Available online at www.sciencedirect.com
ScienceDirect
Insect Biochemistry and Molecular Biology 36 (2006) 759–768
Insect Biochemistry and Molecular Biology
www.elsevier.com/locate/ibmb

Endocrine control of TAG lipase in the fat body of the migratory locust, *Locusta migratoria*

Lutz Auerswald*, Gerd Gäde

Zoology Department, University of Cape Town, Rondebosch 7701, South Africa

Received 20 March 2006; received in revised form 13 June 2006; accepted 17 July 2006

Abstract

Aspects of the role and activation of the enzyme triacylglycerol lipase (TAG lipase) in the fat body of the migratory locust *Locusta migratoria* were investigated. TAG lipase is under the hormonal control of the three endogenous adipokinetic peptides of the migratory locust, Locmi-AKH-I, Locmi-AKH-II and Locmi-AKH-III. Injection of low doses (5–10 pmol) of each peptide causes an increase in lipase activity. The activation of lipase is time dependent: an elevated activity was recorded 15 min after injection of 10 pmol Locmi-AKH-I and maximum activation was reached after 45–60 min. The activation of TAG lipase is also dose-dependent. Doses of 2 pmol of each Locmi-AKH had no effect, whereas 5 pmol caused a significant activation. Maximum activation is reached with a dose of 10 pmol. Analogues of the second messengers cAMP (cpt-cAMP) and IP_3 (F-IP_3) both activate the enzyme glycogen phosphorylase whereas only cpt-cAMP, but not F-IP_3, activates TAG lipase; cpt-cAMP elevates the lipid levels in the haemolymph. Activation of lipase is specific to the three endogenous AKH peptides: 5 pmol of the endogenous peptide Locmi-HrTH and 10 pmol of corazonin failed to activate lipase. High doses of octopamine did not activate lipase nor did they elevate the lipid concentration in the haemolymph. TAG lipase is stimulated by flight activity but activation is slower than that of glycogen phosphorylase: after 30 min of flight or after 5 min of flight plus 1 h of subsequent rest, activity of TAG lipase is increased, but not immediately after 5 min of flight. In contrast, glycogen phosphorylase is activated significantly after 5 min of flight. These activation patterns of the two enzymes mirror-image the concentration of their substrates in the haemolymph: there is a significant decrease in the concentration of carbohydrates after 5 min of flight, whereas no change of the concentration of lipids can be measured after such short time of flight activity; however, a subsequent rest period of 1 h is sufficient to increase the lipid concentration.

Keywords: Triacylglycerol; Lipase; Locust; Insect; AKH; Glycogen phosphorylase; Second messengers; Octopamine

1. Introduction

Principle energy substrates for the contraction of flight muscles of insects are lipids, carbohydrates and the amino acid proline or a combination of these three substrate classes (Gäde and Auerswald 2003). Locusts, which are known for their capability of long-distance flight, typically use lipids to power such long flights (Beenakkers et al. 1981), although the amino acid proline and carbohydrates also contribute to the energy supply during locust flight. Flight metabolism in locusts is divided into three chronological phases:

(1) In the first phase, during the transition from resting metabolism to flight, the concentration of proline in haemolymph and flight muscles declines rapidly because proline 'sparks' the Krebs cycle in mitochondria of the flight muscles (Worm and Beenakkers 1980). Mobilization of energy reserves by peptide hormones (see below) is also initiated as flight stimulates the release of adipokinetic hormones from the corpora cardiaca into the haemolymph (van der Horst 2003).
(2) In the next phase, which lasts up to approximately 30 min into the flight, glucose that derives from

*Corresponding author. Tel.: +27 21 650 3624; fax: +27 21 650 3301.
E-mail address: lauerswa@botzoo.uct.ac.za (L. Auerswald).

0965-1748/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ibmb.2006.07.004

glycogen in the flight muscles and trehalose in the haemolymph, serves as the major substrate (Worm and Beenakkers 1980). These immediate energy reserves are depleted fast, however, because the demand exceeds the supply of carbohydrates soon after the onset of flight. From this stage onwards, energy substrates have to be mobilized for use from reserves that are mainly stored in the fat body.

(3) This happens in the third phase of flight metabolism when mainly lipids are oxidized, although there is still a minor contribution of carbohydrates (van der Horst et al. 1978, Worm and Beenakkers 1980). Substrates, which are mainly stored in the fat body as glycogen and triacylglycerides, are mobilized by a complicated cascade of events; these are initiated by the stimulus of flight (see van der Horst 2003).

