# On the Release of the Three Locust (*Locusta migratoria*) Adipokinetic Hormones: Effect of Crustacean Cardioactive Peptide and Inhibition by Sugars

James E. Flanigan and Gerd Gäde

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

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An existing test to monitor the rate of adipokinetic hormone release from the corpora cardiaca (CC) of *Locusta migratoria in vitro* was improved, so that a constant basal rate of release was achieved and the amount of released Lom-AKH-I, II and III could be quantified by HPLC. This test system was subsequently used to demonstrate that a small peptide, which has been found in a few insect species including *L. migratoria*, crustacean cardioactive peptide (CCAP), induces release of all three AKHs. Moreover, 80 mM trehalose reduces CCAP-induced release of AKHs *in vitro*, and 160 mM glucose reduces this release even further. Glucose also had a greater inhibitory effect than trehalose on the spontaneous release and inhibited the high potassium-stimulated release of AKH from the CC *in vitro*. Eighty mM sucrose, on the other hand, had no effect on the release of AKH. The effect of trehalose and glucose could be due to their use as an energy source, with trehalose first having to be converted to glucose. Whatever the stimulus, the three AKHs are released in the same proportions as they are found in the CC, which *in vivo* would make Lom-AKH-I, the most abundant AKH, the major effector of the biological effects of AKHs in adult locusts.

## Introduction

The migratory locust (*Locusta migratoria*) synthesises three adipokinetic hormones (Lom-AKH-I, II and III, according to the nomenclature of Raina and Gäde (1988)), whose major metabolic actions are the release of lipid and carbohydrate from the fat body (Goldsworthy, 1983; Gäde, 1996). The AKHs are produced in the glandular part of the corpora cardiaca (CC), and are packaged into secretory granules until released into the haemolymph by exocytosis. The ratio of Lom-AKH-I, II, and III in the CC of *L. migratoria* is roughly 14:2:1, with about 100–150 pmoles of Lom-AKH-I in young adults (Oudejans *et al.*, 1991; Oudejans *et al.*, 1993; Sharp-Baker *et al.*, 1966). Flight is a natural stimulus for the release of AKHs from the CC, with the main fuel for long-distance flight in *L. migratoria* being lipid released from the fat body through the action of the AKHs. The physiological reason for *L. migratoria* having three AKH hormones is not well understood.

Recently, two peptides have been identified that induce the release of Lom-AKH from the CC in a dose-dependent manner *in vitro* and which therefore may regulate Lom-AKH release in locusts *in vivo* as well. The first, locustatachykinin I (Lom-TK-I), was isolated from the brain and CC of *L. migratoria* and was shown to stimulate the release of AKH from isolated CC *in vitro* (Nässel *et al.*, 1995). A feature of Lom TK-I- stimulated release of AKH is that it is inhibited by concentrations of the disaccharide trehalose found in resting locusts (80 mM), but not by the concentration of trehalose that occurs during long-distance flight (40 mM) (Passier, 1996). The second peptide was isolated from the brain of the desert locust (*Schistocerca gregaria*) and identified as crustacean cardioactive peptide (CCAP). It was shown to stimulate release of AKH from CC in *L. migratoria* and *S. gregaria* (Veelaert *et al.*, 1997). CCAP was first identified in the crab *Carcinus maenua* (Stangier *et al.*, 1987) and was subsequently found in the insects *L. migratoria* (Stangier *et al.*, 1989) and *Manduca sexta* (Lehman *et al.*, 1993), with a diverse range of biological actions.

In the present study the CCAP- stimulated release of all three AKHs present in the CC of *L. migratoria* was investigated, measuring the amounts of each AKH released and testing whether release of AKHs is inhibited by trehalose and whether other sugars such as the disaccharide

Reprint requests to Prof. G. Gäde.
Fax: +27 21 650 3330.
E-mail: ggade@biotzoo.uct.ac.za

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sucrose and the monosaccharide glucose have the same effect.

