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Modulation of cardiac rhythm by allatostatins in the cockroach *Blattella germanica* (L.) (Dictyoptera, Blattellidae)

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Abstract

Cardiac rhythm was measured in *Blattella germanica* females during the reproductive cycle. The rate increased from day 0 to 1, remained constant during the vitellogenic period and fell by about 20% during the period of oothecal transport. The effects of allatostatins, allatostatin analogues and corazonin were tested on semi-isolated heart preparations. Allatostatins showed a rapid, reversible and dose-dependent cardioinhibitory activity. *Blattella* allatostatin 1 (BLAST-1: LYDFGL–NH_2), was the most active, eliciting 76% inhibition at 10^{-7} M and even 19% inhibition at 10^{-9} M. BLAST-2 (DRLYSFGL–NH_2), BLAST-3 (AGSDGRLYSFGL–NH_2) and BLAST-4 (APSSAQRLYGFGL–NH_2) were less active. An analogue of BLAST-2 with C-terminus in acid form and a pseudopeptide analogue of BLAST-2 with a methyleneamino $\Psi[CH_2NH]$ peptide bond surrogate between residues L^3 and Y^4 were inactive. Corazonin elicited rapid, reversible and dose-dependent cardioacceleratory activity. When tested together with BLAST-1, corazonin overrode the cardioinhibitory effect of allatostatin. Our previous results had shown that high levels of allatostatin were maintained during the period of oothecal transport. This and the fact that physiological concentrations of allatostatins produce physiological levels of inhibition, suggest that allatostatins are involved in the modulation of cardiac rhythm in this cockroach.

Keywords: Blattella germanica; Cardiac rhythm; Allatostatin; Antimyotropic activity

1. Introduction

Reproductive cycles in cockroaches begin with a previtellogenic period, followed by vitellogenesis, and finish with egg chorionation and oviposition when eggs are packaged into an ootheca. Some cockroach species drop the ootheca immediately on formation, while others, such as *Blattella germanica*, transport the ootheca until the emergence of the young (Roth, 1970). In *B. germanica*, females in the period of oothecal transport are characterised by a generally depressed physiological state, with low rates of juvenile hormone production (Gadot et al., 1989; Maestro et al., 1994), low food consumption (Lee and Wu, 1994; Osorio et al., 1998) and low locomotory activities (Lee and Wu, 1994).

Our preliminary observations carried out on *B. germanica* seemed to indicate that cardiac rhythm also decreased after the formation of the ootheca, and the occurrence of myomodulatory and cardiomodulatory neuropeptides in insects (see Gäde, 1997 for a review) suggested that the heart beat rate in this species could be hormonally regulated. In addition, circumstantial evidence (see below) leads to the hypothesis that arthropod allatostatins with the characteristic C-terminal sequence YXFGL–NH_2 are prime candidates for such regulation. These allatostatins were first identified by their inhibitory action on the production of juvenile hormone in the corpora allata of cockroaches (Woodhead et al., 1989; Pratt et al., 1989). Thereafter, peptides belonging to the same family were reported in other insect orders (see Stay et al., 1994 for review), although allatostatins did not inhibit the production of juvenile hormone in all of them. What would seem to be a more general function of allatostatins is related to their antimyotropic activity affecting gut motility, which has been described in Dictyoptera (Duve et al., 1995; Lange et al., 1995), Diptera (Duve and Thorpe, 1994; Duve et al., 1996) and Lepidoptera (Duve et al., 1997).

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In *B. germanica*, four allatostatins (BLAST-1 to 4) have been identified (Bellés et al., 1994) and up to 13 (including the four isolated from tissue extracts) have been deduced from the cDNA codifying for the corresponding prohormone (Bellés et al., 1999). They inhibit juvenile hormone production (Bellés et al., 1994) and the release of vitellogenin from the fat body (Martín et al., 1996). Immunocytochemical studies have revealed the occurrence of allatostatins in different areas of the gut, stomatogastric and central nervous systems, including some neurohemal areas from which the peptides might be released to the haemolymph (Maestro et al., 1998). In addition, allatostatin concentration measured by ELISA in brain, midgut and haemolymph changes during the reproductive cycle, reaching its highest levels in the period of oothecal transport (Vilaplana et al., in press).

