca. 25 μL (Romaña et al., 1995) Then, the specimen was immobilized dorsal side up on a wax plate, the wings were cut off so the dorsal vessel was visible through the cuticle and the heart beat rate was directly counted. Heartbeat frequency was recorded every min for 5 min. For recording heartbeat frequency in the case of dsRNA-treated animals (see Section 2.6 below), the procedure was similar, although the specimens were not injected with any peptide solution. In these cases we recorded heartbeat rate for each specimen at least three times between minutes 5 and 10.

2.5. Tissue extraction, RNA isolation, cDNA preparation, polymerase chain reaction (PCR), and Southern-blot analysis

Brains and midguts from 5- and 12-day-old females (the later ones carrying the oothecae) were dissected and immediately frozen in liquid nitrogen. RT-PCR followed by Southern blotting was used to estimate mRNA levels. cDNA was synthesized from total RNA as previously described (Maestro and Belles, 2006). One microgram of total RNA was used for reverse transcription in the case of midguts, whereas in the case of brains, the whole RNA from one brain was used. To estimate mRNA levels semi-quantitatively, a non-saturating number of cycles in the PCR system were used. Primers used for amplifying LMS were: forward, 5′-CAGCATCAGAATGAAGTACGTCAG-3′, reverse, 5′-AACATTTGTTTACTGTACTGGCCAA-3′. As a reference, the same cDNAs were amplified with a primer pair specific for Actin-5C (Maestro et al., 2005). cDNA probes for Southern blot analyses were generated by PCR with the same primer pairs and labeled with fluorescein, using the Gene Images random prime-labeling module (Amersham Biosciences).

2.6. RNA interference

Systemic RNAi *in vivo* in females of *B. germanica* was performed as previously described (Maestro and Belles, 2006; Maestro et al., 2009). A 635-bp fragment spanning positions 95–729 of the BgLMS cDNA (Vilaplana et al., 2004) was used to generate a dsRNA (dsLMS). As a control, a heterologous sequence of 307 bp from the polyhedrin of *Autographa californica* nucleopolyhedrovirus (dsMock) was used. A dose of 2 μg was injected into the abdomen of freshly molted adult females. Recording of heartbeat rate *in vivo* and tissue dissections were performed at the indicated ages.

3. Results

3.1. LMS inhibits heartbeat frequency on semi-isolated heart preparations

To gain an initial insight into the possible role of LMS on *B. germanica* heart beating, we studied the effect of various concentrations of the synthetic peptide (10^{-6}, 10^{-7} and 10^{-8} M) on semi-isolated heart preparations. As a negative control, we tested pQDVVHSFLRF-OH at 10^{-6} M. Results (Fig. 1) show that heartbeat frequency remained more or less constant (around 30 beats/15 s), although tending to increase slightly, during the first 20 min, whereas the administration of LMS produced a fast, dose-dependent and reversible inhibition. The doses of 10^{-6} and 10^{-7} M elicited 49% and 25% inhibition, respectively. Heartbeat frequency at a dose of 10^{-8} M tended to be reduced (by 14%) but did not significantly differ from that measured in the pre-treatment. Treatments with pQDVVHSFLRF-OH did not elicit any apparent response (Fig. 1).

3.2. LMS reduces heartbeat frequency in vivo

The effect of LMS on heartbeat frequency was studied *in vivo* on 5-day-old adult virgin females. Assuming that the average volume of haemolymph in a female of this age is of ca. 25 μL (Romaña et al., 1995), we injected the appropriate dose in 1 μL solution to reach final haemolymph peptide concentrations of 10^{-6}, 10^{-7} and 10^{-8} M, just as in the experiments carried out on semi-isolated heart preparations. Also in this case, we used pQDVVHSFLRF-OH at 10^{-6} M, as a negative control, and additional control specimens that received only 1 μL water were used. Results (Fig. 2) indicated that the treatment *in vivo* with LMS also produced a fast, dose-dependent and reversible decrease in heartbeat frequency. Differences were most striking 1 min after the treatment, when the dose of 10^{-6} M elicited an 86% decrease, whereas that of 10^{-7} M gave 55%. Neither the dose of 10^{-8} M of LMS, nor that of 10^{-6} M of pQDVVHSFLRF-OH elicited significant differences with respect to water controls (Fig. 2).

