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Relationship of Adipokinetic Hormone I and II to Migratory Propensity in the Grasshopper, *Melanoplus sanguinipes*

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This report examines three aspects of adipokinetic hormone (AKH) involvement in migratory flight behavior in the grasshopper, *Melanoplus sanguinipes.* The titer of hemolymph AKH I during long-duration tethered flight was examined using radioimmunoassay (RIA) after narrow bore RP-HPLC. The hemolymph fraction containing AKH I was assayed using commercially available anti-Tyr[1]-AKH I serum. Titer determinations of hemolymph AKH were done at rest and after various periods of flight. The amount of AKH I released from the corpora cardiaca during flight was estimated. When resting levels of AKH I and II in corpora cardiaca (CC) of migrants and non-migrants were examined with HPLC, no significant differences in AKH levels were detected between non-migrants, animals that had flown for 1 h to identify them as migrants, and animals that had flown to exhaustion (i.e., voluntary cessation). CC levels of both AKH I and II were less in this species than in locusts. When the lipid mobilization in response to AKH I and II was compared in migrants (animals that had self-identified as migrants in a 1-h tethered flight test) and non-migrants (animals that would not perform a 1-h flight in a tethered flight test), the adipokinetic response to AKH I was greater in migrants than in non-migrants, possibly indicating differences in level of sensitivity or number of receptors in the target tissues. AKH II had little effect on hemolymph lipid levels in either flight group, and may not play a significant role in lipid mobilization in this species. Arch. Insect Biochem. Physiol. 55:33–42, 2004.

KEYWORDS: radioimmunoassay; corpora cardiaca; adipokinetic hormone; lipids; migration; RP-HPLC

INTRODUCTION

The North American migratory grasshopper *Melanoplus sanguinipes* Fabricius (Orthoptera: Acrididae) is an important pest species on rangeland in the western United States and Canada (Hewitt, 1977). Outbreak migrations of this species have occurred several times (Parker et al., 1955), and non-circuit migratory flights are common even in non-outbreak years (McAnelly, 1985). Though *M. sanguinipes* displays no consistent morphological differences between fliers and non-fliers (Parker et al., 1955), some individuals will make long, undistracted, tethered flights, and there is a close correspondence between duration of tethered flight in the laboratory and migratory behavior in the field (McAnelly and Rankin, 1986). As with several other insect migrants, individual *M. sanguinipes* tend to fly for only a few moments or for several hours at a time. Those that perform at least 60 min of tethered flight in a single flight test are classified as migrants (McAnelly, 1985). Three 60-min flight tests on succeeding days can reliably distinguish all migrants from non-migrants in a population (McAnelly, 1985; Kent and Rankin, 2001). It may be useful to point out that the flight

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experience imposed on the grasshoppers in these experiments is different from the round-about flights used in locust AKH experiments. The round-about tests do not assess individual *tendency* to migrate though they do stimulate flight activity in the group of animals being subjected to the experience. The individual tethered flight tests used here are behavioral assays designed to distinguish migrants from non-migrants as well as to allow performance of long duration flight. This flight test system allows us to distinguish differences due to, or effects of, migratory *propensity* from those due to actual *performance* of long-duration flight.

Lipid reserves in thorax and eviscerated abdomen are significantly reduced after flight to voluntary cessation in *M. sanguinipes* (Kent et al., 1997; Kent and Rankin, 2001), indicating that lipid is an important flight fuel in this species as it is in locusts (Goldsworthy, 1983). Also as in locusts, hemolymph carbohydrate is rapidly consumed during the early stages of flight, while hemolymph lipid is elevated during the same period. Glycogen stores are relatively larger in *M. sanguinipes* than in locusts and are mobilized over a relatively longer period of time (the first 1–2 h of flight) (Kent et al., 1997).

The locust adipokinetic hormones (AKHs, at least three have been isolated) are members of a family of short peptides with similar amino acid sequences that have various metabolic functions in different insects (Gäde et al., 1977). In locusts and in *M. sanguinipes*, AKH I is responsible for lipid mobilization during flight. The AKHs are produced in the glandular lobes of the CC, a neurohemal/endocrine gland that both stores brain neurosecretions and produces neurohormones. We have identified only AKH I and II in *M. sanguinipes*, with amino acid sequences identical to those of the corresponding locust hormones, Lom-I and Scg-II, respectively (Taub-Montemayor et al., 1997, 2002). Injection of exogenous AKH or extract of *M. sanguinipes* CC elevates hemolymph lipid but does not affect hemolymph carbohydrate (Kent et al., 1997).

