Available online at www.sciencedirect.com
ScienceDirect
Journal of Insect Physiology 54 (2008) 367–377
Journal of Insect Physiology
www.elsevier.com/locate/jinsphys

Regulation of carbohydrate metabolism and flight performance by a hypertrehalosaemic hormone in the mosquito *Anopheles gambiae*

Christian Kaufmann[a,b,*], Mark R. Brown[a]

[a]*Department of Entomology, University of Georgia, Athens, GA 30602, USA*
[b]*Institute of Zoology, University of Neuchâtel, Neuchâtel, NE 2007, Switzerland*

Received 6 June 2007; received in revised form 20 October 2007; accepted 22 October 2007

Abstract

The role of adipokinetic hormones (AKHs) in the regulation of carbohydrate and lipid metabolism and flight performance was evaluated for females of the African malaria mosquito, *Anopheles gambiae*. Injection of various dosages of synthetic Anoga-AKH-I increased carbohydrate levels in the haemolymph and reduced glycogen reserves in sugar-fed females but did not affect lipid levels. Anoga-AKH-I enhanced the flight performance of both intact and decapitated sugar-fed females, during a 4 h flight period. Anoga-AKH-II had no effect on carbohydrate or lipid levels or flight performance, thus its function remains unknown. Targeted RNA-interference lowered Anoga-AKH receptor expression in sugar-fed females, consequently injections of Anoga-AKH-I failed to mobilize glycogen reserves. Taken together, these results show that a primary role for the neurohormone, Anoga-AKH-I, is to elevate trehalose levels in the haemolymph of female mosquitoes.

Keywords: Diptera; Adipokinetic hormone; Neuropeptide; Metabolism; Flight

1. Introduction

Energy expenditure while flying, running, and swimming requires the mobilization of metabolites in insects, and the storage and release of carbohydrates, lipids, proteins, and amino acids are strongly under endocrine control (Gäde, 2004). Neuropeptides in the adipokinetic hormone (AKH) family are important regulators of energy metabolism, and more than 40 peptide isoforms in this family—herein called AKHs—have been extensively characterized across the insect orders (Gäde et al., 1997; van der Horst, 2003; Gäde, 2004). The number of AKHs known to exist in a particular insect species varies from one in the lower orders, such as Odonata, to as many as four in Blattodea and Orthoptera, and in the higher order of Diptera, only one or two AKHs are known. AKHs occur as octa-, nona-, or decapeptides and are characterized by a pyroglutamate at the amino-terminus, two conserved aromatic amino acid residues at position 4 (typically a phenylalanine) and a tryptophan at position 8, and an amidated carboxy-terminus. The blocked termini make it only accessible to endopeptidases. Present in all life stages, these peptides are synthesized and secreted from a distinct region of the corpora cardiaca (CC), a neurohaemal gland connected to the brain that also contains intrinsic neurosecretory cells. Other cell types in the brain and ganglia may also synthesize such peptides, as shown by immunocytochemistry in females of the yellow fever mosquitoes *Aedes aegypti* (Brown and Lea, 1988), and of the African malaria mosquito *Anopheles gambiae* (Kaufmann and Brown, 2006), as well as in other insect species (Schooneveld et al., 1985; Kodrik et al., 2003).

The primary endocrine function of AKH is to mobilize metabolites from storage to the haemolymph, and this has been demonstrated in several different insects. In the migratory locust, *Locusta migratoria*, injection of species-specific AKH mobilized lipids, an adipokinetic or hyperlipaemic effect (van der Horst, 2003). In the American cockroach, *Periplaneta americana*, it increased the trehalose in the haemolymph, a hypertrehalosaemic effect (Scarborough et al., 1984). Similarly, it mobilized proline

---

*Corresponding author. Institute of Zoology, University of Neuchâtel, Neuchâtel, NE 2007, Switzerland. Fax: +41 55 410 6555.
E-mail address: chrigi.kaufmann@gmail.com (C. Kaufmann).

0022-1910/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jinsphys.2007.10.007

in dung beetles, *Scarabaeus* spp. and the fruit beetle, *Pachnoda sinuata*, revealing a hyperprolinaemic effect (Gäde and Auerswald, 2002). In larvae of *D. melanogaster*, manipulation of AKH gene expression altered not only circulating levels of trehalose and lipid, but also affected general locomotor activity and survival of imagos during starvation (Lee and Park, 2004).

The expression of two AKH genes (Anoga-AKHs) and a putative G protein-coupled receptor for AKH (Anoga-AKHR) in female *A. gambiae* (Kaufmann and Brown, 2006) suggest that at least one of the AKHs may be involved in the mobilization of metabolites for flight, as known for other insects (Gäde and Auerswald, 2002; van der Horst, 2003). Both carbohydrates and lipids are used as flight substrates in females of this species (Kaufmann and Briegel, 2004). Other dipteran AKHs have been identified (Table 1), and their bioactivity reported for the blow fly, *Phormia terraenovae* (Gäde et al., 1990), the horse fly (*Tabanus* spp., Woodring and Leprince, 1992), the tsetse fly, *Glossina morsitans* (Pimley, 1984; Mwangi and Awiti, 1989), and the fruit fly, *D. melanogaster* (Lee and Park, 2004). In this study, we first tested the synthetic Anoga-AKHs for effects on metabolite mobilization in female *A. gambiae*, and then examined whether these peptides also affected female flight performance. In addition, this *in vivo* approach was combined with targeted RNA interference of Anoga-AKHR gene expression to link the endocrine effect of an Anoga-AKH to the putative AKHR.