The aim of this study was to quantify the changes in cardiac rhythm during the reproductive cycle, and to determine whether allatostatins might be involved in the modulation of these changes. Therefore, the dynamics of cardiac rhythm of *B. germanica* females throughout the reproductive cycle was studied, and the effects of different allatostatins and analogues on heart beat rate were tested. The effect of corazonin (Veenstra, 1989) and the combined effect of allatostatins and corazonin were also tested. Corazonin is a cardioacceleratory peptide originally identified in the cockroach *Periplaneta americana* (Veenstra, 1989). Thereafter, Predel et al. (1994) showed that it elicits cardioacceleratory activity in other cockroaches, including *B. germanica*. In addition, corazonin has been described in the cockroach *Nauphoeta cinerea* and the moth *Manduca sexta* (Veenstra, 1991), whereas the gene coding for the same peptide has been reported in *Drosophila melanogaster* (Veenstra, 1994). These findings suggest that corazonin may be present in *B. germanica* and that it might also be involved in the modulation of cardiac rhythm in this species.

2. Materials and methods

2.1. Insects

Adult female *B. germanica* were obtained from a colony fed on dog chow and water, and reared in the dark at 30±1°C and 60–70% r.h. Freshly moulted virgin females were isolated and used at the appropriate physiological ages, which were assessed by measuring the basal oocyte length. In general, virgin specimens were used, but the experiments with females in the period of oothecal transport were carried out with mated females, because they retain the ootheca during the entire period of embryogenesis. In these females, the presence of spermatozoa in the spermathecae was assessed, thus indicating that mating had occurred.

2.2. Measurements of cardiac rhythm in vivo

In order to record cardiac rhythm during the reproductive cycle, CO_2-anaesthetised specimens were immobilised dorsal side up on a paraffin wax plate. Then, wings were cut off so the dorsal vessel was visible through the cuticle, thus the heart beat rate could be directly quantified. Cardiac rhythm was recorded (number of beats in 15-s period) immediately after immobilisation, and 5 min later.

2.3. Synthetic peptides

BLAST-1 (LYDFGL–NH_2), BLAST-2 (DRLYSFGL–NH_2), BLAST-3 (AGSDGRLYSFGL–NH_2), BLAST-4 (APSSAQRLYGFGL–NH_2), and the analogue of BLAST-2 with the C-terminus in acid form (BLAST-2-COOH) were synthesised as previously described (Bellés et al., 1994). The pseudopeptide analogue of BLAST-2 with a methyleneamino peptide bond surrogate between residues L^3 and Y^4 (DRLΨ[CH_2NH]YSFGL–NH_2: BLAST-2-PTMA) was synthesised as previously described (Piulachs et al., 1997). Corazonin (*p*QTFQYSRGWTN–NH_2) was from Bachem (Bubendorf, Switzerland).

2.4. Semi-isolated heart preparations

For semi-isolated heart preparations we used 5-day-old female *B. germanica*, according to Baumann and Gersch (1982) with slight modifications. The specimens were pinned ventral side up on a small depression (40 mm diameter, 5 mm deep) made on a wax plate, and the dorsum with the heart attached was dissected free. The heart preparations were covered with 500 µl of Ringer saline (water solution containing 9 g/l NaCl, 0.8 g/l KCl, 0.8 g/l $NaHCO_3$ and 0.8 g/l $CaCl_2$), which was oxygenated with an air flow (1 l/min) supplied at 2–3 mm above

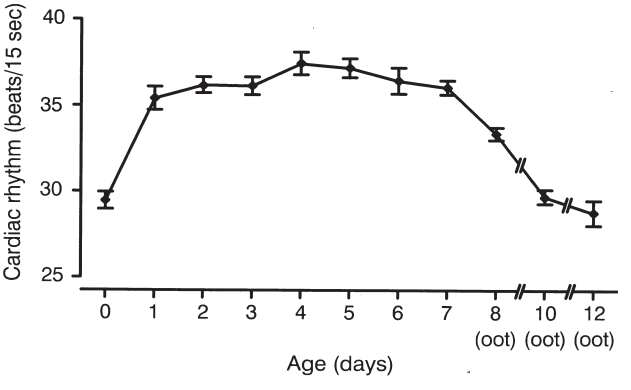

Fig. 1. Cardiac rhythm during the first reproductive cycle in female *B. germanica*. Measurements were recorded in immobilised specimens with the wings cut off in order to visualise the heart through the cuticle. Results are expressed as the mean±SEM (n=8–18). (oot): females transporting the ootheca.

