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Stimulation of locomotion in *Pyrrhocoris apterus* (Heteroptera: Pyrrhocoridae) is wing-morph independent and correlated with lipid mobilization by adipokinetic hormone

Radomír SOCHA, Dalibor KODRÍK and Rostislav ZEMEK

Institute of Entomology, Academy of Sciences, Branišovská 31, CZ-370 05 České Budějovice, Czech Republic;
e-mail: socha@entu.cas.cz

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Abstract. The effects of 5 pmols of adipokinetic hormone (Lom-AKH-I) on both the locomotion and mobilization of lipids were studied in 10-day-old diapausing adult females of the short-winged (brachypterous) morph of *Pyrrhocoris apterus* (L.). The results revealed that AKH stimulation of locomotion in this bug is wing-morph independent. The stimulatory effect of AKH on locomotion was shown to be positively correlated with its effect on lipid mobilization.

INTRODUCTION

The stimulatory effect of adipokinetic hormone (AKH) from *Locusta migratoria* (Lom-AKH-I), an insect neuropeptide from the adipokinetic hormone/red pigment concentrating hormone family (Gäde, 1997), on insect locomotion was firstly demonstrated in the long-winged (macropterous) adult females of the flightless firebug, *Pyrrhocoris apterus* (L.) (Heteroptera) (Socha et al., 1999). Since there are wing morph-related physiological differences in *P. apterus* (Šula et al., 1998), there is a question as to whether the AKH stimulates locomotion only in the macropterous morph with reproduction arrest of non-diapause type or also in the diapausing short-winged (brachypterous) morph.

The other question is the mechanism of the stimulatory effect of AKH on locomotion. A hypothetical model of dual roles (metabolic and neuromodulatory) of this hormone was already proposed (Socha et al., 1999). The presumption that AKH influences the locomotion via mobilization of lipids was tested in the present study by determination of the temporal course of the lipid level in the haemolymph and its correlation with locomotion data.

MATERIAL AND METHODS

The laboratory stock of brachypterous *P. apterus* used in the present study originated from bugs collected in the Czech Republic (České Budějovice, 49°N). The bugs were reared on linden seeds and water ad libitum at a constant temperature of 26 ± 1°C under a long-day (LD, 18L : 6D) photoperiod, allowing continuous breeding. Animals destined for diapause were reared from eggs under a short-day (SD, 12L : 12D) photoperiod at 26 ± 1°C. For more details on rearing methods see Socha et al. (1997).

The 10-day-old adult females of the brachypterous morph destined for determination of adipokinetic responses were injected with either adipokinetic hormone from *L. migratoria* (Lom-AKH-I; Calbiochem) (experimental group) or saline solution (control group). Treated females were injected with 5 pmol of the hormone (dose was derived from our pilot experiments) in a total volume of 2 μl of 20% methanol in Ringer saline and control females of the same age were injected with the solvent only. This volume was injected with a syringe (5 or 10 μl; Hamilton Co., Reno, Nevada) through the metathoracal-abdominal intersegmental membrane into the thorax.

Locomotion was measured in 42 bugs in both the experimental and control group. For detailed description of the method see Socha et al. (1999). The obtained time series data were analyzed by application of the unstructured multivariate approach (Diggle, 1992). Lipid concentration in the haemolymph was determined by the modified sulpho-phosphovanilin test (Zöllner & Kirsch, 1962). The haemolymph samples were collected just before and at various times, ranging between 0.5 and 9 h after the injection, so that the data constituted a time series. The optical densities, recorded at 546 nm in a spectrophotometer (UV 1601 Shimadzu), were converted with the aid of a calibration graph, based on known aliquots of oleic acid to mg lipids per ml haemolymph using the Microsoft Excell program. Results were expressed as a mean of haemolymph lipid elevations (difference of lipid levels after and before injection) ± SD. The results of adipokinetic responses were plotted and the curve was fitted by the Fig. P program (Biosoft, version 6.0, Cambridge, U.K.). For statistical analyses the Instat program (GraphPad Software, version 1.15, San Diego, CA, U.S.A.) was used.