## Materials and Methods

### *Insects*

Migratory locusts, *Locusta migratoria*, were reared under crowded conditions, at 32 °C, 40% humidity, on a 12 h dark:light cycle as described previously (Gäde, 1992). They were fed on grass supplemented with oats. Males were used 3–4 weeks after the final moult.

### *AKH release from CC in vitro*

The method was derived from Veelaert *et al.* (1997) and Nässel *et al.* (1995). Locusts were decapitated, the CC removed as intact as possible and stored in 100 µl of insect saline buffer (150 mM NaCl, 10 mM KCl, 4 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM Hepes; pH 7.0 at room temperature). The wells of a plastic microtiter plate were used for incubation. Figure 1 shows a flow diagram of the procedure. For the first series of experiments, the CC were grouped in pools of 10, in the second series they were pooled in groups of 5. See Fig. 1 for directions. After the last CC was removed, the glands were rinsed three times with 100 µl of insect saline buffer and left to incubate for one hour to allow the rate of release of AKH to reach a stable level. The saline was collected using a Hamilton syringe. This was done by inspection under a dissecting microscope, so that the CC would not be accidentally damaged. All incubation media and CC extracts were stored in Eppendorf tubes containing 50 µl of 2.5 M acetic acid and frozen at −80 °C until quantitated by HPLC (see below) which was done always within a week of collection. The CC were then incubated in 20 µl of insect saline buffer for precisely 30 min following which the saline was collected as above. A second incubation of 30 min followed, then a third 30 min incubation with the insect saline buffer containing CCAP. A concentration of 1 µM CCAP was used to give maximum stimulation of release of AKH. In a first series of experiments, the CC were then washed 5 times with 100 µl saline to remove the CCAP, then incubated for 30 min in insect saline buffer and then a second incubation with insect saline buffer containing 1 µM CCAP followed. In a second series, this secondary treatment with CCAP was omitted. After the last CCAP stimulation, the CC were finally incubated for 30 min in saline that contained 50 mM KCl to evoke a near maximal release (i.e. 110 mM NaCl, 50 mM KCl, 4 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM Hepes; pH 7.0). At the end of the experiment the CC were taken up in 100 µl of water and stored frozen as the other samples. The AKHs remaining inside the CC were extracted by sonicating for 2 x 10 seconds. After centrifuging, the supernatant was collected, and the pellet was resuspended in 0.1% trifluoroacetic acid (TFA) in water and sonicated again. After centrifugation, the supernatants were combined. In the first series of experiments, a parallel experiment was run with insect saline buffer containing 80 mM trehalose, with only one stimulation with CCAP. In the second series, three parallel experiments were run with insect saline buffer containing 80 mM trehalose, 80 mM sucrose and 160 mM glucose.

### *HPLC analysis of AKHs*

The amounts of Lom-AKH-I, -II and, in the first series of experiments, Lom-AKH-III, contained in the samples were quantitated by reverse phase HPLC, using a Nucleosil 100 C-18 column (i.d. 4.6 mm x 250 mm length) with a 1 ml per min flow rate and equipment as described previously (Gäde, 1985). Solvent A contained 0.11% TFA in water while solvent B contained 60% acetonitrile and 0.1% TFA. A linear gradient was run from 43% B to 50.5% B in 15 min to separate Lom-AKH-I and -II. When Lom-AKH-III was also measured, the amount of solvent B was increased to 74% in one minute, then increased to 79% B in 10 minutes. A column washing step of 4 min of 99% B followed each run. Fluorescence was monitored at excitation 276 nm and emission 350 nm. Synthetic AKH samples were run to identify and quantify peak areas. The peaks were integrated and the results were expressed as pmoles of peptide released per CC per 30 min incubation.

Ten CC in the incubation wells were just sufficient to measure the release of Lom-AKH-III in non-stimulating conditions. Recovery of peptide from the wells was over 95% (as demonstrated by pilot experiments; data not shown), the recovery may have been aided by repeated incubations in the same well.