Such a stimulus causes the release of neuropeptides from the corpora cardiaca, where they are synthesized and stored (Gäde 1996, 2004). The peptides belong to the family of adipokinetic hormone (AKH)/red pigment-concentrating hormones (RPCH). In *Locusta migratoria*, three endogenous peptides are present that affect metabolism: Locmi-AKH-I (pGlu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH_2), Locmi-AKH-II (pGlu-Leu-Asn-Phe-Ser-Ala-Gly-Trp-NH_2) and Locmi-AKH-III (pGlu-Leu-Asn-Phe-Thr-Pro-Trp-Trp-NH_2)(see Gäde and Auerswald 2003). In addition, a fourth inactive peptide denoted locust hypertrehalosaemic hormone because of its activity in cockroaches (Locmi-HrTH; pGlu-Val-Thr-Phe-Ser-Arg-Asp-Trp-Ser-Pro-NH_2) has been found (Siegert 1999). The three active AKH peptides have different potencies to mobilize energy reserves and their half lives vary too (van der Horst et al. 2001). These differences in potency led to the proposal that Locmi-AKH-I (by far the most abundant AKH) is the major lipid-mobilizing hormone, whereas Locmi-AKH-II may primarily be responsible for carbohydrate mobilization, and Locmi-AKH-III is thought to control energy supply during non-flight activities (van der Horst et al. 2001). Locmi-HrTH does not have any effect on mobilization of metabolite reserves (Siegert 1999).

The key enzyme during carbohydrate mobilization by AKH peptides is glycogen phosphorylase (Gäde 1981), whereas triacylglycerol (TAG) lipase is responsible for the provision of lipids in the haemolymph of *L. migratoria* (van der Horst et al. 2001). Trehalose released from the fat body does not accumulate in the haemolymph, probably because consumption and mobilization are balanced. The carbohydrate concentration in the haemolymph is, therefore, not useful for observing the hypertrehalosaemic effect (see Gäde and Auerswald 2003) but the activation of glycogen phosphorylase in the fat body of locust has been extensively studied (see, for example, Gäde 1981; Vroemen et al. 1995, 1997). Only scattered information, however, is available on the action of TAG lipase in the fat body of insects in general. The most coherent body of information is available for the moth *Manduca sexta* (see Arrese et al. 2001) which has also been shown to use mainly lipids for sustained flight. Moreover, activation of lipase by an endogenous AKH peptide was corroborated for the fruit beetle, *Pachnoda sinuata* (Auerswald et al. 2005). This beetle uses carbohydrates and proline as major flight substrates and lipase activation is necessary for the re-synthesis of proline (Gäde and Auerswald 2002; Auerswald et al. 2005). For locusts, only fragments of information on TAG lipase have been published, such as ideal in vitro assay conditions for lipase from the fat body of *L. migratoria* (Hirayama and Chino 1987) or, differences in lipase activation in gregarious versus solitary phases in the desert locust, *Schistocerca gregaria* (Ogoyi et al. 1998). Most of the knowledge on activation of TAG lipase in the fat body of locusts derives from the indirect measurement of its action, via monitoring the concentrations of lipids in the haemolymph. This is a much easier method to perform compared with the laborious procedure of the lipase assay as outlined recently (Auerswald et al. 2005).

TAG lipase in the fat body initiates the lipolytic process with the liberation of fatty acids from TAGs, leading to the formation of stereo-specific sn-1,2-diacylglycerols (see Ryan and van der Horst 2000). These diacylglycerols are released into the haemolymph, where they are loaded onto lipophorin and transported to the flight muscles where they are unloaded and the fatty acids oxidized to gain energy (Beenakkers et al. 1985; Kanost et al. 1990).

The present study was designed to provide some more detailed information on the mode of activation of TAG lipase in the fat body of *L. migratoria* by the three endogenous AKH peptides to complement knowledge already available on the hypertrehalosaemic and hyperlipaemic action of these peptides and to relate this activation of TAG lipase to flight activity.

2. Materials and methods

2.1. Insects

Two to four weeks old adult male locusts (*Locusta migratoria*) were taken from our own colony; they were reared as previously described (Gäde 1992).

2.2. Bioassays

The locusts were placed under plastic funnels (7 cm diameter) 2 h prior to experimentation at ambient temperature of 25 °C. For measurement of the activation state of TAG lipase, locusts were injected with the appropriate substances (peptides, octopamine, etc.) and were killed after a certain period. The fat body was subsequently dissected and treated as described below. For determination of lipid concentration in the haemolymph, 1 μl of haemolymph was taken with the help of a microcapillary from a puncture of the membrane at the base of a hind leg