Table 1
AKH family members known for Diptera

Name	Sequence	Species
Tabat-AKH	**pQ**L**TF**TPG**W**a	*T. atratus*[a] and other *Tabanus* spp.[b]
Tabat-HoTH	**pQ**L**TF**TPG**W**GYa	*T. atratus*[a] and other *Tabanus* spp.[b]
Phote-HrTH	**pQ**L**TF**SPD**W**a	*P. terraenovae*[c], *D. melanogaster*[d], and *N. bullata*[e]
Anoga-HrTH (Anoga-AKH-I)	**pQ**L**TF**TPA**W**a	*A. gambiae*[f]
Anoga-AKH-II	**pQ**V**TF**SRD**W**NAa	*A. gambiae*[f], *A. aegypti*[g], and *C. pipiens*[h]
Aedae-AKH-I	**pQ**L**TF**TPS**W**a	*A. aegypti*[g] and *C. pipiens*[h]

Bold letters illustrate conserved amino acids.

[a] Jaffe et al. (1988) also HoTH, hypotrehalo saemic hormone.

[b] AKHs in *T. calens T. lineola T. proximus T. suluciforns* same as in *T. atratus* (Woodring and Leprince, 1992).

[c] Gäde et al. (1990).

[d] Schaffer et al. (1990).

[e] Verleyen et al. (2004).

[f] Kaufmann and Brown (2006).

[g] Predicted in the *A. aegypti* genome data base, Aedae-AKH-I: GeneID, AAEL011996; and AKH-II same in *A. gambiae* and *A. aegypti*: GeneID, AAEL010950.

[h] Predicted in the *C. pipiens* genome data base, Aedae-AKH-I or Culpi-AKH-I: GeneID, 145648990; and AKH-II same in *A. gambiae*, *A. aegypti*, and *C. pipens*: GeneID, 145648990.

2. Material and methods

2.1. Mosquitoes

The colony of *Anopheles* (*Cellia*) *gambiae s.s.* (Giles), strain 16CSS from Lagos, Nigeria, was maintained at $26 \pm 1\,^{\circ}\mathrm{C}$ under long-day conditions (16 h light, 8 h dark). Larvae were raised in trays ($24 \times 16 \times 6$ cm) with 350 ml distilled water and fed pulverized Tetramin® daily (Timmermann and Briegel, 1993). Imagos (200–300 in cages of $24 \times 19 \times 18.5$ cm) had access to 10% fructose solution or distilled water, depending on experimental conditions. For experiments, large females with wing lengths between 3.2 and 3.5 mm (measured from the alula to the tip, including the fringes) were used. For egg production and experiments, females were given blood meals from a human arm.

2.2. Injection of Anoga-AKHs

Synthetic Anoga-AKH-I and Anoga-AKH-II (Table 1) were dissolved first in dimethyl sulfoxide (DMSO) and then diluted to 2.5% DMSO in *Aedes* saline (7.5 g NaCl, 0.35 g KCl, and 0.21 g $CaCl_2$ in 1 l nanopure H_2O; adjusted to pH 6.5). For control injections, 2.5% DMSO in *Aedes* saline alone was used. Known amounts of peptide in 0.25 μl of DMSO/saline were injected with fine glass needles into the first abdominal segment of females to ensure the least damage. In blood-fed female mosquitoes, haemolymph volume doubles as water is drawn from the midgut and excreted in the first hour (1–2.4 μl in *Aedes aegypti*, Clements, 1992), so an injection of 0.25 μl is within physiological tolerance and resulted in $<10\%$ mortality in control and experimental females. The needles were made from borosilicate glass capillaries (TW100-6, World Precision Instruments) on a Flaming/Brown Micropipette puller (Model P-97, Sutter Instruments Co.). After injection, females were caged, held at 27 °C in a humidified chamber, and not given access to fructose solution or water.

2.3. Biochemical analyses

To quantify nutrient reserves, two abdomens from experimental sugar-fed females were pooled, and for experimental blood-fed females, a whole individual or haemolymph from two females was used. Haemolymph was collected by incubating two individuals together in 100 μl *Aedes* saline on ice. Each female was carefully opened at the segmental line between the last two abdominal segments to allow haemolymph to bleed or diffuse into the saline. After 10 min, 90 μl of the haemolymph solution was collected for assay. Sugar, glycogen, and total lipids were measured in the samples using a modified version of Van Handel and Day (1988). For the separation of sugar and lipids 1.6 ml $CHCl_3$–MeOH (v:v, 1:1) and 0.6 ml of distilled H_2O were used. Glycogen in the precipitate and sugar in the aqueous fraction were measured with the hot anthrone