3.3. RNAi treatment decreases LMS mRNA levels

Freshly molted adult females were treated with dsLMS, maintained without males and killed on day 5 of adult life, to dissect out the brain and the midgut for RT-PCR analysis. A second group was similarly treated, but maintained with males and killed on day 12, during the period of ootheca transport. All the later females

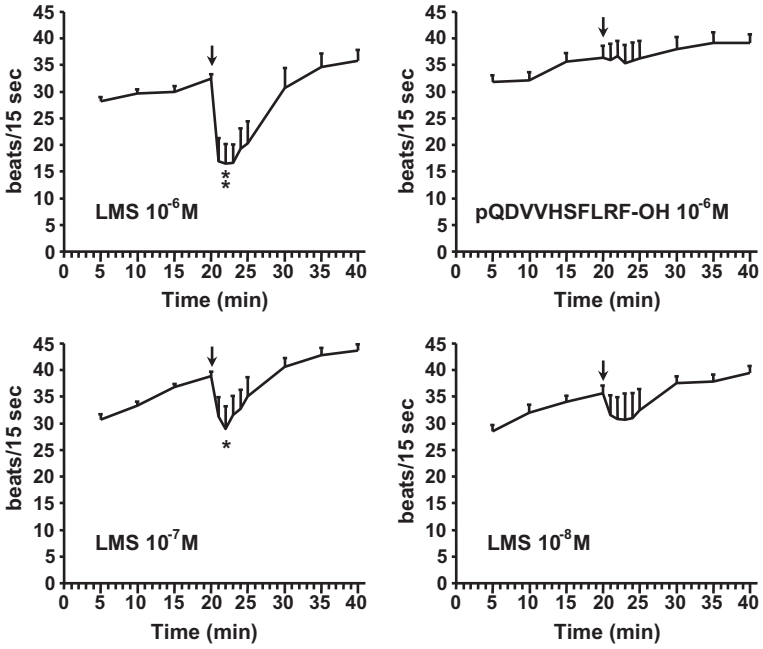

Fig. 1. Effect of LMS (10^{-6}, 10^{-7}, 10^{-8} M) and pQDVVHSFLRL-OH (10^{-6} M) on heartbeat rate in 5-day-old *Blattella germanica* semi-isolated heart preparations. The peptides were applied (arrow) after a 20 min stabilization period. Results are expressed as the mean ± SEM (n = 5–7). Asterisks indicate significant differences between values before and after the treatment (Student's t-test: $^{*}P < 0.05$; $^{**}P < 0.005$).

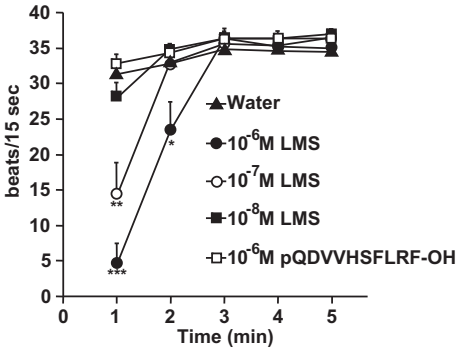

Fig. 2. Heartbeat rate in 5-day-old *Blattella germanica* females after treatment *in vivo* with LMS (10^{-6}, 10^{-7}, 10^{-8} M), pQVVHSFLRL-OH (10^{-6} M) or water. Heartbeat rate was counted every minute for 5 min immediately after peptide injection. Results are expressed as the mean ± SEM (n = 8–13). Asterisks indicate significant differences between peptide and control treatments (Student's t-test: $^{*}P < 0.05$; $^{**}P < 0.0005$; $^{***}P < 0.0001$).