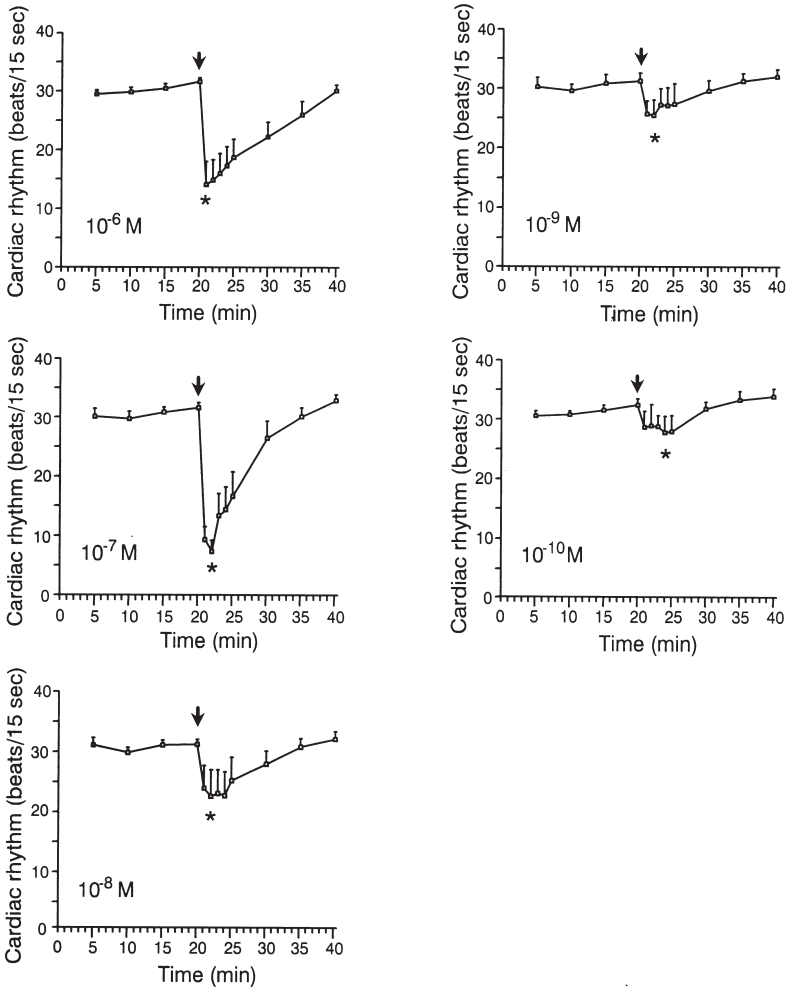

Fig. 2. Effect of BLAST-1 (10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10} M) on the heart beat rate in 5-day-old female *B. germanica*. The peptide was applied (arrow) to the dorsal vessel preparation after a stabilisation period of 20 min. Results are expressed as the mean±SEM (n=8). The asterisk indicates that the mean of the differences between the values before and after the treatment was significantly different from zero (t-test, $p<0.05$).

the liquid surface. After 20 min (which is the time necessary to reach a constant cardiac rhythm; unpublished observations), 150 µl of the saline solution was removed, and the same volume containing the desired concentration of peptide, was added. In the case of allatostatins, cardiac rhythm was recorded every minute for 5 min and then every 5 min for 15 min. In the case of corazonin, a supplementary measurement, 30 s after treatment, was recorded. Every single specimen was used for only one series of measurements. Maximum activation or inhibition percentages were calculated comparing the last value before treatment with the lowest (inhibition) or highest (activation) value due to the treatment.

3. Results

3.1. Cardiac rhythm during the reproductive cycle

The rate of cardiac rhythm during the gonadotrophic cycle as well as that in females carrying the ootheca are shown in Fig. 1. Cardiac rhythm increased from day 0 (29.5±0.5 beats/15 s) to day 1 (35.4±0.7 beats/15 s), remained constant during the vitellogenic period (values between 36.0 and 37.5 beats/15 s), decreased 3 h after the formation of the ootheca, on day 8 (33.4±0.4 beats/15 s), and reached the lowest rate 96 h after the formation of the ootheca (28.8±0.7 beats/15 s).

