Journal of Insect Physiology 54 (2008) 1404–1412

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Age-dependent changes of fat body stores and the regulation of fat body lipid synthesis and mobilisation by adipokinetic hormone in the last larval instar of the cricket, *Gryllus bimaculatus*

Anurag N. Anand, Matthias W. Lorenz *

Department of Animal Ecology 1, University of Bayreuth, 95440 Bayreuth, Germany

ARTICLE INFO

Article history:
Received 5 April 2008
Received in revised form 30 July 2008
Accepted 4 August 2008

Keywords:
AKH
Energy stores
Food intake
Hormonal regulation
Triacylglycerol lipase

ABSTRACT

Data on the hormonal regulation of the formation and mobilisation of fat body stores are presented and discussed in relation to general parameters of last instar larval development such as growth, food intake, and moulting. Crickets feed voraciously during the first half of the last larval stage. With the onset of feeding, fat body lipid synthesis increases, leading to increasing lipid stores in the fat body with a maximum reached on day 5. Lipid (42% of fat body fresh mass) is the main constituent of the fat body stores, followed by protein (6%) and glycogen (2%). During the second half of the last larval stage, feeding activity dramatically decreases, the glycogen reserves are depleted but lipid and protein reserves in the fat body remain at a high level except for the last day of the last larval stage when lipid and protein in the fat body are also largely depleted. The process of moulting consumes almost three quarters of the caloric equivalents that were acquired during the last larval stage. Adipokinetic hormone (AKH) inhibits effectively the synthesis of lipids in the larval fat body. Furthermore, AKH stimulates lipid mobilisation by activating fat body triacylglycerol lipase (TGL) in last larval and adult crickets. Both effects of AKH are weaker in larvae than in adults. This is the first report on the age-dependent basal activity of TGL in larval and adult insects. In addition, for the first time, an activation of TGL by AKH in a larval insect is shown.
© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

The insect fat body is of central importance for maintaining metabolic homoeostasis. In addition to many other functions, the fat body synthesises lipid, glycogen, and protein (Keeley, 1985) which can be broken down during periods of high energy demand. The larval stages generally serve to gain body size and weight and to accumulate substrates that allow the imago to emerge with significant fat body stores (Gilbert and Chino, 1974; Beenakkers et al., 1985). In insects that do not feed as adults, it is these energy stores that provide fuel for flight and reproduction (Gilbert and Chino, 1974; Arrese et al., 2001). By contrast, insects that feed as adults do not rely completely on energy stores built up during the larval stages. Nevertheless, an appreciable amount of stored energy reserves is usually carried over from the last larval stage, increased by intense feeding during early adult life, and later mobilised to fuel flight and reproduction (Hill and Goldsworthy, 1968; Walker et al., 1970). The two-spotted cricket, *Gryllus bimaculatus*, belongs to the latter group, which starts adult life with significant amounts of lipid, glycogen, and protein in the fat body (Lorenz and Anand, 2004). Due to intense feeding (Woodring et al., 2007) and a high rate of lipid synthesis (Lorenz, 2001; Lorenz and Anand, 2004), the amount of lipid in the fat body is more than doubled within a short time and reaches a maximum on day 2 after adult emergence. In addition to lipids, the major component of the fat body, protein and glycogen also increase dramatically during these first 2 days of adult life. Furthermore, considerable amounts of energy-rich substrates are transferred to the developing flight muscles. Thereafter, the fat body stores are mobilised to fuel vitellogenic egg growth and, about 2 days later, the flight muscles start to histolyse and probably provide additional substrates for oogenesis (Lorenz and Anand, 2004; Lorenz, 2007).