RESULTS

The ratio between the means of locomotion of AKH-treated and control females of *P. apterus* gradually increased within 5 h after injection of the hormone and then subsequently decreased (Fig. 1). Time series analysis revealed highly significant (D = 48.14, df = 9, $P < 0.001$) differences between locomotion of treated and control females. The results thus demonstrated the stimulatory effect of AKH on the locomotion of diapausing brachypterous females.

The temporal course of the adipokinetic response in AKH-treated females (Fig. 2) exhibited a similar pattern as found in the locomotion ratio. The elevation of lipids in haemolymph in AKH-treated females ranged from 3.5 to 14 mg/ml, with a maximal response recorded at the 4th hour after the treatment. The differences between the responses of AKH- and saline-treated females were statistically significant (t-test, $P < 0.05$), except for the value measured at 0.5 h after treatment.

The linear regression applied to the relationship between locomotion ratio and increase of lipids in haemolymph of AKH-

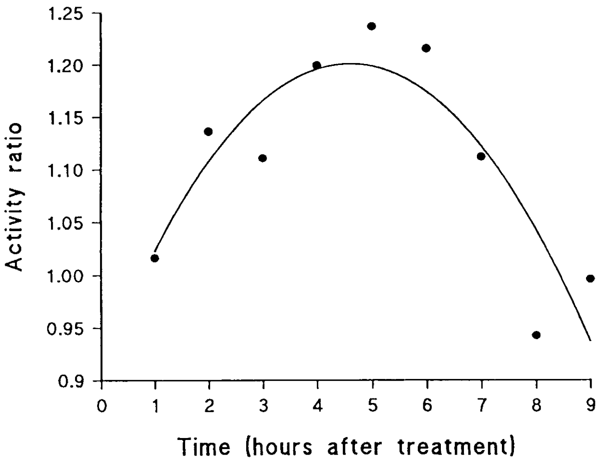

Fig. 1. Ratio of locomotion (mean number of infrared beam crosses per female and hour) between AKH-treated (injected with 5 pmol of the Lom-AKH-I) and control (injected with the solvent only) females of *P. apterus*. Data were fitted by 2nd order polynomial regression.

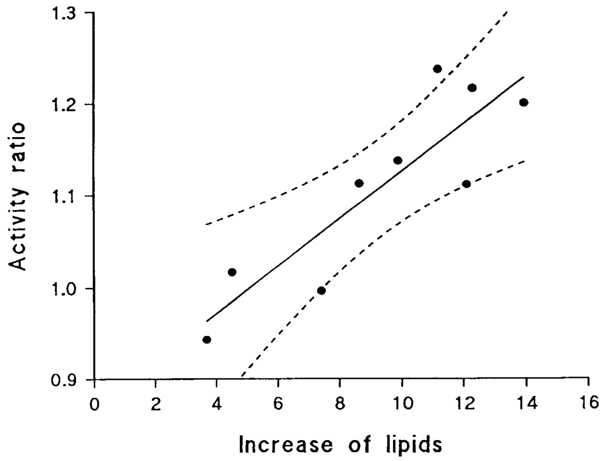

Fig. 3. Relationship between locomotion ratio and increase of lipids in haemolymph of AKH-treated females of *P. apterus*. Solid line: regression line in the form y = 0.0256x + 0.8690 (r = 0.8813, P = 0.0017); dashed line: 95% confidence intervals.

treated females of *P. apterus* (Fig. 3) was able to explain 77.7% of the total variance.