3.2. Effect of allatostatins on the cardiac rhythm

To study the effect of allatostatins on cardiac rhythm, various concentrations of BLAST-1, 2, 3 and 4, BLAST-2-COOH and BLAST-2-PTMA were assayed on semi-isolated heart preparations. Results are shown in Figs. 2–4, and the respective percentages of inhibition are summarised in Fig. 5. In all cases, cardiac rhythm remained constant, between 29 and 31 beats/15 s, during the first 20 min, whereas the application of allatostatins, in general, produced a fast, dose-dependent and reversible inhibition.

BLAST-1 was the most active, eliciting 76% inhi-

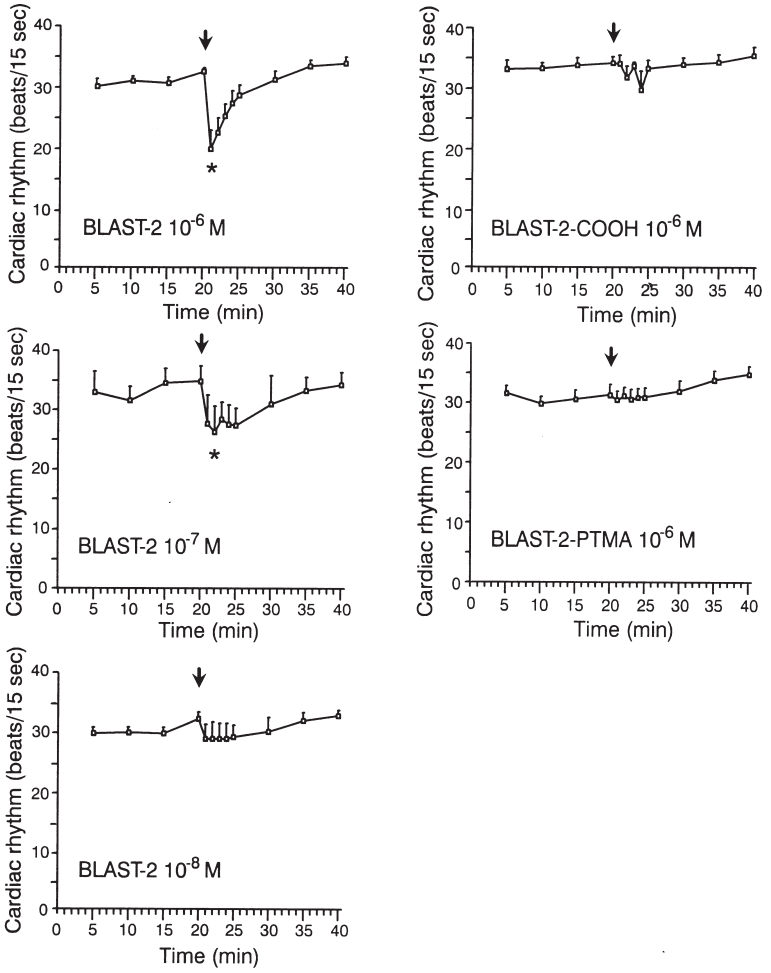

Fig. 3. Effect of BLAST-2 (10^{-6}, 10^{-7}, 10^{-8} M), BLAST-2-COOH (10^{-6} M) and BLAST-2-PTMA (10^{-6} M) on the heart beat rate in 5-day-old female *B. germanica*. The peptides were applied (arrow) to the dorsal vessel preparation after a stabilisation period of 20 min. Results are expressed as the mean±SEM (n=7–9). The asterisk indicates that the mean of the differences between the values before and after the treatment was significantly different from zero (t-test, $p<0.05$).

bition at 10^{-7} M and around 19% at 10^{-9} M (Figs. 2 and 5). BLAST-2, 3 and 4 were also active, but not as active as BLAST-1, eliciting an inhibition of 27%, 28% and 37% respectively at a concentration of 10^{-7} M (Figs. 3–5). The analogue of BLAST-2 with the C-terminus in acid form (BLAST-2-COOH) and the pseudopeptide analogue with a methyleneamino bond between residues L^3 and Y^4 (BLAST-2-PTMA) did not show any significant inhibitory activity when both were tested at a concentration of 10^{-6} M (Figs. 3 and 5).

