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Pleiotropic effects of the neuropeptides CCAP and myosuppressin in the beetle, *Tenebrio molitor* L.

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Abstract Biological activities of crustacean cardioactive peptide (CCAP; PFCNAFTGCa) and leucomyosuppressin (Lem-MS; pQDVDHVFLRFa) were studied in heterologous bioassays in the larvae and adults of *Tenebrio molitor*. CCAP exerted a reversible and dose-dependent cardio-stimulatory effect on the semi-isolated heart of the experimental beetles at a concentration of $>10^{-7}$ M and induced an effect similar to the endogenic cardio-stimulatory peptide, proctolin. Injections of CCAP (10^{-9}–10^{-3} M) into 4-day-old adult reproductive females increased the concentration of soluble proteins in hemolymph in comparison to the saline injected controls. Electrophoretic analyses indicated significant increase in the level of two proteins 130 and 170 kDa, and a partial increase of the level of 67-kDa protein. The studies indicated that CCAP increased also free hemolymph sugar concentration in young larvae and adults of the mealworm beetle. The cardio-inhibitory peptide Lem-MS exerted the opposite effect: at concentration 10^{-7}–10^{-6} M, it significantly decreased the heartbeat frequency. The induced changes were dose-dependent and reversible, but at higher concentrations ($>10^{-5}$ M) the stimulatory effect disappeared. Injections of the Lem-MS into young larvae at concentrations 10^{-9}–10^{-3} M, also increased the free hemolymph sugar level similarly to the CCAP. This work demonstrates the pleiotropic effects of CCAP and Lem-MS in *Tenebrio molitor*.

Keywords Carobohydrates · Cardioactive peptide · Chronotropic activity · Hyperglycemic activity · Myoactive factors · Soluble proteins · *Tenebrio molitor*

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Introduction

The crustacean cardioactive peptide (CCAP) (PFCNAFT-GCa) was originally isolated from the neurohaemal structures and the pericardial organs of the shorecrab *Carcinus maenas* (Stangier et al. 1988). So far, in insects, this peptide has been identified in neurohormonal system of the beetle *Tenebrio molitor* and several other insects like *Drosophila melanogaster*, *Locusta migratoria*, *Manduca sexta* and *Spodoptera eridania* (Stangier et al. 1989; Cheung et al. 1992; Furuya et al. 1993; Dulcis et al. 2005). The peptide CCAP has no structural homology to the other neuropeptide families and belongs to a family of cardioactive peptides (CAPs) because of its ability to stimulate heartbeat. This cyclic nonapeptide with the sulfide bridge has been shown to stimulate contractions of the crayfish heart (Stangier et al. 1987) and also induce a rapid increase in the frequency and amplitude of phasic contraction of the myocardium of *Tenebrio molitor* (Furuya et al. 1993). This molecule is an endogenous signal substance in a few species of insects and crustaceans (Dircksen 1998). In *M. sexta* adults the CAPs, and possibly CCAP, triggers an increase of the heart rate during long flights (Tublitz 1989), modulates oviduct contractions in *L. migratoria* (Donini et al. 2001; Donini and Lange 2002), and hindgut activity in *M. sexta* (Tublitz et al. 1992). It also increases hemolymph circulation and facilitates the transport of energy substrates

from the fat body to the flight muscles (Tublitz 1989). In locusts *Locusta migratoria* and *Schistocerca gregaria* the CCAP has been identified as the adipokinetic hormone (AKH) release inducing factor (Veelaert et al. 1997; Vullings et al. 1999). The AKHs are synthesized and released from the corpora cardiaca (CC). They generally mobilize lipids and carbohydrates from the fat body during a long flights and control a lot of accompanying processes on cellular, organ a organismal levels (see Kodrík 2008). This implies that CCAP also acts as a hypertrehalosaemic hormone. Another study suggests that the CCAP is involved in the regulation of ecdysis behavior (review: Dircksen 1998; Gammie and Truman 1999; Dulcis et al. 2005). In an immunocytochemical study, CCAP-immunoreactive neurons were found in the brain and nerve tissues (ventral nerve cord) of flies, moths, cockroaches and also beetles from *Tenebrionidae* family (Breidbach and Urbach 1996; Predel and Wegener 2006).