The intermediary metabolism of the fat body is regulated by hormonal signals. Juvenile hormones and ecdysteroids are involved mainly in the regulation of vitellogenin synthesis and release (Hardie, 1995), although they also act on the formation and possibly on the release of fat body lipids (Zhao and Zera, 2002). The most important hormonal regulators of intermediary metabolism, however, are neuropeptides of the adipokinetic/hypertrehalosaemic hormone (AKH) family (Gäde et al., 1997; Van der Horst, 2003). The study of action of these hormones on intermediary metabolism has mainly focused on lipid or carbohydrate mobilisation in adult

* Corresponding author. Tel.: +49 921 552655; fax: +49 921 552784.
E-mail address: matthias.lorenz@uni-bayreuth.de (M.W. Lorenz).

0022-1910/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jinsphys.2008.08.001

flying insects. In addition to their activating role in catabolic processes, and in analogy to the vertebrate hormone glucagon, AKHs also inhibit anabolic pathways in the insect fat body, such as the synthesis of lipids (Gokuldas et al., 1988; Lee and Goldsworthy, 1995; Ziegler, 1997; Lorenz, 2001) or proteins (Carlisle and Loughton, 1979; Asher et al., 1984; Cusinato et al., 1991). A number of studies focused on the energy-mobilising function of AKH in larval stages where the action of AKH is comparable to that in adult insects, but the maximal metabotropic effect is lower with the result that higher doses of AKH are required to obtain the same energy-mobilising effect as in adults (Mwangi and Goldsworthy, 1977; Van Marrewijk et al., 1984; Ziegler et al., 1990, 1995; Woodring et al., 2002). Furthermore, it was found that in insects that mainly use lipid as a flight fuel, such as *Manduca sexta*, *Locusta migratoria*, and *G. bimaculatus*, AKH mobilises carbohydrate stores in larvae, leading to increased sugar titres in the haemolymph. Therefore, AKH should also be regarded as an important regulator of homoeostasis in insect larvae (Oudejans et al., 1993; Oda et al., 2000). Such an increase in haemolymph sugars is much weaker or not detectable in adults (Van Marrewijk et al., 1984; Ziegler et al., 1990; Woodring et al., 2002), indicating a somewhat higher relative importance of carbohydrate metabolism in the larval stages of these insects.

The role of AKHs in larval insects, however, has not been studied intensively. Because the amount of fat body reserves first accumulated in the last larval stage and later carried over to the adult stage is very important for the fitness of the imago, the present research was conducted to shed some light on the possible functions of AKH in last larval instar females of *G. bimaculatus*. As a first step, general parameters of larval development, age-dependent accumulation of energy-rich substrates in the fat body, and the capacity of the fat body to synthesise lipids were determined. This served as a basis for the subsequent experiments in which the effects of AKH on synthesis and mobilisation of lipids in the larval fat body were investigated and compared with the effects of AKH in adult females.

2. Materials and methods

2.1. Experimental animals

Two-spotted crickets, *Gryllus bimculatus* de Geer (Ensifera, Gryllidae) were taken from our lab colony. The origin of the founder animals and the maintenance of the colony is described in detail elsewhere (Lorenz et al., 1997, 2004). Newly ecdysed (within 30 min after moult) female last instar larvae (LL) were collected between 08:00 and 09:00 h Central European Summer Time (CEST) and designated 0-day-old. Thus the insects described in this paper as "day 0" are between 0 and 24 h after the previous ecdysis, and so on. The staged LL were reared in groups of 10–40 in 3 or 6 l transparent plastic containers ("Fauna-Box"; Savic, Heule, Belgium) under the same conditions as described above an fed with a 2:4:1 mixture of breeding diet for rats (No: 1311), maintenance diet for rabbits (No. 2021), and breeding/maintenance diet for cats (No. 5031; Altromin, Lage, Germany). The experiments were performed between 08:00 and 09:00 h CEST, therefore, 1-day-old LL were 24 ± 0.5 h old, 2-day-old LL were 48 ± 0.5 h old, etc. For some experiments, where 12-, 18- and 36-h-old animals were used, these were collected from the colony between 14:00 and 15:00 h or 20:00 and 21:00 h CEST, respectively. Experiments with 6-h-old animals were started between 14:00 and 15:00 h CEST, using animals that had been collected between 08:00 and 09:00 h CEST.