In contrast to locusts, the tendency of *M. sanguinipes* to perform long-duration flight is not greatly influenced by environmental cues. Rather, genotype seems to exert a strong influence on the flight behavior (McAnelly and Rankin, 1986) of this species; the heritability of liability of performing long-duration flight is 0.5–0.6 (Kent and Rankin, 2001). Selection on migratory incidence produces a correlated response in the duration of migratory flight (Kent and Rankin, 2001), suggesting that the tendency to migrate is in some way related to the physiological capacity to do so. Although, as noted above, lipid is probably the primary fuel for migratory flight in this species (Kent et al., 1997), total body lipid reserves do not differ significantly in resting migrants and non-migrants nor does body size nor wing length (Kent et al., 1997; Kent and Rankin, 2001).

In this report, we have compared AKH action and titers in *M. sanguinipes* migrants vs. non-migrants and with those reported in locusts. To follow AKH titer changes during flight using a radioimmunoassay (RIA), we employed anti-Tyr^{1}-Lom-AKH I serum to examine AKH I titer changes in animals that had performed flights of various durations from 30 min to 6 h. We have also investigated whether there is any difference between migrants and non-migrants in the amount of AKHs stored in the CC at rest and examined differences in the adipokinetic *response* to AKH I or II in migrants vs. non-migrants after hormone injection.

MATERIALS AND METHODS

Experimental Animals

M. sanguinipes used for this study were reared as described previously (Kent et al., 1997). They were lab-reared, first-generation offspring of grasshoppers collected on the San Carlos Indian Reservation near Globe, Arizona.

Flight Assay

Grasshoppers were assayed using a tethered-flight apparatus (McAnelly and Rankin, 1986). A small stick was attached to the pronotum of each grasshopper with wax, and the insect was then suspended in front of a fan. The fan, an electric heater,

and incandescent lamps simulated conditions of wind speed, illumination, and temperature that are associated with migratory flights in the field (Parker et al., 1955).

Hemolymph Collection

Hemolymph was collected before and 30, 60, 90, 180, or 360 min after flight from 4–6-day-old adult males by puncturing the dorsal neck membrane between the head and pronotum. On the assumption that multiple bleeding could affect subsequent AKH titer and flight behavior, each hopper was bled at just one time point and then removed from the flight apparatus. Hemolymph that bled freely from the puncture was collected in a graduated glass micropipette, the amount collected being limited to 5 µl in flown animals. In rest animals, 25 µl hemolymph were pooled from several animals.

Hemolymph Extraction and Separation of *M. sanguinipes* AKH I by HPLC

The extraction technique was modified from the method of Gäde et al. (1984). Each hemolymph sample was dissolved in 400 µl 50 % methanol and centrifuged at 5,000*g* for 15 min. The supernatant was filtered through a Millipore Ultrafree-MC 0.1-µm pore size centrifugal filter at 5,000*g* for 15 min. The pooled filtrates from each hemolymph sample were frozen in siliconized Eppendorf tubes, dried completely under vacuum in a centrifugal concentrator, and stored at –80°C until analyzed.

M. sanguinipes AKH I was separated from potentially interfering substances by performing narrow bore RP-HPLC on all hemolymph samples. Dried samples were dissolved in 50 µl 12% acetic acid prior to injection. Narrow bore RP-HPLC was performed according to a modified method of Gäde et al. (1984) and Taub-Montemayor et al. (2002) on a 1.0 × 250 mm 300 micron C18 column (Separation Methods Technologies). Absorbance at 210 nm and 280 nm was recorded with an Amersham-Pharmacia SMART System UV-M, and absorbance peak areas were integrated with the system software. Fractions that matched with Lom-AKH I fractions were collected and stored at –80°C until analyzed by RIA for titer determination.

Radioimmunoassay

Hemolymph fractions that contained *M. sanguinipes* AKH I after HPLC were dried and reconstituted with 100 µl RIA buffer (sodium phosphate buffer, pH 7.4, 0.05 M NaCl, 0.1% BSA, 0.1% Triton X-100, and 0.01% NaN_3). Synthetic Lom-AKH I standards (Peninsula Laboratories, Inc.) were diluted in 100 µl RIA buffer with a concentration range of 1 to 1,280 pg. Standards and samples were incubated with 100 µl of anti-Tyr^1-Lom-AKH I antiserum (Peninsula Lab) at 4°C for 16–24 h. Following incubation (day 2), [^{125}I] AKH I (Peninsula Lab; 10,000–15,000 dpm) in 100 µl RIA buffer was added, mixed well, and again incubated for 16–24 h at 4°C. On day 3, pretitrated 100 µl goat anti-rabbit IgG serum and 100 µl normal rabbit serum (Peninsula Lab) were added, vortexed, and incubated at room temperature for 90 min. Five hundred microliters of RIA buffer were added to these tubes and centrifuged at 3,000 rpm for 20 min at 4°C. The supernatant was carefully aspirated, pellets were taken up in 4 ml liquid scintillation cocktail, and levels of ^{125}I were counted using a Beckman LS 400 scintillation counter.