Leucomyosuppressin (pQDVDHVFLRFa) was isolated from the cockroach *Leucophaea maderae* (Holman et al. 1986) and belongs to the family of myosuppressins. The neurohormones belong to the FMRF-related peptides with hormonal, neurotransmitters and neuromodulatory activities in insects. These peptides were isolated from cockroaches (Holman et al. 1986; Predel et al. 2001), locusts (Robb et al. 1989; Schoofs et al. 1993), dipterous insects (Duve et al. 1992; Nichols 1992) and butterflies (Kingan et al. 1990). The mass spectrometry analysis of extracts from *Tenebrio molitor* nerve tissue, and deduced sequences from the *Tribolium castaneum* genome, proved the presence of myosuppressins also in *Tenebrionidae* (Weaver and Audsley 2008; Li et al. 2008).The mode of their physiological actions in the visceral muscles is not uniform. They can produce inhibitory or stimulatory effects, and also both together, depending on the insect species, tested organ or concentration of bioanalogue used (Cuthbert and Evans 1989; Duve et al. 1992; Peeff et al. 1993, Dulcis et al. 2005; Mercier et al. 2007). In, *Tenebrionidae* the Lem-MS acts as a strong cardioinhibitor in semi-isolated heart preparations (Skonieczna and Rosiński 2004). Apart from the myoinhibitory activities, Lem-MS can also control the release of AKH-HrTH from *corpus cardiacum* (Veelaert et al. 1998; Vullings et al. 1999) and influence the movement and excretory functions of Malpighian tubules (Coast 1998). The action of CCAP and Lem-MS seems to be connected with the guanosine-protein coupled receptors (GPCRs) and acts through the second messenger activation (in review Mercier et al. 2007).

However, the broad physiological functions of these two peptides are still ambiguous in other group of insects, and much less is known about their actions in beetles. In this paper, we present the pleiotropic effect of leucomyosuppressin and CCAPs, which act as classical myotropic

peptides, and have the same functions in modulation of protein and carbohydrate levels in *Tenebrio molitor*.

Materials and methods

Insects

A stock culture of *T. molitor* was reared as previously described (Rosiński et al. 1979). The age of mealworm parents is fundamental for the developmental process of their offspring (Ludwig and Fiore 1960; Ludwig et al. 1962), hence, all insects in our experiments were derived from parents that were less than 1 month old. Pupae from a stock culture were sexed according to Ullmann (1973) and kept separately until adult emergence. Adults that emerged within a 6–12 h interval were transferred to plastic boxes (8 cm L, 6 cm W, 6 cm H) and were fed on oats with the addition of 15% (w/w) fresh carrot slices.

Synthetic hormones

Cardioactive nonapeptide (CCAP) and leucomyosuppressin (Lem-MS) were obtained from Bachem AG (Germany). Proctolin and BSA (bovine serum albumin) and other chemicals were procured from Sigma Chemical Company, St Louis, MO, USA.

Injections

All injections were administered to anesthetized (using CO_2) larvae or females of *Tenebrio*. The peptides to be tested were dissolved in 90% acetonitrile (Sigma, USA). Directly before experiments, all tested peptides were dried in vacuo (Jouan RC 10.22, Germany), dissolved in physiological saline for *Tenebrio* (Butz, 1957) and injected in a volume of 2 µl in adequate concentration range through the ventral membrane between the second and the third abdominal segments toward the head. Daily injections into mealworm females were administered once a day, on day 2 after adult emergence, with Hamilton syringe (5 µl; Hamilton Co. Germany). In, *Tenebrio* females the gonadotropic phase continues for 4 days and terminates with egg laying. The first 2 days of this phase are the most important for ovarian development and vitellogenin accumulation. Therefore, all injections were performed on day 2 of this phase to check the stimulatory role of CCAP and/or Lem-MS in protein synthesis. Control insects were similarly injected with the same volume of physiological saline. Proctolin (protein bioassay) and bovine serum albumin (carbohydrate bioassay) were administered in a suitable dose in a manner similar to CCAP and Lem-MS and were used as an internal standard during the experiments.

Hemolymph protein analysis

Four days after adult emergence, hemolymph samples (2 μl) were taken from a foreleg, diluted in 100 μl ice-cold physiological saline and centrifuged at 10,000g for 4 min. The supernatants were stored at −20°C and used for protein analysis according to Bradford assay (1976). The total protein concentration of the hemolymph extracts was determined using bovine serum albumin as a standard.