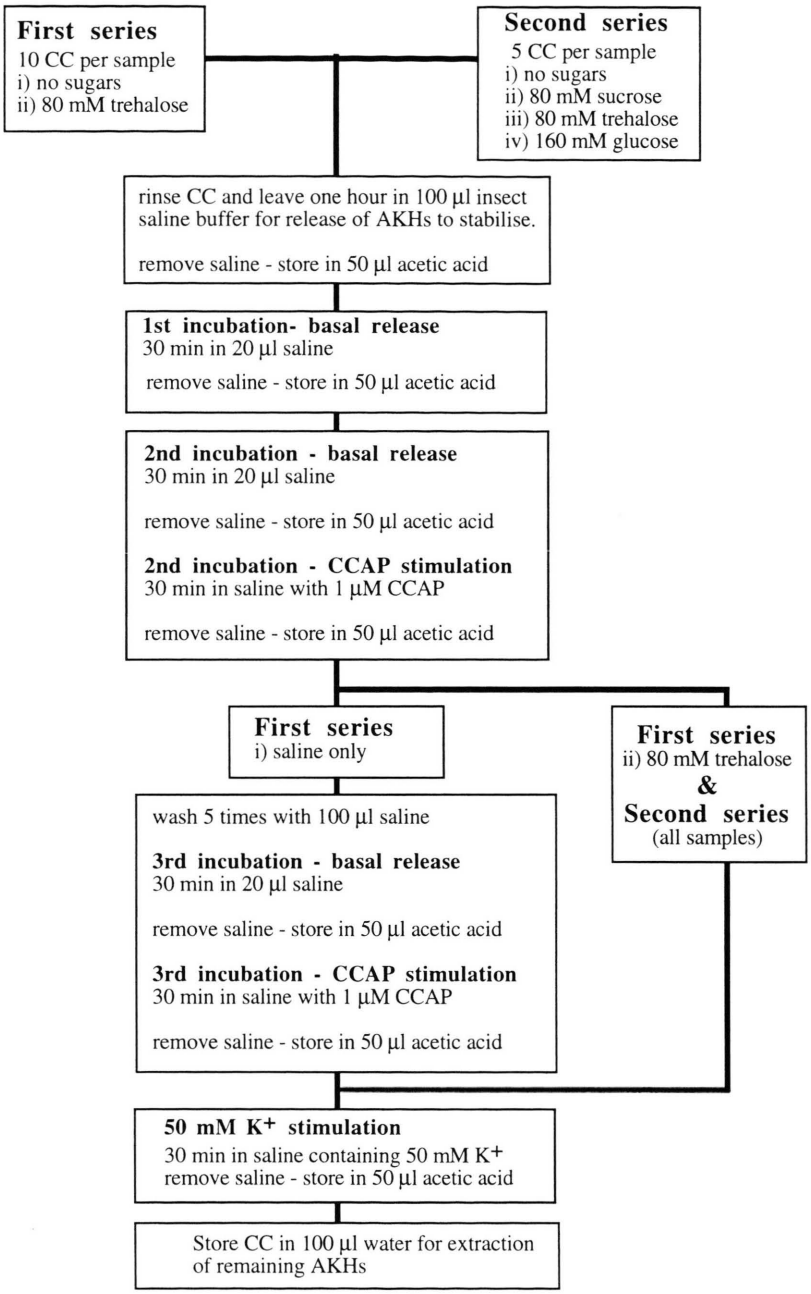


Fig. 1. Flow diagram of the experimental procedure for the treatment of collected CC in the first and second series of experiments.

## Results

After a stabilisation period of one hour the basal release of AKHs was maintained at a constant rate. Furthermore, after stimulation with CCAP, the incubated CC would return to the original basal level of AKH release when the CCAP was washed out (Table I). After washing, re-stimulation with CCAP produced the same level of stimulated release of AKHs. CC stimulated with CCAP showed an increase in release of each AKH by a factor of 2–2.7 (Table I).