Measurement of Hemolymph Volume

Four- to six-day-old males were injected ventrally between the second and third abdominal segments with [^{3}H] methoxyinulin (15,000 dpm in 1 µl insect saline) using a graduated glass micropipette with a fine tip (Levenbrook, 1958). The abdomen was stretched and relaxed three times to disperse the injected material in the hemocoel. Preliminary tests indicated that 30 min were sufficient to distribute the radiolabel. Hemolymph was collected after 30 min by puncturing the dorsal neck membrane between the head and pronotum. One microliter of hemolymph that bled freely from the puncture was collected in a graduated glass micropipette and used for measurement of radioactiv-

ity. The dilution factor obtained in this way provided a measurement of the total hemolymph volume of each animal. The mean hemolymph volume in these experiments was 62.4 ± 8.2 µl (n = 8).

CC Dissection and Extraction

Heads were severed from the prothorax with a razor blade, placed posterior surface down on a glass slide, and sectioned with two downward cuts: one through the centerline of the eyes and one immediately above the labrum. The center section was fixed upright on a dissecting dish. Under a dissection microscope at 8× magnification, blocks of tissue were lifted out, exposing the esophagus and the CC-corpora allata gland complex dorsal to it. At 15×, the preparation was moistened with a drop of insect saline (0.13 M NaCl, 0.005 M KCl; Stone and Mordue, 1980); the glands and esophagus were extended by pulling gently on the esophagus and circumesophageal nerves. The nerves connecting the gland complex to the brain were cut with a jeweler's scissors or lancet (B&D Ultrafine II); the esophagus and glands were removed to a dish of insect saline. At 25×, the gland complex and attached tissue were teased free from the esophagus and removed to fresh saline for final dissection.

The corpora cardiaca extraction technique was modified from the methods of Gäde et al. (1984). Each CC complex was disrupted in 400 µl 50% methanol for 3 min with a Fisher 60 sonic dismembrator. The crude extract was centrifuged at 5,000*g* for 15 min. The supernatant was filtered through a Millipore Ultrafree-MC 0.1-µm pore size centrifugal filter at 5,000*g* for 15 min. The pellet from the first centrifugation was resuspended in 400 µl 50% MeOH and centrifuged and filtered as above. The pooled filtrates from each CC complex were frozen in siliconized Eppendorf tubes, dried completely under vacuum in a centrifugal concentrator, and stored at –80°C until analyzed.

Quantification of CC AKH Content

AKH I and II were separated and analyzed by on-line micro-capillary-LC-ESI-MS (Aglient LC 1100 series; on-line with a Bruker Esquire LC-MS) performed on CC extracts or synthetic hormone aliquots (Peninsula Lab) according to the methods of Taub-Montemayor et al. (2002). Chromatograms containing the total-ion-current (TIC) and base-peak-current were integrated for peak areas using the Esquire-LC DATA analysis software package version 3.0 (Bruker Instruments). The TIC peaks corresponding to the individual peptide hormones were identified by their precursor ion mass peaks as determined via analysis of the synthetic peptide standards. The peaks were detected by their base peak ion current, their areas integrated and compared with a series of areas obtained for known amounts (ranging from 1.5 to 50 pmol) of synthetic AKH I and II. To create the calibration curves, multiple injections were performed for each selected amount of the synthetic AKHs. Peak areas were then plotted against the amount of peptide to develop the standard curves for each AKH. Linear regression analysis was then used to predict amounts from peak areas determined for each AKH. Experiments performed to determine the linearity of response using both analytical systems, narrow bore RP-HPLC and micro-capillary-LC-ESI-MS (Taub-Montemayor et al., 2002) showed that narrow bore RP-HPLC maintained linearity in the range from 5 to 100 pmol, and the latter system showed linearity in the range 0.5 to 50 pmol. Since our previous experiments indicated that the peptides investigated were present in the glands around and below 26 pmol, we can assume that our experimental protocol is within the linear range of absorbance. The operational mode of our analytical instruments was verified prior to each set of analyses by running multiple injections of standard peptides. The efficiency of our extraction method and its ability to quantitatively recover the peptides from the glands was verified via spiking experiments. For this purpose, we selected individuals from the same physiological stage. Multiple sets of gland pairs (a total of 4 per set) were extracted and then divided into two halves, and one half was spiked with the synthetic Lom-AKH I peptide. Both samples were injected separately and quantified.