Heartbeat bioassay

Peptides were assayed according to in vitro microdensitometric method of Rosiński and Gäde (1988a, b) on semi-isolated hearts from adult males and females of *T. molitor*. The adults were used for the experiments because of regularity of the heartbeat, and uni-directional flow of haemolymph in the aorta. The experimental beetles were decapitated and the abdomen prepared for the assay, so that the final preparation consisted of the dorsal vessel (the heart), alary muscles, internal body muscles, the tracheae, and the dorsal cuticle. Based on frequency and regularity of heart-beat, the heart preparations were selected for bioassay, and then were superfused in *Tenebrio* saline (247 mM NaCl, 19 mM KCl, 9 mM CaCl$_2$, 5 mM glucose, 5 mM HEPES, pH 7.0). After 20 min of stabilization in the saline, an open perfusion system was used. In this system, an injection port for tested peptides was located 70 mm above the superfusion chamber. The semi-isolated heart was perfused during bioassay with fresh saline at the rate of about 140 μl/min so that the injected peptide would reach the heart after about 15–20 s. Saline always flowed from the open point to the perfusion chamber onto the caudal portion of the heart. The saline was removed by suction with a Whatman paper at the cephalic end of the preparation. The activity of the heart was recorded automatically using a microdensitometer MD-100 (Carl Zeiss, Germany). The apparatus equipped with photocell counts optically every contraction of the heart, and being connected with microdensitometer register system, generates the cardiogram on paper. The application system with the injection port well fitted to a Hamilton syringe, works as an open system, which was designed to enable samples to be added without causing a change in pressure. All assays were performed at room temperature and many pulse applications of different samples could be assayed in a single preparation. The control frequency of the heartbeat was monitored for the first 20 min of assay, when the heart was perfused with saline. In the experiments the heartbeat was monitored always for 1 min before, and after peptide injection. Peaks (contractions) were scored from obtained myograms for every 1 min, by chart speed 1 cm/10 s. Eventually, the frequency of the heartbeat was expressed as number of contractions per minute. This value was then converted into percentage changes in heartbeat frequency in comparison to the control frequency. The activities of tested peptides are presented in figure as mean values ± SEM from seven separate determinations, and significant differences from the control were established by Student t-test (*$P < 0.05$).

Carbohydrate bioassay

The hyperglycemic activity of CCAP and Lem-MS was studied following the injection of peptides into larvae and young females just after ecdysis. Briefly, the peptides were injected into the experimental beetles as mentioned above and the hemolymph samples were taken just before and in suitable time after the injection. The level of free sugar in hemolymph was estimated for each sample separately (Rosiński and Gäde 1988a). During the experiments all insects were incubated separately. The control insects were injected with physiological saline. For comparison, different developmental stages of insects were used (larvae and young females) because of the differences in carbohydrate metabolism.

The hemolymph samples (2 μl) were mixed with 500 μl of 70% ethanol and shaken several minutes to obtain full extraction of free sugar. The extracts were centrifuged at 10,000$\times g$ for 4 min and the supernatants were used for free sugar concentration analysis (Dubois et al. 1956) using trehalose as a standard.

Polyacrylamide gel electrophoresis (PAGE)

The hemolymph proteins were studied using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli (1970) using 10% polyacrylamide gels. Samples were mixed with sample buffer (1:1) containing 125 mM Tris–Hcl, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, pH 6.8 and heated for 3 min at 95°C in a water bath before being loaded onto the gels. After heating, 0.5% bromophenol blue was added (1 μl) and electrophoresis was run at 30 mA. The gels were destained in a mixture of acetic acid and methanol (destaining solution I: 10% acetic acid and 50% methanol; destaining solution II: 7% acetic acid and 5% methanol). Molecular masses of the studied proteins were estimated using protein standards for SDS-PAGE (Sigma). Gels stained with Coomassie Brilliant Blue were scanned on Alpha Vista II scanner by using the Silver Fast-Se v6.0 2r32 software (LaserSoft Imaging). The concentration of the proteins and the evaluation of their molecular masses were done by using of the Total-Lab 1.1 software (demo version; Nonlinear Dynamics).