3.3. Combined effect of corazonin and allatostatin

Corazonin showed fast, dose-dependent and reversible cardioacceleratory activity when tested on semi-isolated heart preparations. Percentages of activation were 22% and 15% at concentrations of 10^{-7} and 10^{-8} M respectively (Fig. 6).

When corazonin and BLAST-1 were tested together at a concentration of 10^{-7} M each, cardiac rhythm showed a 14% activation, that is a significant 8% less than when corazonin was applied alone (Fig. 6). Conversely, when tested together at a concentration of 10^{-8} M each, the activation observed (14%) was almost the same as that found when corazonin was tested alone (15%) (Fig. 6).

4. Discussion

The cardiac rhythm during the reproductive cycle of female *B. germanica* increased from the day of emergence to the first day of adult life, maintained constant rates during the whole vitellogenic period, and decreased when forming the ootheca, remaining at low rates for at least 4 days within the period of oothecal transport. Although this pattern is cyclic and approximately parallel to the gonadotrophic cycle (Bellés et al., 1987), dif-

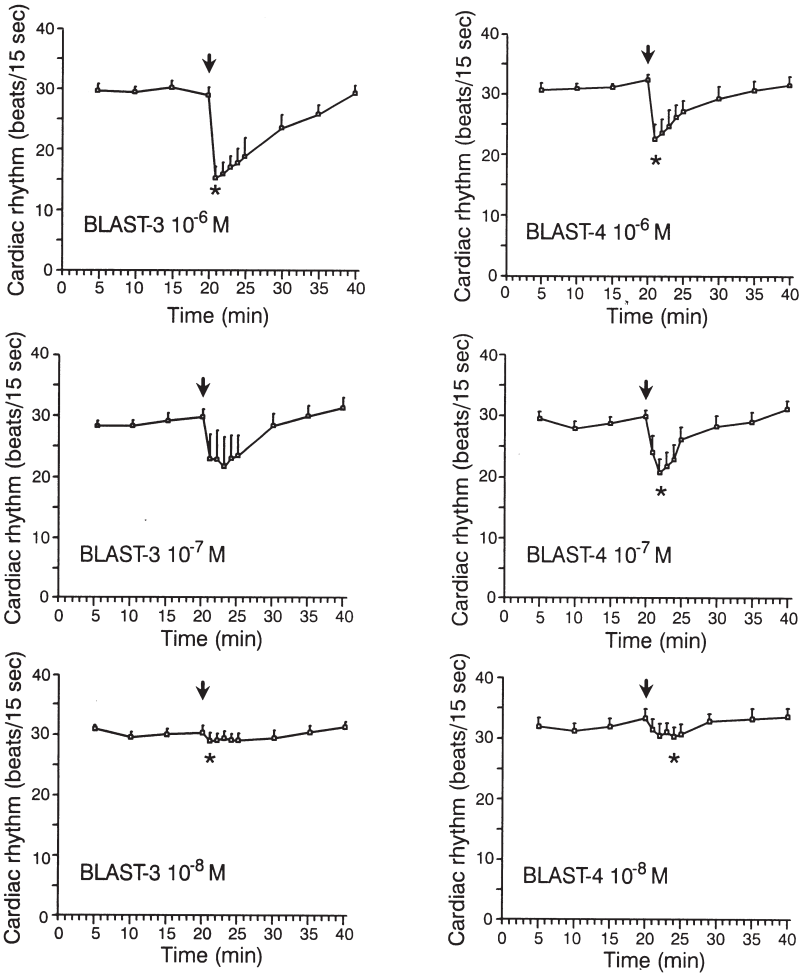

Fig. 4. Effect of BLAST-3 (10^{-6}, 10^{-7}, 10^{-8} M) and BLAST-4 (10^{-6}, 10^{-7}, 10^{-8} M) on the heart beat rate in 5-day-old female *B. germanica*. The peptides were applied (arrow) to the dorsal vessel preparation after a stabilisation period of 20 min. Results are expressed as the mean±SEM (n=7–8). The asterisk indicates that the mean of the differences between the values before and after the treatment was significantly different from zero (t-test, $p<0.05$).