Injections and Measurement of Hemolymph Lipid

Either synthetic Lom-AKH I or Scg-AKH II (Peninsula Lab) was dissolved in distilled water and diluted to make 2 pmol/µl and 20 pmol/µl concentrations. Injection needles were made from graduated glass micropipettes drawn over a flame. Four- to six-day-old males received 1 µl of AKH solution delivered as follows: a glass needle was introduced into a puncture made with a sterile pin in the membrane between the third and fourth abdominal sternites, and the dose was delivered slowly under mouth pressure through a blow-tube. The abdomen was stretched and relaxed three times to disperse the injected material in the hemocoel.

Hemolymph was collected after 60 min by puncturing the dorsal neck membrane between the head and pronotum. One microliter of hemolymph that bled freely from the puncture was collected in a graduated glass micropipette. Hemolymph lipid concentration was measured colorimetrically using a vanillin-reagent assay (Stone and Mordue, 1980), with cholesterol (1 mg/ml in methanol) as standard.

RESULTS

AKH Titers

The success of radioimmunoassay is dependent on the development of a good antibody and separation of the desired peptide from other interfering substances that can affect the peptide-antibody binding. To separate *M. sanguinipes* AKH I from potentially interfering substances, narrow bore RP-HPLC was performed on all hemolymph samples. The fraction that had the same retention time as Lom-AKH I was further analyzed by RIA for titer determination. At the time these experiments were done, we had only identified *M. sanguinipes* AKH I in the relevant fraction. Subsequent work has revealed the additional presence of *M. sanguinipes* AKH II in the fraction with the same retention time as Lom-AKH I in this study (Taub-Montemayor et al., 2002). Thus, the hemolymph HPLC fraction that we used in this work probably contained both *M. sanguinipes* AKH I and II. The results show that the method was sensitive to detecting as little as 8 pg Lom-AKH I and Scg-AKH II had no affect on the binding between [^{125}I] Lom-AKH I and antiserum (Fig. 1). Since *M. sanguinipes* AKH II has the same amino acid sequence as Scg-AKH II, the RIA results likely reflect only *M. sanguinipes* AKH I titer, rather than a combination of AKH I and II. Thus, we believe the RIA actually identified only *M. sanguinipes* AKH I in this study.

The concentration of Lom-AKH I required for half-maximal inhibition of binding of [^{125}I] Lom-AKH I analog was found to be approximately 153 pg (0.13 pmol). The assay we used can detect as

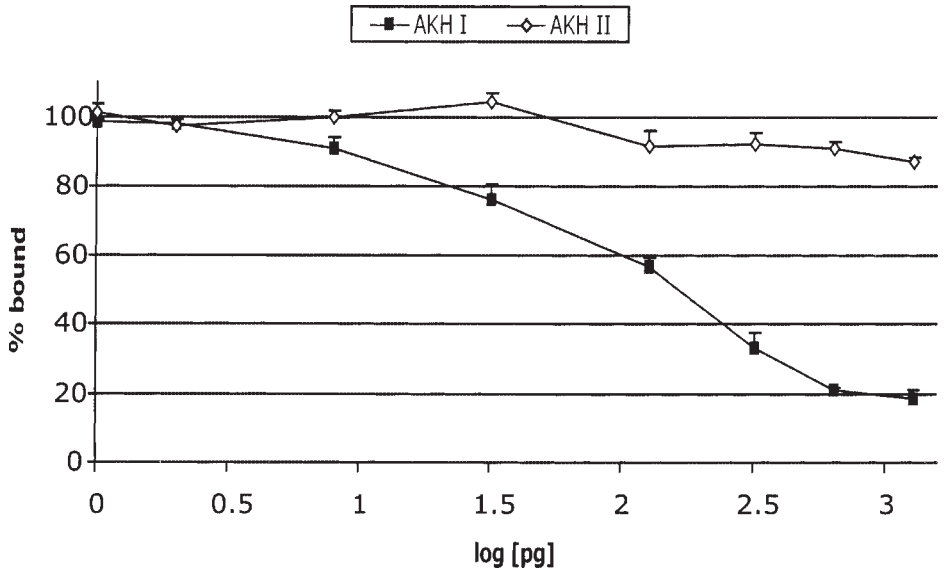

Fig. 1. Standard curve for RIA assay of AKH. Displacement of [^{125} I] AKH analog by Lom-AKH I and Scg-AKH II (n = 10 for AKH I and AKH II). Error bars show standard error. The non-radioactive AKH I and II (1–1,280 pg) were pre-incubated with the antiserum for 24 h at 4°C and [^{125}I] AKH was added.