DISCUSSION

We reported earlier that AKH stimulates locomotion in *P. apterus* macropterous females with a non-diapause reproductive arrest (Socha et al., 1999). Our present results revealed that AKH stimulates locomotion also in the brachypterous morph of *P. apterus*. Thus, we experimentally proved that the AKH effect on locomotion is a more ordinary phenomenon of this hormone and does not represent a feature connected with macroptery. However, the comparison of the data on locomotion obtained for macropterous (Socha et al., 1999) and brachypterous females (present paper) of this bug treated with the same dose of AKH indicates some differences in the response to this hormone. In macropterous females the stimulation of locomotion culminated at the 2nd hour after treatment, while in brachypterous females the peak occurred between the 4th and 6th hours after injection. In addition, the ratio of locomotion between the AKH-treated and control females was higher in the macropterous morph (about 1.8) than that in the brachypterous morph (about 1.25). We suppose that this reflects differences in several physiological characteristics (e.g. feeding, drinking, digestion, and carbohydrate and lipid contents) which are, in turn, associated with the life strategies of these two wing morphs (Šula et al., 1998). The higher capability of macropterous females to mobilize lipids from the fat body might ensure their high locomotion. The lipids appear to provide a substantial part of their energetic demands for this purpose since the glycogen content in their fat bodies is very low, 20% of the value found in reproductive brachypterous females, and 4% of the value found in diapausing brachypterous females (Šula et al., 1998). On the contrary, the diapausing brachypters exhibit substantially lower locomotion (Socha & Zemek, unpublished data) probably because they are preparing for hibernation and for this purpose they accumulate energetic reserves (Socha et al., 1998).

We found a highly significant effect of treatment by AKH on the level of lipids in the haemolymph of the brachypterous females. The temporal course of the stimulatory effect of AKH on the locomotion was positively correlated with its effect on lipid mobilization. This finding is in accordance with the metabolic pathway of our hypothetical model describing the possible mechanism of AKH effect on locomotion via mobilization of lipids (Socha et al., 1999).

The higher locomotion response to AKH in macropterous than in diapausing brachypterous females is in accordance with higher lipid concentration in the haemolymph (Šula et al., 1998) and faster mobilization of lipids in macropters after injection of hormone (unpubl. data). Similarly, a substantially higher lipid level during rest and more intensive mobilization of lipids after AKH injection were found in crowded (gregarious) than in isolated (solitary) phases of *L. migratoria* (Ayali & Pener, 1995). These data also coincided with a much faster increase of lipid levels in haemolymph during flight in gregarious than in solitary locusts (Ayali at al., 1996). To date, the phase- or wing morph-related differences in adipokinetic responses were reported only for *L. migratoria* (Ayali et al., 1996), *Schistocerca gregaria* (Schneider & Dorn, 1994; Ogoyi et al., 1996), some noctuids (Fescemyer, 1993), and *P. apterus* (Socha & Kodrík, 1998).

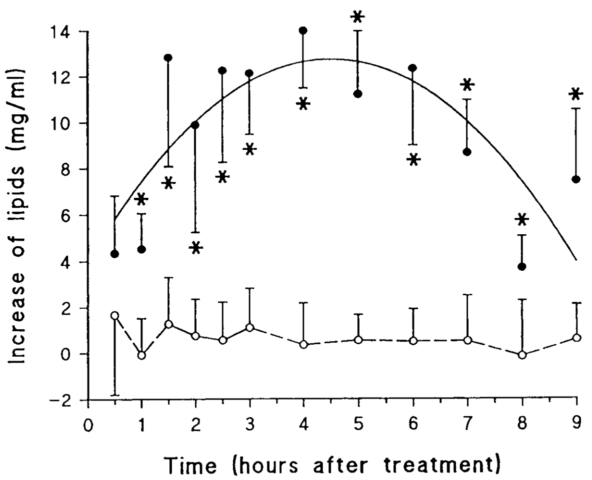

Fig. 2. The temporal course of adipokinetic response in 10-day-old diapausing brachypterous females of *P. apterus* injected with 5 pmol of Lom-AKH-I expressed as elevation of haemolymph lipid level. Controls represent effect of injected saline. Statistically significant differences at the 5% level are indicated by * (experimental vs. control). Note that each point represents an average of independent samples from 6–11 bugs. Data of AKH-treated females were fitted by 2nd order polynomial regression.

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