To examine the effect of trehalose on release of AKH the second incubation and subsequent stimulation with CCAP from Table I is used and is combined with the 50 mM $K^+$ stimulation results. Eighty mM trehalose reduced the basal and the CCAP- stimulated release of AKH to about one-third, while it had no effect on 50 mM $K^+$ – stimulated release (Table II). Trehalose did not seem to limit the basal level of release of Lom-AKH-III, but the amount of Lom-AKH-III measured in basal release was at the detection limit of our experimental set up.

Table I. The amount of AKH released by CC in incubation: effect of repeated incubation and repeated CCAP stimulation.

| | Basal release | CCAP stimulated |
|---|---|---|
| *Lom-AKH-I* | | |
| First 30 min incubation | 1.53 ± 0.63 | – |
| Second 30 min incubation | 1.47 ± 0.40 | 3.54 ± 0.67 |
| Third 30 min incubation | 1.29 ± 0.50 | 3.50 ± 0.95 |
| *Lom-AKH-II* | | |
| First 30 min incubation | 0.31 ± 0.12 | – |
| Second 30 min incubation | 0.32 ± 0.07 | 0.74 ± 0.25 |
| Third 30 min incubation | 0.25 ± 0.50 | 0.77 ± 0.42 |
| *Lom-AKH-III* | | |
| First 30 min incubation | 0.14 ± 0.12 | – |
| Second 30 min incubation | 0.10 ± 0.05 | 0.24 ± 0.08 |
| Third 30 min incubation | 0.12 ± 0.10 | 0.24 ± 0.09 |

Values are mean ± SD of pmoles of AKH released per CC in 30 min incubation.
N is 5–6, with 10 CC per sample.
In each case the second and third release values are not significantly different from the first values by ANOVA and paired t-test.
CCAP stimulation always produced an increase in release of AKH, $p<0.001$ by paired t-test.

The ratio of Lom-AKH-I, Lom-AKH-II and Lom-AKH-III released by the CC was always in the same approximate proportions and matches the ratio contained in the CC itself (Table III). Lom-AKH-I makes up about 75% of the AKH content of the CC and also about 75% of the AKH that is released from the CC *in vitro*. These numbers are 18% and 6% for Lom-AKH-II and III, respectively. The total amount of AKH contained in the CC was calculated by adding the amount released in each incubation to the amount extracted from the CC at the end of the experiment. A small amount of AKH may have been lost in the washing steps. The locusts used in the second series of experiments had 20% more Lom-AKH-I and II present in their CC compared to those of the first series (Table IV). There was one month separating the two experiments, so a new generation of locusts was used for the second series. Because of the different amounts of AKH in the two groups and because of different rates of basal release (see below), the results from the two groups are kept separate.

In the second series of experiments the effect of different sugars on the release of AKHs was measured; 80 mM trehalose was repeated and 80 mM sucrose and 160 mM glucose was included. Only 5 CC were used in each incubation and Lom-AKH-III was not measured. The basal release of AKH was lower than in the first series of experi-

Table II. Effect of 80 mM trehalose on the release of AKHs from CC in incubation.

| | Basal release | Stimulated release 1 μM CCAP | Stimulated release 50 mM $K^+$ |
|---|---|---|---|
| *Lom-AKH-I* | | | |
| No sugars | 1.47 ± 0.40 | 3.54 ± 0.67 | 22.8 ± 1.8 |
| 80 mM trehalose | 0.46 ± 0.21 | 1.16 ± 0.35 | 22.3 ± 7.5 |
| *p* (unpaired t-test) | 0.003 | 0.035 | ns |
| *Lom-AKH-II* | | | |
| No sugars | 0.32 ± 0.07 | 0.74 ± 0.25 | 6.18 ± 0.88 |
| 80 mM trehalose | 0.10 ± 0.04 | 0.21 ± 0.07 | 4.90 ± 1.17 |
| *p* (unpaired t-test) | 0.005 | 0.001 | 0.03 |
| *Lom-AKH-III* | | | |
| No sugars | 0.10 ± 0.05 | 0.24 ± 0.08 | 1.77 ± 0.28 |
| 80 mM trehalose | 0.07 ± 0.06 | 0.11 ± 0.10 | 2.03 ± 0.86 |
| *p* (unpaired t-test) | ns | 0.05 | ns |