Results

Myotropic activity of Lem-MS and CCAP

The studies demonstrate cardioregulatory action of CCAP and leucomyosuppressin on the semi-isolated heart of the beetle, *Tenebrio molitor*. The effects of tested peptides are reversible and dose-dependent. Figure 1 demonstrates the typical myogram responses of *T. molitor* to CCAP and Lem-MS. Figure 2 displays the action of tested peptides in the first minute of assay after peptide application. Because of different duration on each preparation, this period was optimal to count average dose-dependent effects on heart, so the presented data did not show the maximal heart rate after peptide application. Because of variability of used preparations the results presented in Fig. 2 are the average values, which do not strictly coincide with the example shown in Fig. 1. The most important interaction of tested peptides was reached immediately after application. The significant effects of CCAP were observed in the concentration range 10^{-7}–10^{-3} M (Fig. 2).

Strong cardiostimulatory effect at concentration over 10^{-7} M of the CCAP seems to be similar to the effect of another well known endogenic peptide from beetle to proct-
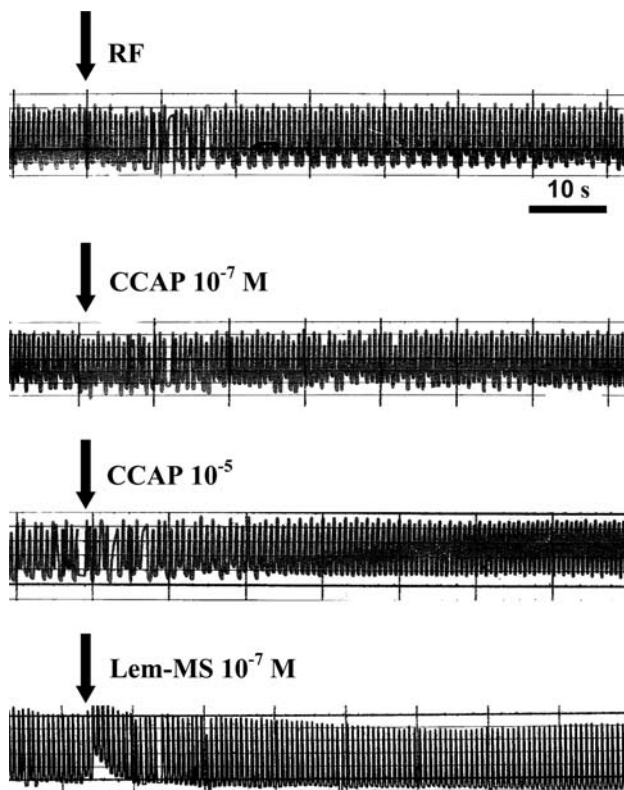

Fig. 1 Myograms displaying typical responses on the spontaneous activity in heart of *Tenebrio molitor* to CCAP and Lem-MS in the first minute of assay after application. Peptide applications indicated by arrow. *RF* applications of saline solution

olin, and is concomitant with an increase of frequency (positive chronotropic effects) of myocardial contractions. This property is well demonstrated on the attached myograms, where we can compare typically the action of CCAP with physiological saline RF (Fig. 1).

In contrast to CCAP, leucomyosuppressin decreased the frequency of the *Tenebrio molitor* heartbeat. Figure 1 demonstrates the typical response of heart muscles to Lem-MS (negative chronotropic effect). The strongest, but still not complete, cardioinhibitory action of the heart was demonstrated by Lem-MS application at a concentration 10^{-5} M (Fig. 2). At higher concentrations of the peptide the effect was much more effective, and even stopped the heart completely. The effects of Lem-MS were also dose-dependent and reversible. It is interesting to note that a few minutes' washing with the physiological saline, after each peptide application, restored the heartbeat activity to control levels.

Effect of CCAP and Lem-MS on hemolymph proteins

The average concentration of total soluble protein in the hemolymph of 4-day-old females, injected with physiological solution on day 2 of the reproductive phase, was 30 mg ml^{-1}. Successive injections of the mealworm females with CCAP at concentration range 10^{-9}–10^{-3} M, during the same time interval, caused significant changes in the protein content ($P < 0.05$; Fig. 3). The total soluble protein was elevated to about 65 ± 0.4 mg ml^{-1} and was the highest at concentration 10^{-3} M (77.6 ± 0.3 mg ml^{-1}) compared with controls. The injections of Lem-MS and proctolin, during the same period and doses, also increased the protein content in hemolymph, but the changes were not statistically significant ($P > 0.05$; Fig. 3).