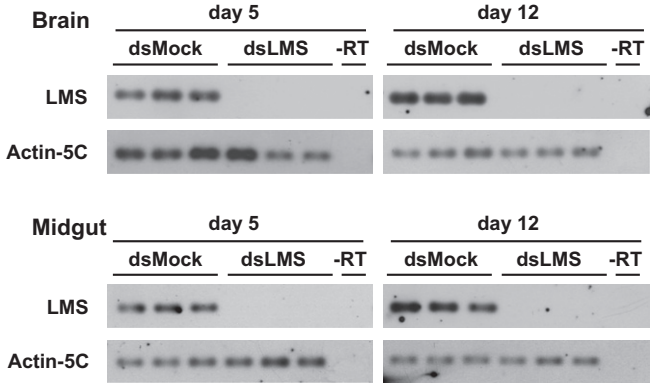

Fig. 3. Silencing LMS expression in *Blattella germanica* females. Specimens were treated immediately after the imaginal molt. LMS mRNA in brain and midgut from dsMock- and dsLMS-treated specimens were analyzed using RT-PCR, followed by Southern-blot. Actin-5C mRNA levels were used as a reference. –RT: negative controls without the reverse transcriptase step.

formed oothecae correctly around day 7, as is usual in our colony (Aguilar et al., 2003). Equivalent experiments were carried out with dsMock. Tissues were processed, and results (Fig. 3) showed that LMS mRNA was virtually undetectable in both brain and in midgut tissues in dsLMS-treated specimens on day 5. Expression in dsLMS-treated specimens was still undetectable on day 12. Expression of LMS mRNA was well apparent in dsMock-treated specimens (Fig. 3).

3.4. LMS knockdown increases heartbeat frequency

Using the same experimental design, on day 5 and day 12 of adult life we measured heart beating in adult females that had been treated with dsLMS or dsMock when freshly emerged. Results (Fig. 4) showed that on day 5, heartbeat frequency in dsLMS-treated specimens (37.6 + 0.4 beats/15 s, n = 23) tended to be higher than in the dsMock-treated group (36.8 + 0.4 beats/15 s, n = 22), but differences were not statistically significant (t test, p = 0.0653) (Fig. 4). On day 12, in animals carrying oothecae, heartbeat frequency in dsLMS-treated specimens (35.2 + 0.4 beats/15 s, n = 25) was significantly higher (t test, p = 0.0153) than in the dsMock-treated specimens (33.5 + 0.5 beats/15 s, n = 24) (Fig. 4).

4. Discussion

Experiments on semi-isolated heart preparations showed that LMS reduces heartbeat frequency in a dose dependent manner, with threshold concentrations between 10^{-8} and 10^{-7} M. These concentrations can be considered physiological for a peptide serving as a circulating hormone. Heterologous assays following this system *in vitro* with the desert locust, *S. gregaria*, used concentrations of LMS of 10^{-6} M to effectively decrease heartbeat frequency (Cuthbert and Evans, 1989). Later, it was shown that the

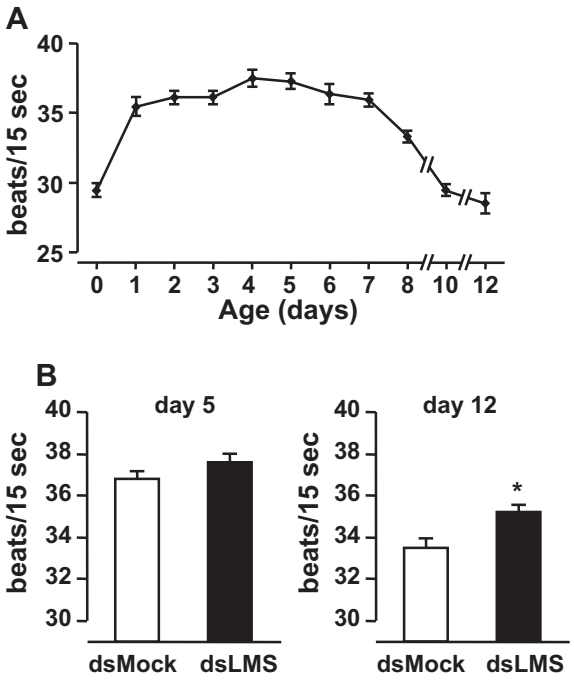

Fig. 4. Effect of dsLMS treatment on heartbeat rate in *Blattella germanica* females. (A) Cardiac rhythm during the first reproductive cycle (data from Vilaplana et al., 1999). (B) Heartbeat rate in dsMock- and dsLMS-treated adult females. Specimens were treated immediately after the imaginal molt and heartbeat rate was measured *in vivo* at days 5 and 12. Results are expressed as the mean ± SEM (n = 22–25). Asterisk indicates significant differences (Student's t-test: $^{*}P < 0.05$).