Values are mean ± SD of pmoles of AKH released in 30 min incubation per CC. N is 5–6 for each sample, with 10 CC per sample. Ns = not significant.

Table III. Percentage of each AKH in CC and released into the media.

| | Lom-AKH-I | Lom-AKH-II | Lom-AKH-III |
|---|---|---|---|
| Total CC content | 74 ± 2 | 19 ± 2 | 7 ± 0.7 |
| Released in basal incubation | 77 ± 5 | 17 ± 4 | 5 ± 2.7 |
| Released with CCAP stimulation | 78 ± 6 | 16 ± 5 | 6 ± 3.7 |
| Released with 50 mM $K^+$ stimulation | 74 ± 2 | 20 ± 2 | 6 ± 0.9 |

Values are mean ± SD. The total of I + II + III = 100%. N = 6 with 10 CC per sample. There was no significant difference between the four groups (by ANOVA).

Table IV. Total AKH content of CC.

| | Lom-AKH-I | Lom-AKH-II | Lom-AKH-III |
|---|---|---|---|
| First series ($n$=11) | 146.8 ± 24.1 | 36.0 ± 7.1 | 14.3 ± 2.2 |
| Second series ($n$=19) | 178.7 ± 30.9 | 46.3 ± 9.3 | Nd |
| $p$ (t test) | 0.003 | 0.002 | – |

Values are mean ± SD of pmoles AKH per CC. 10 CC per single sample for first series and 5 CC per sample for second series. Nd = not done.

ments and trehalose did not significantly reduce it (Table V). However, trehalose did reduce the CCAP- stimulated release of AKHs. Glucose had a greater inhibitory effect than trehalose on release of AKH, with lower amounts of AKHs released in the basal release and the CCAP- and $K^+$ – stimulated release. Sucrose had no effect on the release of AKHs: the results for media containing sucrose are the same as media without any sugars (Table V).

The amount of AKH that is released by the action of CCAP on the CC is calculated by subtracting the amount of AKH released in basal incubation from the amount released in CCAP-stimulated incubation. This clearly shows that trehalose and glucose reduce the level of CCAP stimulation, with 80 mM trehalose reducing release of the AKHs by approximately 3–4 fold, and 160 mM glucose by 4–5 fold (Table VI). Each of the AKHs measured is affected in the same proportion.

## Discussion

### *Amount of AKH released*

Previous studies on the release *in vitro* of Lom-AKH-I upon stimulation with CCAP (Veelaert *et al.*, 1997) and locustatachykinin I (Nässel *et al.*, 1995; Passier, 1996) presented the data on the amount of peptide released as a proportion of the basal release. The reason for this was the large

Table V. Effect of the sugars trehalose, sucrose and glucose on the release of AKHs from CC in incubation.