Injecting of females with 10^{-5} M CCAP and Lem-MS 2 days after adult emergence modulated protein composition in the hemolymph, but only the CCAP-elicited changes were significant. Both qualitative and quantitative changes were recorded with the most evident and significant increase of amounts of 130- and 170-kDa proteins and the slight increase of amount of the 67-kDa one (Fig. 4; Table 1).

Effect of CCAP and Lem-MS on free hemolymph carbohydrates

The experiments demonstrated that the CCAP and Lem-MS increase the free hemolymph sugar concentration in young larvae (Fig. 5a, b), but only CCAP elevates them in the adults (Fig. 6). In the young larvae, the response to both injected peptides was time and dose dependent. The injections at dose of 10^{-6} M caused significant elevation of the carbohydrate concentration after 1 h. The maximum

Fig. 2 Dose-responses in the heartbeat frequency of *T. molitor* to the CCAP and Lem-MS in the first minute of assay after peptide application. *RF* responses of the hearts to saline solution. *Symbols* represent mean values ± SEM, *n* = 7. Significant differences (*$P < 0.05$) from the control (saline) are indicated by *asterisk* (Student *t*-test)
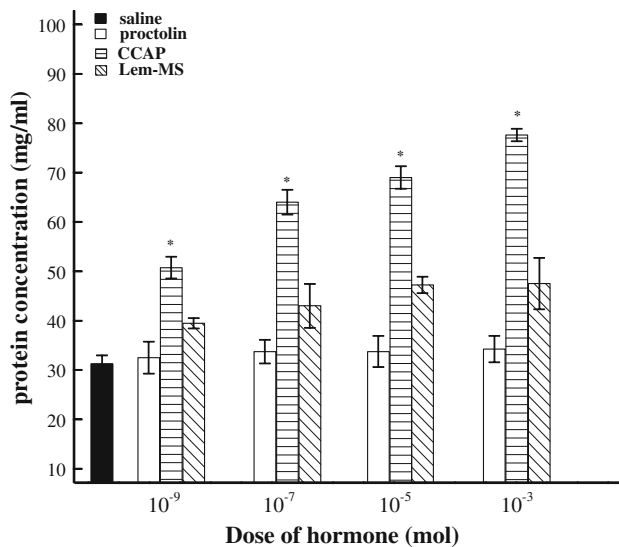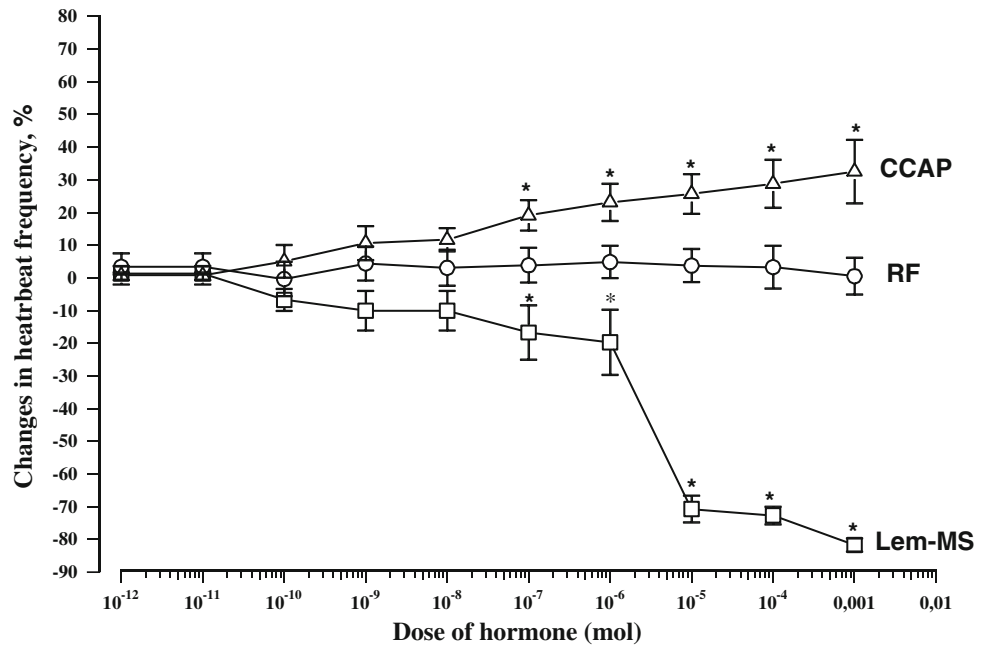

Fig. 3 Changes in total protein concentration of hemolymph in 4-day-old females of *Tenebrio* injected with saline and different dose of CCAP, Lem-MS and proctolin (as internal standard) 2 days after adult emergence. Columns represent mean values ± SEM, *n* = 15. Significant differences (*$P < 0.05$) from the control (saline) are indicated by asterisk (Student *t*-test)

hyperglycemic response occurred 90–120 min after the injection (Fig. 5a). This time-dependent response reduced after reaching its peak, yet the effects persisted until 3 h postinjection.