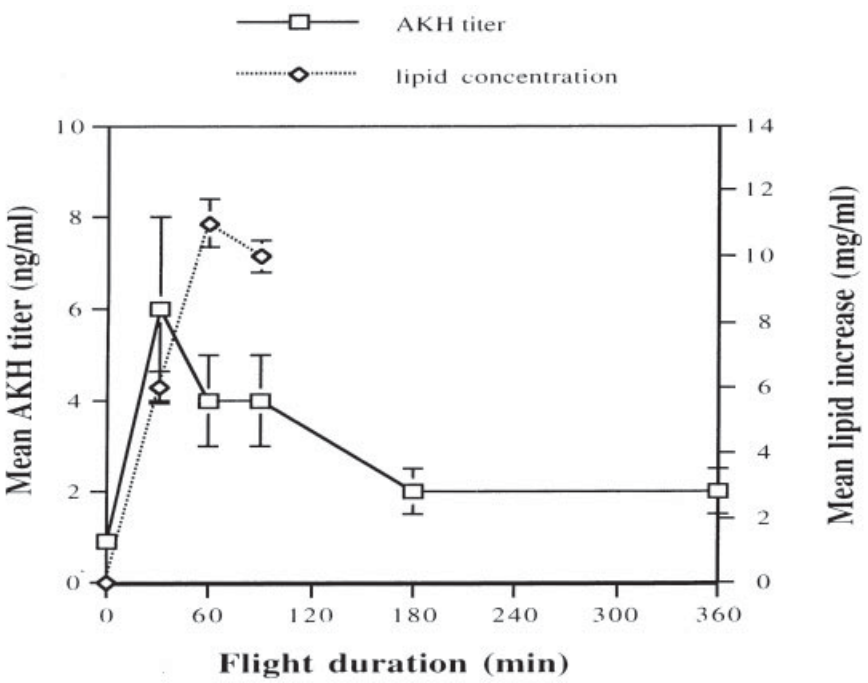

Fig. 2. Change in AKH titers and lipid concentrations during flight. Titer for AKH I (squares) increases during the first 30 min of flight and then subsequently declines. The increase in lipid (diamonds) observed in the hemolymph correlates with the increase in AKH I titer but with a slight delay. AKH titer was examined before and 30, 60, 90 min, 3 h and 6 h after flight. Hemolymph was collected once for each animal. N = 5 for each time point and error bars show standard error. Hemolymph lipid increase data were taken from Kent et al. (1997).

little as 8 pg of Lom-AKH I (Fig. 1). It was sufficiently sensitive to measure AKH in a 25-µl sample of hemolymph from unflown controls and in 5-µl samples of hemolymph from flown animals.

M. sanguinipes AKH I titer was examined at rest and after 30, 60, 90, 180, and 360 min of flight (Fig. 2). The titer before flight was relatively low (0.8 ± 0.2 ng/ml). It increased up to 5.7 ng/ml (5.7 ± 1.4 ng/ml, mean ± S.E) after 30 min of flight, then dropped at 60 and 90 min to 4.2–4.3 ng/ml (4.2 ± 0.3 and 4.3 ± 0.3 at 60 and 90 min, respectively). After 3 and 6 h of flight, it had dropped to 2.7 ng/ml (2.7 ± 0.2 ng/ml) and 2.2 ng/ml (2.2 ± 0.3 ng/ml), respectively.

Storage of AKH in CC

We identified migrants and non-migrants by the 1-h assay. After a resting period of 7 days, we measured total AKH content of the CC (by HPLC) in resting non-migrants, resting migrants, and migrants flight-tested to voluntary cessation. No significant difference in the content of one pair of CC was observed between resting migrants and non-migrants (effect of migratory propensity) nor between migrants flown to voluntary cessation and resting migrants (effect of flight performance) (N = 35 females, 28 males; males were all 15 days old and females were 8, 12, 16, 20, days old). Once we had developed methods to resolve *M. sanguinipes* AKH I and II by HPLC, we repeated these experiments. No significant differences were found between 1-h fliers (migrants) and non-migrants in AKH content of CC for either AKH I or II (Fig. 3). The mean amount of AKH I per individual gland pair for both long-flier and non-flier combined was 26.5 ± 3.16 pmol (mean ± S.E., n = 15) and of AKH II 6.7 ± 1.43 pmol (mean ± S.E., n = 15). These data are obtained from RP-HPLC fractions of extracts of CC dissected from adults of both sexes following flight testing. They reflect LC peaks corresponding in retention time to synthetic AKH I and II standards. The amount of AKHs were estimated from 210 nm absorbance peaks relative to a standard curve prepared using known amounts of synthetic AKH I and II (Fig. 4).