| | Basal release | Stimulated release 1 µM CCAP | Stimulated release 50 mM $K^+$ |
|---|---|---|---|
| *Lom-AKH-I* | | | |
| No sugars | 0.89 ± 0.38 | 3.12 ± 1.11 | 44.3 ± 7.70 |
| Sucrose | 0.93 ± 0.30 | 3.22 ± 1.10 | 30.2 ± 11.8 |
| Trehalose | 0.73 ± 0.31 | 1.59 ± 0.36[a] | 32.3 ± 12.3 |
| Glucose | 0.46 ± 0.18[a] | 0.95 ± 0.18[b] | 26.5 ± 8.30[c] |
| *Lom-AKH-II* | | | |
| No sugars | 0.31 ± 0.17 | 0.75 ± 0.21 | 9.85 ± 2.10 |
| Sucrose | 0.32 ± 0.09 | 0.84 ± 0.26 | 8.68 ± 3.70 |
| Trehalose | 0.23 ± 0.09 | 0.40 ± 0.26[a] | 8.22 ± 2.95 |
| Glucose | 0.14 ± 0.08[a] | 0.25 ± 0.06[b] | 6.89 ± 2.20[c] |

Values are mean ± SD of pmoles of AKH released in 30 min incubation per CC. N is 6 for each sample, with 5 CC per sample.
Statistical test by ANOVA.
[a] Significantly lower than no sugars and sucrose sample, $p < 0.05$.
[b] Significantly lower than no sugars, sucrose and trehalose sample, $p < 0.05$
[c] Significantly lower than no sugars sample, $p < 0.05$.

Table VI. AKH released due to the stimulation by CCAP, showing the effect of different sugars.

| | **AKH released by CCAP stimulation** | | |
|---|---|---|---|
| | Lom-AKH-I | Lom-AKH-II | Lom-AKH-III |
| *First series* | | | |
| No sugars | 2.06 ± 0.77 | 0.42 ± 0.20 | 0.14 ± 0.05 |
| 80 mM trehalose | 0.70 ± 0.42[a] | 0.11 ± 0.09[a] | 0.04 ± 0.05[a] |
| *Second series* | | | |
| No sugars | 2.23 ± 1.37 | 0.45 ± 0.25 | – |
| 80 mM sucrose | 2.29 ± 1.02 | 0.52 ± 0.20 | – |
| 80 mM trehalose | 0.87 ± 0.29[b] | 0.17 ± 0.12[b] | – |
| 160 mM glucose | 0.48 ± 0.20[c] | 0.11 ± 0.09[c] | – |

Values are mean ± SD of pmoles of AKH released in 30 min incubation per CC. N is 5–6 for each sample, with 10 CC per sample for the first series and 5 CC per sample for the second series. Statistical test by ANOVA and t-test.
[a] Significantly lower than no sugars sample.
[b] Significantly lower than no sugars and sucrose sample, $p < 0.05$.
[c] Significantly lower than no sugars, sucrose and trehalose sample $p < 0.05$.

variability in the basal rate of released AKH. We found that after being left to stabilise at room temperature the CC *in vitro* produced a constant and reproducible basal rate of release of the three AKHs and the actual pmoles of hormone released could be used to compare different experimental parameters. Furthermore, stimulation of the rate of release with CCAP could be done at least twice on the same batch of CC with the same result. As each preparation contained 5 or 10 CC the variation between experiments was probably due to the preparation rather than any great difference in the basal rate of release. Before stabilisation, the CC incubated *in vitro* released a large amount of AKHs immediately i.e. up to 10 pmol Lom-AKH-I per CC (personal observation), probably due to damage to the glandular cells. Considering the amount of AKHs contained in the CC (see below) even a small amount of damage to the glandular cells would cause a large increase in apparent basal release.

For the actual amount of Lom-AKH-I released, Nässel *et al.* (1995) gave one example when 0.83 pmol of Lom-AKH-I were released spontaneously per CC per 30 min, with 2.8 pmol released when maximally stimulated by Lom-TK I. Passier (1996) stated that under non-stimulating conditions a CC released approximately 2 pmoles Lom-AKH-I in 30 min. Lom TK-I doubled the release of Lom-AKH-I under normal conditions, while 80 mM trehalose decreased the basal release to half the rate and completely inhibited the effect of Lom TK-I. Our results show a similar amount of Lom-AKH-I release, except that the stimulating effect of CCAP was not completely abolished by trehalose. The proportionate increase of AKH release by CCAP stimulation, i.e. 2–2.7 fold, is the same as that found by Veelaert *et al.* (1997).