The hyperglycemic activity of CCAP and Lem-MS was also dose dependent (Fig. 5b). The CCAP injection was a bit, but not significantly more effective and increased the
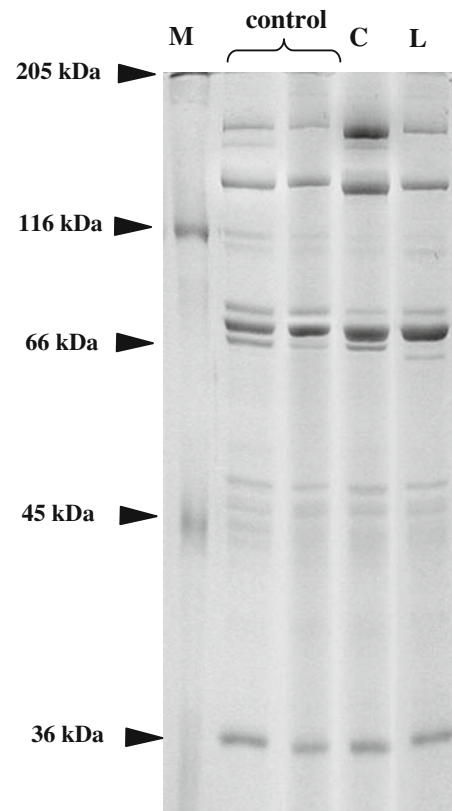
Fig. 4 SDS-PAGE of hemolymph of 4-day-old female *Tenebrio* injected with 10^{-5} M CCAP and 10^{-5} M Lem-MS 2 days after adult emergence. Control females were injected with saline. *C* CCAP, *L* Lem-MS, *M* molecular standards (205 kDa myosin, 116 kDa β-galactosidase, 66 kDa albumin, 45 kDa ovalbumin, 36 kDa glyceraldehyde-3-phopsphate dehydrogenase)

Table 1 Densitometric analysis of electrophoretogram of hemolymph proteins from 4-day-old female *Tenebrio* injected with 10^{-5} M CCAP and 10^{-5} M Lem-MS 2 days after adult emergence. Numbers represent mean values $\pm$ SEM, $n = 10$ (analysis of ten separate gels)

	MW (kDa)			Peak surface			Peak height		
Saline	171.4	130.9	67.2	2,052 $\pm$ 0.9	2,280 $\pm$ 1.2	2,052 $\pm$ 1.1	88,5 $\pm$ 1.1	137,5 $\pm$ 0.7	164,7 $\pm$ 0.96
CCAP	170.5	130.7	66.7	*2,888 $\pm$ 0.74	*2,964 $\pm$ 0.55	2,200 $\pm$ 0.95	*109,4 $\pm$ 1.3	*153,4 $\pm$ 0.88	185,9 $\pm$ 1

Significant differences ($P < 0.05$) experimental versus control (saline) are indicated by asterisk MW (kDa)—molecular weights in kilodaltons; peak surface and peak height—as results of densitogram analysis
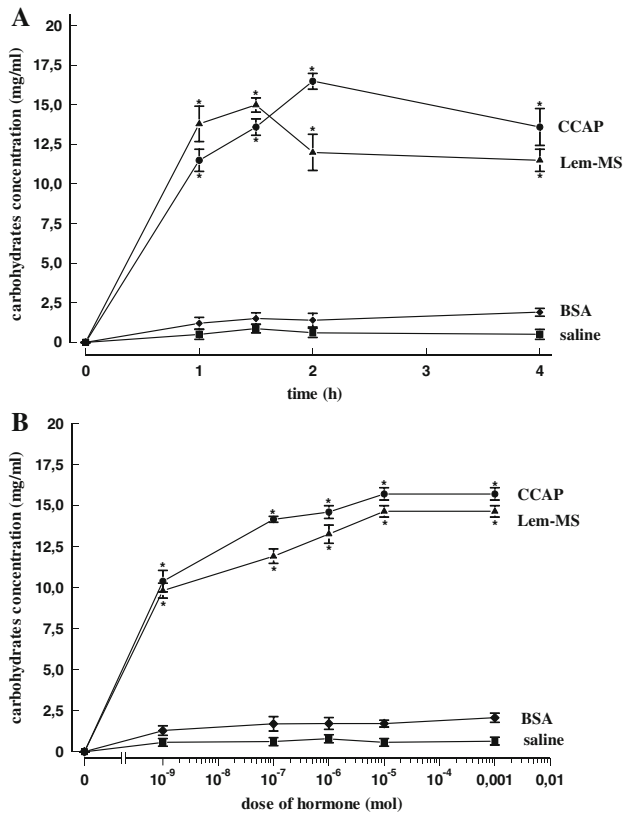