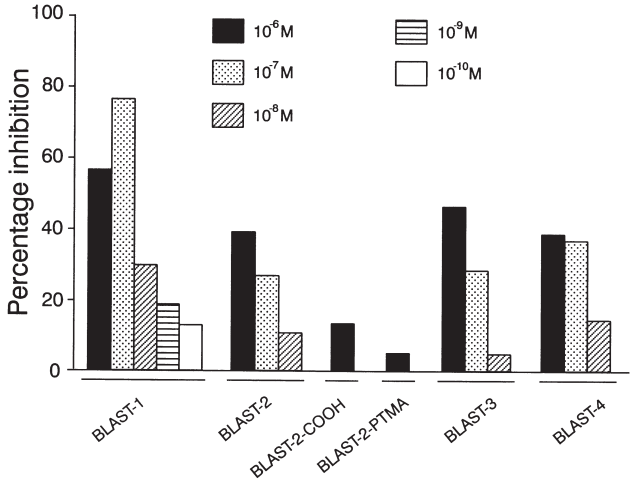

Fig. 5. Percentage of inhibition of the heart beat rate induced by BLAST-1, BLAST-2, BLAST-2-COOH, BLAST-2-PTMA, BLAST-3 and BLAST-4 on semi-isolated heart preparations in 5-day-old female *B. germanica* (see Figs. 2–4).

ferences between maximal and minimal heart beat rates (4-day-old females and 96 h after the formation of the ootheca, respectively) were rather modest (around 23%).

Using semi-isolated heart preparations from 5-day-old female *B. germanica*, it was shown that allatostatins induced a fast, dose-dependent and rapidly reversible cardioinhibitory activity. BLAST-1 was the most potent peptide, eliciting a decrease in heart beat rate of around 20% at a concentration of 10^{-9} M. Interestingly, this percentage drop could be considered physiological, given that it is similar to that occurring between vitellogenic and postvitellogenic stages (see above). BLAST-2, 3 and 4 elicited less obvious inhibitory effects, whereas the analogue of BLAST-2 with the C-terminus in acid form (BLAST-2-COOH) and the pseudopeptide analogue BLAST-2-PTMA were inactive at a dose of 10^{-6} M. The inactivity of BLAST-2-COOH stresses the importance of the amide group at the C-terminus for cardioinhibitory activity, as occurs in other allatostatin activities, such as inhibition of juvenile hormone pro-