Compared with the total amount of AKHs present in the CC only a small amount is released during the basal incubation and during the CCAP-stimulated incubation. But this is not surprising considering the potency of the AKH hormones. Dose-response curves have been constructed for each AKH in *L. migratoria*. Only 0.1–0.2 pmol of Lom-AKH-I is enough to mobilise lipids in the locusts, and maximum response is reached with 2–3 pmol (Gäde, 1990; Lee and Goldsworthy, 1996). The amount of Lom-AKH-I released by CC *in vitro* with 80 mM trehalose, about 0.5–0.7 pmol, is large enough to cause a significant lipid mobilisation. Therefore it is questionable whether this actual amount is released *in vivo*. As basal release was inhibited further by glucose, there is a possibility of greater control over release of AKHs from the CC.

The ratio of Lom-AKH-I: II: III released from the CC was always in the same proportion as the total CC content. Lom-AKH-I and II are co-localised in the same secretory granules (Diederen *et al.*, 1987) and so are released in the same proportionate concentrations. The mRNA for each of the three AKH precursors are co-localised in the neurosecretory cells of the CC (Bogerd *et al.*, 1995)

and so Lom-AKH-III may also be released in a constant proportion to the other AKHs. It is therefore possible that any released AKH will have approximately 3–5 times as much Lom-AKH-I than II and 10 times as much Lom-AKH-I than Lom-AKH-III.

The possible physiological reason for having three AKH hormones has been of considerable interest lately. *L. migratoria* is the only insect found to have three AKHs; several insect species have two while most have only one AKH (Gäde, 1996; Gäde, 1997; Gäde *et al.*, 1997). The three AKHs of *L. migratoria* have overlapping actions but with different potencies, which may vary with age. With regard to lipid mobilisation Lom-AKH-I is by far the most potent; in adults aged 2–4 weeks, Lom-AKH-I has an $ED_{50}$ of 0.8 pmol, compared to 6.5 pmol for Lom-AKH-II and 1.9 pmol for Lom-AKH-III (Goldsworthy *et al.*, 1997). If CC were stimulated to release the amount of AKH that is stimulated by CCAP, for example during flight, then Lom-AKH-I alone would elicit the maximum lipid mobilising response. The amount of Lom-AKH-II and Lom-AKH-III released would, by themselves, produce much less than the maximum and therefore would be irrelevant to lipid mobilisation. The switch to lipid-based metabolism can be attributed to Lom-AKH-I, but finding a role for Lom-AKH-II and III is more difficult. In some tissue responses Lom-AKH-III is the most potent effector, such as increasing cAMP concentration and activating glycogen phoshorylase (Vroemen *et al.*, 1995a), in inhibiting RNA synthesis in the fat body (Kodrik and Goldsworthy, 1995), in inducing calcium influx (Vroemen *et al.*, 1995b) and increasing $[Ca]_i$ in single fat body cells (Goldsworthy *et al.*, 1997). However, consider for example, the acetate uptake assay, an *in vitro* assay based on the inhibition of fatty acid synthesis by AKH hormones. Lom-AKH-III is the most potent inhibitor of this process, with an $EC_{50}$ of 0.04 pmol/300 µl, compared to 0.16 pmol/300 µl for Lom-AKH-I and 0.48 pmol/300 µl for Lom-AKH-II (Goldsworthy *et al.*, 1997) (300 µl being the approximate haemolymph volume of an adult locust). If the AKH hormones are simultaneously released in levels proportionate to their levels in the CC, i.e. at least 10 times as much Lom-AKH-I than Lom-AKH-III, then *in vivo* Lom-AKH-I may still be the major effector of this response. In flight, Lom-AKH-III mRNA increases 4 times over resting levels, while Lom-AKH-I and II only doubled (Bogerd *et al.*, 1995), and as newly synthesised AKHs are preferentially released (Diederen *et al.*, 1987), this could change the ratio of released AKH hormone in long-term flight. On the other hand, Lom-AKH-III has a much faster removal rate from the haemolymph than the other AKHs (Oudejans *et al.*, 1996). Any search for a specialist role of Lom-AKH-II or III should take into account the haemolymph levels of each hormone as well as comparing their potency. It has been suggested that Lom-AKH-III is released constitutively from the CC and may regulate metabolism in the non-flying locust (Vreomen *et al.* 1997). Even if this is so, as Lom-AKH-I and II are released *in vitro* under non-stimulating conditions (see basal release, Tables I and V) they could be released in the non-flying locust as well.