endogenous LMS-like peptide of *S. gregaria* (PDVDHVFLRFamide) also decreased the spontaneous contractions of the heart at a concentration of 10^{-6} M (Robb et al., 1989). Effective concentrations of LMS used in semi-isolated heart preparations of the tenebrionid beetle *T. molitor* were between 10^{-7} and 10^{-6} M (Wasielewski and Skonieczna, 2008), whereas LMS, identified from brain and retrocerebral complex of *Z. atratus*, another tenebrionid, inhibited heart contractions of this insect with threshold concentrations of 10^{-8}–10^{-7} M (Marciniak et al., 2011). Therefore, the active concentrations found in our experiments using semi-isolated heart preparations with *B. germanica* are consistent with the results obtained in other insects using similar methods. These concentrations are also similar to those used in heart preparations of the American lobster, *H. americanus*, and its native LMS-like peptide, pQDLDHVFLRFamide (Stevens et al., 2009). In this crustacean, concentrations tested to inhibit heart beating were between 10^{-9} and 10^{-6} M, the effects were dose-dependent, and the threshold concentrations were 10^{-8}–10^{-7} M (Stevens et al., 2009).

Our experiments also showed that LMS inhibits heartbeat rates *in vivo* in *B. germanica*. This approach *in vivo* has been used in *H. americanus*, in which an initial concentration of ca. 10^{-7} M in the pericardial cavity significantly decreased the heartbeat frequency (Stevens et al., 2009), a concentration that is within the range of effective concentrations used in our experiments *in vivo*. In the case of *H. americanus*, however, the effects of myosuppressin injections into intact specimens were relatively long-lasting, with a recovery of basal heartbeat frequency taking between 25 min to 1 h. In *B. germanica*, return to normal frequency took only between 1 and 2 min. This fast recovery suggests that LMS is rapidly degraded by enzymes in the internal milieu of the intact cockroach. Indeed, LMS was shown to be degraded in diluted haemolymph of the moths *Spodoptera littoralis* and *Lacanobia oleracea*, with a half-life of 13.1 and 65.4 min, respectively, and degradation was faster in diluted contents of the midgut lumen of the two species (half-life of 0.5 min in *L. oleracea*, and 2.2 min in *S. littoralis*) (Matthews et al., 2009). Degradation of LMS in *B. germanica* has not been studied, but a study *in vivo* combining the use of a radioiodinated derivative of the allatostatin DRLYSFGLamide, microdialysis techniques and HPLC analysis with a radioisotope detector, determined that the half-life of this peptide in the internal milieu of the adult female of *B. germanica* is ca. 4 min (Peralta et al., 2000).

The RNAi experiments described herein showed that treatment with dsLMS decreases LMS mRNA to virtually undetectable levels, whereas heartbeat frequency increases significantly in females transporting oothecae. Effectiveness of dsRNA treatment for target transcript reduction is not surprising, given that *B. germanica* has proven to be very sensitive to RNAi *in vivo* (Belles, 2010). The derived increase in heartbeat frequency is modest: only a tendency when measured on day 5, during full vitellogenesis, and a modest although statistically significant increase from 33 to 35 beats/15 sec, when measured on day 12, during the period of ootheca transport. However, this should be expected in a physiological context if we consider that the cardiac rhythm when the insect forms the ootheca is only reduced about 7–8 beats/15 sec (Fig. 4A) (Vilaplana et al., 1999). If we hypothesize that such a reduction is regulated by myoinhibitory peptides, then the increase that we can expect when we reduce the expression of such peptides may be within this order of magnitude. The fact that differences between dsLMS-treated and controls on day 5 (during vitellogenesis, previous to ootheca formation) were non-significant, in contrast with the significant differences observed on day 12 (during ootheca carrying), may be due to the different physiological status of these two ages. For example, the lower heartbeat rate measured in ootheca carrying females might be more affected by inhibitory signals (like LMS), thus more prone to show RNAi effects. An additional possibility may be related to differences in peptide decay rates due to differences in the duration of the treatment. In a similar work studying *B. germanica* preproallatostatin (Maestro and Belles, 2006), mRNA and peptide levels were analyzed after RNAi, and brain mRNA was reduced close to undetectable levels, whereas brain allatostatin peptide showed 48% and 79% reduction at day 6 and day 14, respectively.