Adipokinetic Response to Synthetic Lom AKH I and Scg AKH II

Long-fliers (migrants) exhibited a consistently higher elevation of hemolymph lipid levels in re-

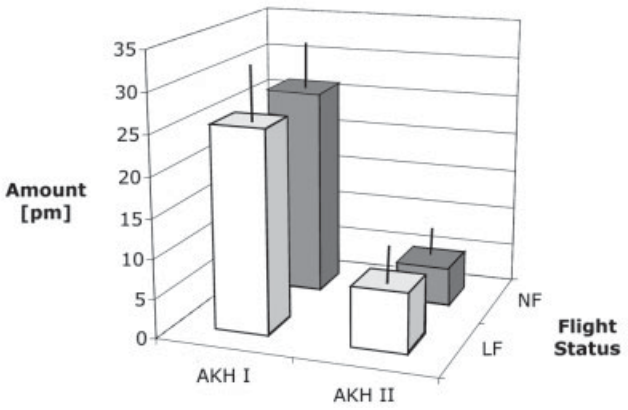

Fig. 3. Comparison of AKH in one pair of CC of flight-tested females. No significant differences were observed for either AKH I or II when comparing migrants and non-migrants (ANOVA: $F_{(1,13)}$ = 0.1918, P = 0.6685 and ANOVA: $F_{(1,13)}$ = 0.6979, P= 0.4185, respectively). Error bars are standard error.

sponse to AKH I injection than non-fliers (non-migrants) as determined by measuring hemolymph lipid increase after injection of synthetic Lom AKH I (Fig. 5). In contrast, injection of 20 pmol Scg AKH II showed negligible lipid concentration increase compared to Lom-AKH I injection, and there was no significant difference in response between non-fliers and long-fliers (Fig. 5). Injection of 2 and 20 pmol of synthetic locust AKH I caused hemolymph lipid increase, the higher dose approximately doubling the response.

DISCUSSION

The rise in hemolymph AKH during flight of *Locusta migratoria* was demonstrated by Cheeseman et al. (1976) and Cheeseman and Goldsworthy (1979) by means of a bioassay. *L. migratoria* is a large insect and has a correspondingly large hemolymph volume (~250 µl) so that in monitoring AKH titer changes during flight, 50–100 µl of hemolymph could be obtained from each individual locust after flight. The hemolymph was then fractionated via Sephadex column (Cheeseman et al., 1976), and the fraction containing AKH was assayed by monitoring the amount of lipid mobilization it elicited when injected into a non-flying

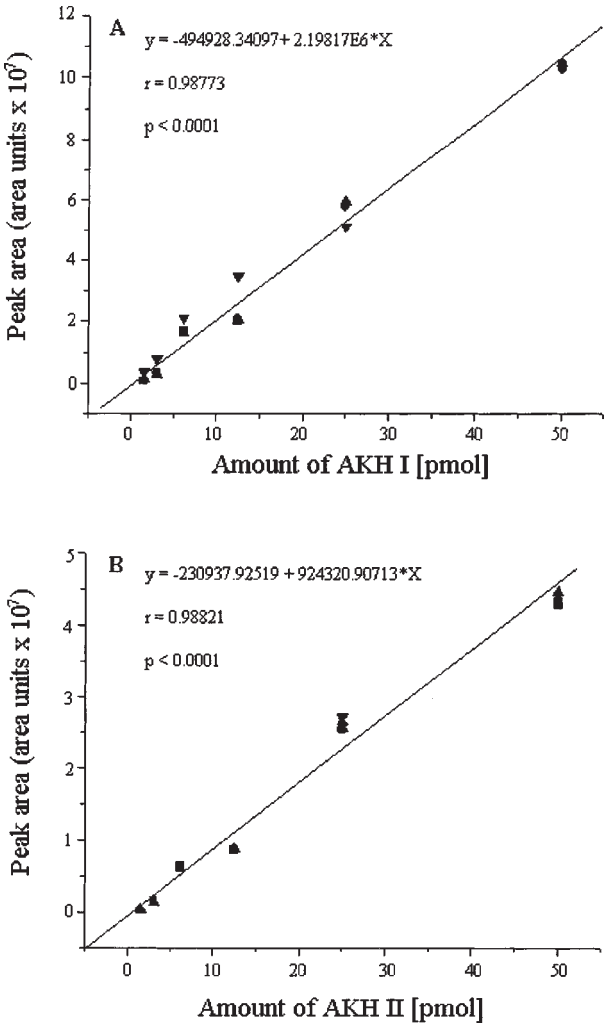

Fig. 4. Standard curves for synthetic AKH I and II used for calibration. The amount of AKH was calculated by using the base peak current observed during LC-MS runs as described in more detail in Materials and Methods. Linear regression for both curves was calculated as R^2 = 0.99.