Under the conditions described, the last larval stage lasted 8–9 days, with the majority of animals moulting during the second half of day 8. A small number of the larvae (about 5–10%) moulted during the night from day 7 to day 8 of the last larval stage, thus they were only 7-day-old at the time of the adult moult. For some experiments, newly ecdysed adult females were collected and reared as described for the last instar larvae.

To obtain pharate adults, i.e. 8-day-old LL which had already formed the new adult cuticle under the old larval cuticle, the rearing boxes with LL at day 8 after the moult were inspected in the afternoon for animals that displayed no or almost no locomotor activity. Approximately 50% of these animals were pharate adults.

2.2. Determination of body weight and food intake

The animals were weighed each day between 08:00 and 09:00 h CEST on a Sartorius LC1201S balance to the nearest mg.

For the determination of food intake, female LL were kept individually in plastic containers (Polarcup No. 590, 16 cm $\times$ 11 cm $\times$ 6 cm, length $\times$ width $\times$ height; Bellaplast AG, Altstätten, Switzerland) with a piece of papier-mâché egg divider as a shelter and a glass vial plugged with cotton wool as a water supply. The diet was supplied in glass Petri dishes (5 cm diameter, 1 cm height) of known weight and they were replaced with new Petri dishes containing fresh food mixture every 24 h. Each cricket received 500 mg of fresh food, which amounts to 458.5 mg dry mass, according to the measured water content ($8.3 \pm 0.1\%$, $n = 30$).

2.3. Biochemical composition of the fat body

The abdominal fat body from one side was dissected from decapitated crickets and transferred to a pre-weighed 1.5 ml safe-lock Eppendorf tube, filled with 20 mg Na_2SO_4 and 200 μl 75% MeOH in water to determine the fat body fresh mass and to estimate lipid, protein, glycogen, and free carbohydrate content as previously described (Lorenz, 2003, 2004) and modified (Lorenz, 2007). Separation of the total lipid extract into lipid classes by thin-layer chromatography was performed as described previously (Lorenz, 2001). The bands containing lipids were scraped off, extracted with hexane and quantified using a modified sulphophosphovanillin essay (Lorenz, 2003).

For the determination of fat body water content, fat bodies were dissected into pre-weighed Eppendorf tubes. The fresh mass was measured and subsequently the fat bodies were dried overnight in a SpeedVac and reweighed (Lorenz and Anand, 2004).

2.4. Acetate incorporation into fat body lipids

The incorporation of acetate into fat body lipids was estimated by incubating half abdominal fat bodies for 60 min in medium 199 (M7653; Sigma–Aldrich, Taufkirchen, Germany) which was modified as described by Lorenz (2001; MM199) and contained approximately 1.8 GBq [1-^{14}C]Na-acetate/mmol (MC 125; Hartmann Analytic, Braunschweig, Germany). To determine age-dependent effects of adipokinetic hormone, one half fat body was incubated in MM199 without further additions (control), whereas the other half fat body was incubated in MM199 containing 10^{-7} M *G. bimaculatus* AKH (Grybi-AKH, pQVNFSTGWamide; H-9230; Bachem, Weil am Rhein, Germany; purified by HPLC).

The dose-dependency of the inhibition of acetate incorporation by AKH was tested using quarter fat bodies incubated for 60 min in MM199. One fat body quarter served as the control, whereas the other three quarters were incubated in medium containing different concentrations of AKH.

2.5. Lipid and carbohydrate mobilisation

Haemolymph (2 μl per animal) was collected immediately before and 180 min after the injection of 2 μl of 20% dimethylsulf-

Download English Version:

https://daneshyari.com/en/article/2841267

Download Persian Version:

https://daneshyari.com/article/2841267

Daneshyari.com