If LMS inhibits heartbeat frequency when the ootheca is formed, then we should expect that LMS expression should be up-regulated at this time. However, LMS mRNA levels in brain tissues of *B. germanica* are quite constant during the first gonadotrophic cycle and first days of ootheca transport (Vilaplana et al., 2004). This suggests that the regulation must be produced at other points, as, for example, in peptide levels, by modulation of the translation of the precursor mRNA, the peptide release or the peptide degradation or at the level of LMS receptor at the heart, among other possibilities. Moreover, the possibility that other peptides contribute to regulation of cardiac rhythm cannot be ruled out. Allatostatins and corazonin are additional obvious candidates to modulate heartbeat frequency in an inhibitory or stimulatory way, respectively (Vilaplana et al., 1999). However, LMS appears to be a good candidate as a physiological regulator of cardiac rhythm from an evolutionary perspective. Indeed, the fact that myosuppressins have cardioinhibitory properties in insects and crustaceans suggests that modulation of heartbeat frequency might have been an ancestral function of this family of peptides in arthropods.

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References

Aguilar, R., Maestro, J.L., Vilaplana, L., Pascual, N., Piulachs, M.D., Belles, X., 2003. Allatostatin gene expression in brain and midgut, and activity of synthetic allatostatins on feeding-related processes in the cockroach *Blattella germanica*. Regulatory Peptides 115, 171–177.

Aguilar, R., Maestro, J.L., Vilaplana, L., Chiva, C., Andreu, D., Belles, X., 2004. Identification of leucomyosuppressin in the German cockroach, *Blattella germanica*, as an inhibitor of food intake. Regulatory Peptides 119, 105–112.

Belles, X., 2010. Beyond *Drosophila*: RNAi in vivo and functional genomics in insects. Annual Review of Entomology 55, 111–128.

Cuthbert, B.A., Evans, P.D., 1989. A comparison of the effects of FMRFamide-like peptides on locust heart and skeletal muscle. The Journal of Experimental Biology 144, 395–415.

Gadot, M., Burns, E., Schal, C., 1989. Juvenile hormone biosynthesis and oocyte development in adult female *Blattella germanica*: effects of grouping and mating. Archives of Insect Physiology and Biochemistry 11, 189–200.

Holman, G.M., Cook, B.J., Nachman, R.J., 1986. Primary structure and synthesis of a blocked myotropic neuropeptide isolated from the cockroach, *Leucophaea maderae*. Comparative Biochemistry and Physiology C 85, 219–224.

Lee, H.-J., Wu, Y.-L., 1994. Mating effects on the feeding and locomotion of the German cockroach, *Blattella germanica*. Physiological Entomology 19, 39–45.

Ma, M., Chen, R., Sousa, G.L., Bors, E.K., Kwiatkowski, M.A., Goiney, C.C., Goy, M.F., Christie, A.E., Li, L., 2008. Mass spectral characterization of peptide transmitters/hormones in the nervous system and neuroendocrine organs of the American lobster *Homarus americanus*. General and Comparative Endocrinology 156, 395–409.

Maestro, J.L., Belles, X., 2006. Silencing allatostatin expression using double-stranded RNA targeted to preproallatostatin mRNA in the German cockroach. Archives of Insect Biochemistry and Physiology 62, 73–79.

Maestro, J.L., Danés, M.D., Piulachs, M.D., Cassier, P., Belles, X., 1994. Juvenile hormone inhibition in corpora allata from ovariectomized *Blattella germanica* (L.) (Dictyoptera, Blattellidae). Physiological Entomology 19, 342–348.