Fig. 6 Hyperglycemic response of freshly ecdysed females of *Tenebrio* injected with saline, BSA (as internal standard), CCAP and Lem-MS. Hyperglycemic response was estimated after 120 min after injection *Symbols* represent mean values $\pm$ SEM, $n = 15$. Significant differences ($P < 0.05$) experimental versus control (saline) are indicated by *asterisk* (Student *t*-test)

Fig. 5 Time (**a**) and dose-dependent (**b**) hyperglycemic response of *Tenebrio molitor* larvae injected with saline, BSA (as internal standard), CCAP and Lem-MS. All bioassays were performed on white, freshly moulted larvae about 150 mg of body mass. Dose-dependent hyperglycemic response was estimated 120 min after injection. *Symbols* represent mean values $\pm$ SEM, $n = 20$. Significant differences ($P < 0.05$) experimental versus control (saline) are indicated by *asterisk*

level of free sugar in the larvae's hemolymph upto ca. 16 ± 0.35 mg ml^{-1}, whereas Lem-MS just to 14.7 ± 0.3 mg ml^{-1}. Similar results were obtained for adults using the injections of the CCAP at the concentration range 10^{-7}–10^{-3} M (Fig. 6). The total content of free sugars was elevated to about 7 ± 0.4 mg ml^{-1} and was the highest at the concentration 10^{-3} M (9.8 ± 0.3 mg ml^{-1}). The application of the same doses of Lem-MS had no effect on increasing the free sugar concentration in the hemolymph of young females (Fig. 6). Very similar results were obtained when bovine serum albumin was injected (Fig. 6).
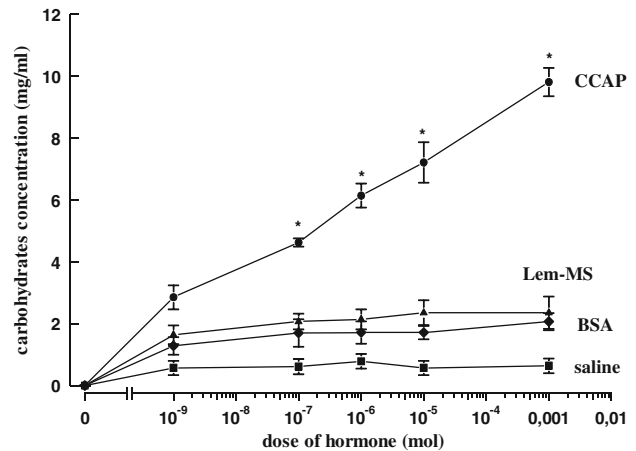
Discussion

Intensive investigation of insect myotropic peptides in the last few years indicated a pleiotropic activity of many of them. Schoofs et al. (2001) found that some peptides from a FMRF-amide like family, which are known for their myotropic activity, also affect ovary maturation and egg development, inhibit food intake and also promote cuticle melanization in *Locusta migratoria* and *S. gregaria*. Also CCAP originally discovered as myotropic factor of *Tenebrio* (Furuya et al. 1993) has been detected in many various insect species (Cheung et al. 1992; Veelaert et al. 1997; Sakai et al. 2004), where regulates many different processes (Veelaert et al. 1997; Coast 1998; Vullings et al. 1999). Recent investigations show that both CCAP and Lem-MS also control the release of adipokinetic/hypertrehalosemic neurohormones from *corpora cardiaca* (Schoofs et al. 2001).