animal. This assay, while fairly sensitive, is indirect and inherently subject to an additional source of variation (the assay animal) relative to a purely biochemical assay. Furthermore, *M. sanguinipes* is a smaller insect than *L. migratoria*, and the volume of hemolymph obtainable from a single bleeding is correspondingly less (5–10 µl); thus, we explored an alternative to bioassay. *M. sanguinipes* AKH I has the same amino acid sequence as that of *L. migratoria* AKH I (Taub-Montemayor et al., 1997), making it possible to use the commercially available Lom-AKH I antiserum for the development

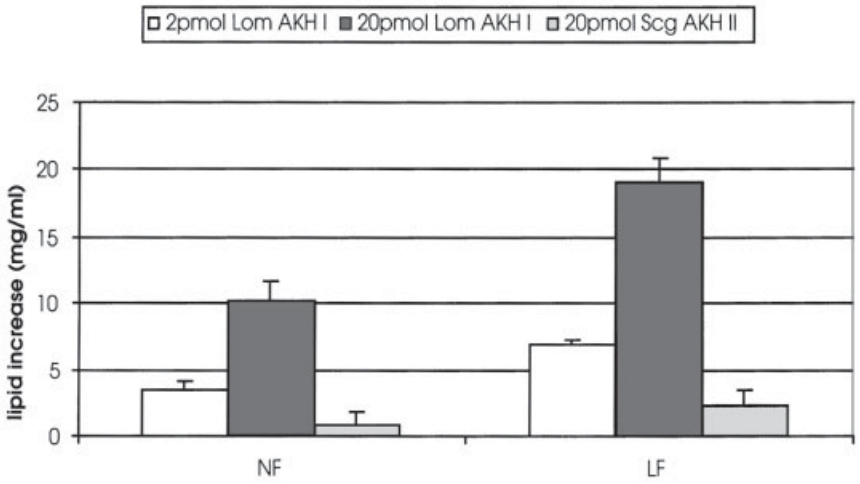

Fig. 5. Adipokinetic response to injected synthetic hormones. The response of lipid increase in the hemolymph was determined 60 min after injection of synthetic Lom AKH I or Scg AKH II. Long-fliers exhibited a higher elevation of hemolymph lipids after injections of Lom AKH I. No significant difference in response to the injection of Scg AKH II was observed when comparing long-fliers (LF) and non-fliers (NF) (ANOVA: $F_{(1,13)} = 1.24$, $P = 0.28$). Overall injections of 2 and 20 pmol of AKH I caused an increase of lipid in the hemolymph, the higher dose approximately doubling the response; 9–11-day-old males and females were used. N=10 for AKH I injections and 7 for AKH II injection. Error bars show standard error.

of an RIA. Since AKH II does not affect hemolymph lipid in *M. sanguinipes*, the fact that the RIA we used did not recognize *M. sanguinipes* AKH II (which has the same sequence as Scg AKH II; Taub-Montemayor et al., 2002), was not a concern in examining the relationship between AKH I titers and increasing hemolymph lipid levels after flight.

Even though partitioning each hemolymph sample by RP-HPLC requires time and labor, it improves the assay sensitivity substantially by separating AKH from interfering substances (Moshitzky and Applebaum, 1990). The *M. sanguinipes* AKH I titer without the RP-HPLC separation step was significantly lower than the titer after separation, indicating that there are indeed interfering substances in hemolymph (data not shown).

AKH titer during flight increased sharply during the first 30 min of flight and subsequently declined after extended flight. This initial increase is consistent with and seems to slightly precede the increase in hemolymph lipid observed by Kent et al. (1997) during the first 30 min of flight in *M. sanguinipes* (see Fig. 3). This pattern is also similar to the hemolymph lipid concentration changes observed in locusts that reach a steady state after about an hour and then slowly decline during extended periods of flight (reviewed in Goldsworthy, 1983). Cheeseman and Goldsworthy (1979) found that the maximal amount of AKH recovered from *L. migratoria* hemolymph as estimated by bioassay was about 5.0 ng/ml, as compared to 5.7 ng/ml after 30 min flight in *M. sanguinipes*; this then decreased to about 2.13 ng/ml by 90 min in *L. migratoria* (as compared to 4.3 ng/ml in *M. sanguinipes*). Recently, Candy (2002) showed that AKH I titer after 60 min flight in *S. gregaria* was around 3.4 ng/ml (2.9 pmol/ml), using RIA.