Maestro, O., Cruz, J., Pascual, N., Martin, D., Belles, X., 2005. Differential expression of two RXR/ultraspiracle isoforms during the life cycle of the hemimetabolous insect *Blattella germanica* (Dictyoptera, Blattellidae). Molecular and Cellular Endocrinology 238, 27–37.

Maestro, J.L., Cobo, J., Belles, X., 2009. Target of rapamycin (TOR) mediates the transduction of nutritional signals into juvenile hormone production. Journal of Biological Chemistry 284, 5506–5513.

Marciniak, P., Kuczer, M., Rosinski, G., 2011. New physiological activities of myosuppressin, sulfakinin and NVP-like peptide in *Zophobas atratus* beetle. Journal of Comparative Physiology B, doi: 10.1007/s00360-011-0563-5.

Matthews, H.J., Audsley, N., Weaver, R.J., 2009. Degradation of leucomyosuppressin by enzymes in the hemolymph and midgut of *Lacanobia oleracea* and *Spodoptera littoralis* (Lepidoptera: Noctuidae) larvae. Peptides 30, 565–570.

Orchard, I., Lange, A., Bendena, W.G., 2001. FMRFamide-related peptides: a multifunctional family of structurally related neuropeptides in insects. Advances in Insect Physiology 28, 268–329.

Osorio, S., Piulachs, M.D., Belles, X., 1998. Feeding and activation of Corpora Allata in the Cockroach *Blattella germanica* (L.) (Dictyoptera, Blattellidae). Journal of Insect Physiology 44, 31–38.

Peralta, E., Vilaplana, L., Pascual, N., Carreño, C., Piulachs, M.D., Andreu, D., Belles, X., 2000. A microdialysis study of allatostatin degradation in *Blattella germanica* (L.) (Dictyoptera, Blattellidae). Physiological Entomology 25, 254–259.

Robb, S., Packman, L.C., Evans, P.D., 1989. Isolation, primary structure and bioactivity of schistoflrf-amide, a FMRF-amide-like neuropeptide from the locust, *Schistocerca gregaria*. Biochemical and Biophysical Research Communications 160, 850–856.

Romaña, I., Pascual, N., Belles, X., 1995. The ovary is a source of circulating ecdysteroids in *Blattella germanica* (L.) (Dictyoptera, Blattellidae). European Journal of Entomology 92, 93–103.

Roth, L.M., 1970. Evolution and taxonomic significance or reproduction in *Blattaria*. Annual Review of Entomology 15, 75–96.

Stemmler, E.A., Cashman, C.R., Messinger, D.I., Gardner, N.P., Dickinson, P.S., Christie, A.E., 2007. High-mass-resolution direct-tissue MALDI-FTMS reveals broad conservation of three neuropeptides (APSGFLGMRamide, GYRKPPFNGSIFamide and pQDLDHVFLRFamide) across members of seven decapod crustaean infraorders. Peptides 28, 2104–2115.

Stevens, J.S., Cashman, C.R., Smith, C.M., Beale, K.M., Towle, D.W., Christie, A.E., Dickinson, P.S., 2009. The peptide hormone pQDLDHVFLRFamide (crustacean myosuppressin) modulates the *Homarus americanus* cardiac neuromuscular system at multiple sites. The Journal of Experimental Biology 212, 3961–3976.

Vilaplana, L., Maestro, J.L., Piulachs, M., Belles, X., 1999. Modulation of cardiac rhythm by allatostatins in the cockroach *Blattella germanica* (L.) (Dictyoptera, Blattellidae).. Journal of Insect Physiology 45, 1057–1064.

Vilaplana, L., Castresana, J., Belles, X., 2004. The cDNA for leucomyosuppressin in *Blattella germanica* and molecular evolution of insect myosuppressins. Peptides 25, 1883–1889.

Wasielewski, O., Skonieczna, M., 2008. Pleiotropic effects of the neuropeptides CCAP and myosuppressin in the beetle, *Tenebrio molitor* L.. Journal of Comparative Physiology B 178, 877–885.