The cardioactive peptide (CCAP) and Lem-MS possess cardiotropic activity, and both can modulate the action of the beetle's heart. They have a stimulatory (CCAP) or inhibitory (Lem-MS) effect on the isolated heart of the

beetle *T. molitor* (Rosiński, 1995; Skonieczna and Rosiński, 2004). In *Locusta migratoria*, Cuthbert and Evans (1989) showed that Lem-MS acts in a dose-dependent manner, like schistomyosuppressin. The cardioinhibitory activity of myosuppressins is probably strongly connected with their C-terminal amino acid sequence -HVFLRF-amide (Wang et al. 1995). The heartbeat activity of tested peptides was studied only in one aspect, namely ability to induce chronotropic disturbances. The observation of other parameters of contractions, like changes of amplitude or basal tension, requires more complicated monitoring systems, and more advanced analysis of obtained data in the same bioassay. In vitro microdensitometric method allows observing long-time duration of effects for tested peptides.

In this work, we found that CCAP and Lem-MS show also hyperglycemic activity. Both neurohormones elevated the free sugar level in larvae, but only CCAP caused such an effect in *Tenebrio* adults (Fig. 6). The physiological mechanism of hyperglycemic activity of CCAP and Lem-MS is unknown, but it is supposed that there is a relationship to vitellogenesis in female body by influencing accumulation of carbohydrates and lipids in developing oocytes. It is not also known if the hormones affect the carbohydrate level directly and/or indirectly, e.g., by the affecting of CC and releasing of the adipokinetic peptides. The *Tenebrio* CC contains two hormone bioanalogues with hypertrehalosemic activity, Tem-HrTH-I (Gäde and Rosiński 1990) and -II (Rosiński 1995), which stimulate the fat body to synthesize and release trehalose into the hemolymph. If we consider the direct mechanism, it is probable that the tested neurohormones act through a different set of fat body receptors than the Tem-HrTHs because the primary structure of CCAP and Lem-MS is significantly different from the structure of Tem-HrTH (Rosinski and Gäde 1988a, b). It is only the hypothesis, that CCAP acts on insect fat body similar to glucagons in mammalian. In this hypothetical action CCAP can activate a phosphorylase cascade, break up of fat body glycogen and elevate of hemolymph carbohydrate. Probably, CCAP can act as extracelluar ligand by binding to appropriate, unknown fat body cell surface receptor. The most probable mode of action is via guanosine protein-coupled receptors (GPCRs) and activation of fat body glycogen phosphorylase. It can generate hyperglycemia in hemolymph. We have found that the injections of CCAP to young *Tenebrio* females increased also the concentration of soluble proteins in hemolymph (Fig. 3). These results indicate that the metabolic activity of CCAP in *Tenebrio* females is connected not only with elevation of carbohydrate level, but also with hemolymph hyperproteinemia. In *Locusta* and *Schistocerca*, CCAP is a releasing factor for neurohormones from AKH/HrTH group (Veelaert et al. 1997). The neurohormones AKH/HrTH take part in the regulation of many biochemical processes of fat body and peripheral tissues (Gäde et al. 1997). Apart from mobilization of carbohydrates and lipids they also influence on protein metabolism. In *Blaberus discoidalis* females, Bld-HrTH stimulates the fat body to synthesize soluble proteins and increases their release into the hemolymph (Keeley et al. 1991). It is quite possible that a similar function exists in *Tenebrio* females, where CCAP may stimulate the release of AKH/HrTH neuropeptides from CC and plays an important role in the regulation of protein and carbohydrate content in hemolymph. We also confirmed myotropic activity of both peptides in our in vitro bioassays with semi-isolated heart. The tissue response depends on neurohormone and its concentration (Figs. 1, 2). Although the beetle's heart is sensitive to high concentrations of CCAP and Lem-MS, a cardiotropic bioassay allowed identification of these peptides as myotropic. Because of the high rate of endogenic contractile activity of myocardium in *Tenebrio molitor*, in vitro assays require non-physiological concentrations of tested peptides to demonstrate their effective action. Previous in vivo studies on the heart of *D. melanogaster* also demonstrated effects of peptide concentrations over 10^{-4} M (Dulcis et al. 2005).

In summary, the present study revealed new metabolic functions of CCAP and Lem-MS other than their myotropic ones and showed their potential role in regulation of many other different processes with essential physiological meaning.

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