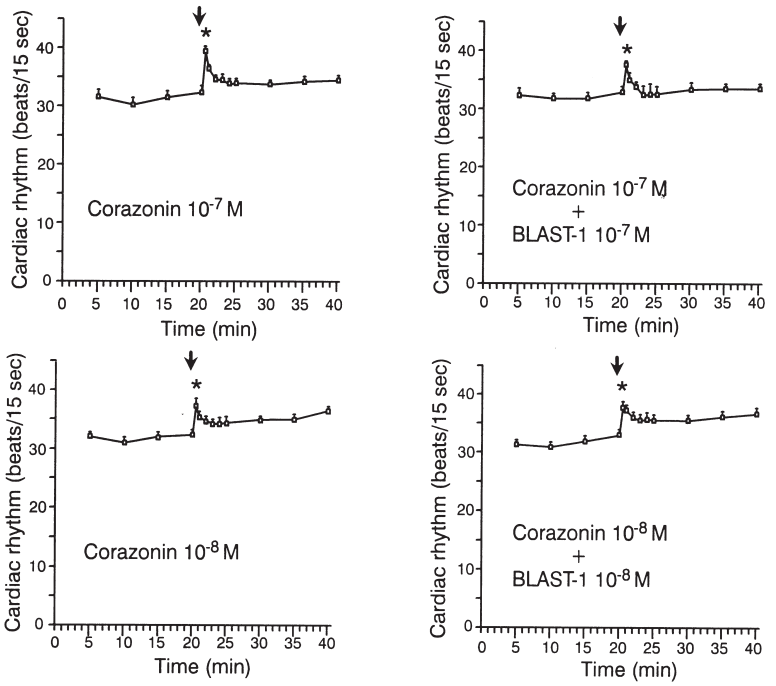

Fig. 6. Effect of corazonin (10^{-7}, 10^{-8} M) and combined effect of corazonin and BLAST-1 (10^{-7} +10^{-7} M, 10^{-8} +10^{-8} M) on the heart beat rate in 5-day-old female *B. germanica*. The peptides were applied (arrow) to the dorsal vessel preparation after a stabilisation period of 20 min. Results are expressed as the mean±SEM (n=8–10). The asterisk indicates that the mean of the differences between the values before and after the treatment was significantly different from zero (t-test, $p<0.05$). The mean of the values after the combined 10^{-7}+10^{-7} M corazonin and BLAST-1 treatment was significantly lower than that of the values after the treatment with corazonin alone at 10^{-7} M (t-test, $p<0.05$), whereas the means of the respective pre-treatment values were not statistically different.

duction or inhibition of gut motility (see Stay et al., 1994). The inactivity of BLAST-2-PTMA indicates that the substitution of the peptide bond L^3–Y^4 by the methyleneamino $\Psi[CH_2NH]$ peptide bond surrogate practically suppresses the cardioinhibitory properties, whereas this substitution did not affect the inhibitory activity on juvenile hormone production so severely (Piulachs et al., 1997).

It is worth noting that the activity elicited by BLAST peptides on heart beat rate is rapidly reversible. This may be due to the catabolic activities of different tissues present in the semi-isolated heart preparations, especially the fat body, that would rapidly degrade the peptides. Therefore, if circulating allatostatins effectively contribute to maintain low heart beat rates in certain periods of the reproductive cycle of *B. germanica*, for example during the period of ootheca transport, then a continuous production of these peptides should be assumed during these periods.

Corazonin elicited fast, dose-dependent and rapidly reversible cardioacceleratory response when tested on semi-isolated heart preparations at doses of 10^{-7} and 10^{-8} M. When corazonin and BLAST-1 were tested together at the same concentration, the cardioacceleratory effects clearly predominated, which indicates that corazonin action overrode the cardioinhibitory properties of allatostatins.

Other insect neuropeptides having cardioinhibitory effects include various FM/LRF-amide peptides, as shown by heart beat rate assays carried out in the locust *Schistocerca gregaria* (Cuthbert and Evans, 1989; Robb et al., 1989). Some of the peptides had a cardioacceleratory effect, whereas others showed a concentration-dependent biphasic effect. However, two of them, SchistoFLRF-amide from *S. gregaria* and leucomyosuppressin previously isolated from the cockroach *Leucophaea maderae*, elicited a purely inhibitory response. The activity thresholds of these peptides occurred between 10^{-9} and 10^{-10} M, and between 10^{-7} and 10^{-8} M, respectively (Cuthbert and Evans, 1989; Robb et al., 1989), which are similar to the thresholds reported here for BLAST-1. Peptides from the same family, including SchistoFRLF-amide and leucomyosuppressin, have also been tested on the blowfly *Calliphora vomitoria*, and only leucomyosuppressin elicited an inhibitory activity but at a concentration of 10^{-6} M (Duve et al., 1993).

In *B. germanica* females, the occurrence of allatostatin-immunoreactive material has been reported in gut, stomatogastric and central nervous systems, including various ganglia of the ventral nerve cord (Maestro et al., 1998), which could innervate the dorsal vessel via segmental nerves. Moreover, the presence of allatostatins has been observed in some neurohemal areas of the brain (Maestro et al., 1998), from which they could be released

to the haemolymph. Indeed, allatostatins have been detected in the haemolymph of *B. germanica* females by ELISA, and their concentration has been estimated to be around 10^{-9} M. In addition, ELISA measurements indicated that brain and midgut levels of allatostatins reached maximum values at the time of ootheca formation which were subsequently maintained until the end of the oothecal transport period (Vilaplana et al., in press). Interestingly, ELISA-measured concentrations of allatostatins are compatible with those of BLAST 1 needed to elicit a decrease in the heart beat rate of around 25% in our semi-isolated heart preparations, and, as stated above, this percentage of inhibition is similar to that found between vitellogenic and postvitellogenic females of *B. germanica*. Taken together, our results suggest that allatostatins are involved in the physiological modulation of the cardiac rhythm in *B. germanica*.

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