If we assume that the half-life of *M. sanguinipes* AKH I during flight is similar to that of Lom-AKH I (35 min; Oudejans et al., 1996), we can roughly estimate the amount of AKH released during flight in *M. sanguinipes*. During the first 30 min after initiation of flight, AKH titer is increased by 4.9 ng/ml. This total increase (ΔI, 4.9 ng/ml) should be total release (ΔR) minus amount of degradation (ΔD) of AKH, i.e., $\Delta I = \Delta R - \Delta D$. For ease of calculation, we assume that the AKH concentration decreases to half of its original concentration in 30

min. At 0 min of flight, the original concentration of AKH is 0.8 ng/ml. If there were no AKH release, titer would be decreased to 0.4 ng/ml after 30 min of flight (ΔD = 0.4 ng/ml). The actual release (ΔR) during 30 min of flight is the sum of the total increase (ΔI, 4.9 ng/ml) plus the amount degraded (ΔD, 0.4 ng/ml), or about 5.3 ng/ml AKH I. Using the same method for the calculation of released AKH I during flight until 90 min, we assume that about 8.9 ng/ml AKH I is released during 90 min of flight. If the total hemolymph volume of *M. sanguinipes* is around 62 µl, then, the total AKH release from the corpora cardiaca is about 552 pg AKH I (0.48 pmol) during the 90 min of flight. Cheesman et al. (1976) estimated that 920–1,500 pg of hormone are released in *L. migratoria* during the 25 min of flight.

When the storage of AKH in CC of *M. sanguinipes* was compared in non-migrants and migrants, there was no significant difference in the stored amount of either AKH I or II in different flight groups (Fig. 3). In addition, in initial experiments to examine total AKH content, we found no significant decrease in total AKH content of the CC as an effect of flight to voluntary cessation, even though AKHs are released during flight. This apparent paradox may have been observed simply because the variances among individuals were too large to permit statistical resolution, and/or because the amount of AKH released during flight was small relative to the total stores in the CC (as shown by our RIA titer determinations). It is also likely that AKH synthesis occurs during flight. For example, in *L. migratoria*, flight activity increased levels of AKH mRNAs in CC (Bogerd et al., 1995), and in a recent report Harthoon et al. (2002) demonstrate that a continuous biosynthesis of AKHs is necessary for maintaining a readily releasable pool of AKH-containing secretory granules during flight.

The AKH content of CC in *M. sanguinipes* was much smaller than in locusts. Siegert and Mordue (1986) quantified the AKH I and II content of CC in *S. gregaria* and *L. migratoria*. They found that AKH I content in adult male and female *S. gregaria* is about 300–1,200 pmol/gland and in *L. migratoria* 100–750 pmol/gland. Amounts for AKH II were determined to be 70–200 pmol/gland and 30–130 pmol/gland, respectively. There was a lot of variability between glands, but amounts of AKHs were consistently higher in females than males in both species. In contrast, *M. sanguinipes* has an average of only 26 pmol AKH I and 7 pmol AKH II per gland, and no differences were observed between the sexes. *M. sanguinipes* body size is 3 to 10 times smaller than that of locusts, but even so the amount of stored AKH seems to be relatively low in this species.

It is interesting that, although long-fliers and non-fliers do not differ significantly in body size, resting lipid reserves, or resting levels of AKH I or II, they *do* differ significantly in their adipokinetic *response* to injections of exogenous AKH I. Injection of 2 and 20 pmol of AKH I induced a consistently greater increase of hemolymph lipid in long-fliers than in non-fliers (Fig. 5). Since lipid is the major fuel for sustained flight, a more intense adipokinetic response in long- fliers of *M. sanguinipes* could be advantageous. Similar results have been observed in other acridids. For example, gregarious *L. migratoria* showed a greater increase of hemolymph lipid than solitary locusts (Ayali and Pener, 1992), and young gregarious *S. gregaria* showed a higher flight-induced hyperlipemic response than isolated young adults, while the difference was insignificant in older insects (Schneider and Dorn, 1994).

In contrast to AKH I, lipid increase by AKH II of this species was almost negligible. It seems that AKH II does not play a major role in lipid metabolism in this species. The mechanism for the difference in the strength of the adipokinetic reaction might be a difference in density of receptors for AKH in the fat body or a difference in the capacity to form low-density lipophorin (LDLp) as in *L. migratoria* (Chino et al., 1992). Future research in our lab will include investigation of the density of receptors for AKH in the fat body and the capacity to form LDLp in long-fliers and non-fliers.

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