### *Inhibitory effect of the different sugars*

Passier (1996) found that 80 mM trehalose reduced the rate of spontaneous release of Lom-AKH-I from isolated CC by about 50% and completely abolished the release of AKH stimulated by Lom TK-I. The effect of trehalose on the potassium-stimulated release of Lom-AKH-I depended on the concentration of potassium. Twenty mM potassium is the threshold for stimulation of release. At potassium concentrations of 20 and 30 mM the 80 mM trehalose reduced the amount of Lom-AKH-I released. Above 30 mM potassium the 80 mM trehalose did not have a significant effect.

We tested whether 80 mM trehalose also inhibits CCAP-stimulated release, and whether trehalose inhibition was specific or whether other sugars would also inhibit AKH release. We found that while 80 mM trehalose did not abolish the effect of CCAP-stimulated release of AKHs, it did reduce it by about 60%, which, considering the amount of AKH released, would have an impact on the mobilisation of lipids if it occurred *in vivo*. The same concentration of sucrose had no effect on any AKH release, which shows that inhibition of release was not due to an osmotic effect on the CC. Glucose had an even greater inhibitory effect than trehalose, reducing CCAP-stimulated release of AKHs by nearly 80%. The glucose concentration of 160 mM was used as an energy equivalent

of 80 mM trehalose. Furthermore, glucose also inhibited spontaneous release in the second series of experiments, where trehalose did not have a significant effect. Finally, while any inhibition of 50 mM $K^+$- stimulated release of AKHs was not statistically significant with trehalose, there was a significant decrease with glucose.

Passier (1996) suggested that the inhibitory effect of trehalose on AKH release could be due to a hyperpolarising effect on the cell membrane of the CC, caused by high ATP levels closing ATP-gated ion channels. If this is correct, then it can be seen why glucose might have a greater inhibitory effect, as glucose would provide more immediate energy for the cells whereas trehalose would first have to be broken down to glucose. The amount of glucose available to CC in a medium containing trehalose would depend on the activity of the cellular trehalase. Sucrose on the other hand would not provide any usable energy to the cells.

Trehalose has also been shown to have an effect on AKH synthesis in CC *in vitro*. In cockroach (*Periplaneta americana*) CC, the synthesis of hypertrehalosemic hormones was increased 5-fold by incorporating 50 mM trehalose into the incubation medium (Khan and Steele, 1992). Higher concentrations of trehalose did not produce greater rates of hormone synthesis. Because glucose at the physiological concentration of 5 mM did not enhance synthesis of the hormones, the authors felt that the enhancement was not due to utilisation of the trehalose as an energy source. However, glucose at a concentration of 140 mM glucose did stimulate about half the glands to synthesise hormone at the same rate as 140 mM trehalose. Sucrose did not have any effect on hormone synthesis. Trehalose and glucose may therefore play a very important role in regulation of AKH synthesis and release in some species of insects, through its utilisation as an energy source.

The maximum amount of Lom-AKH-I released by the effect of Lom TK-I and CCAP is about the same, ranging from 2–4 pmoles in a 30 min incubation, which is a small amount compared to the total AKH content per CC, but sufficient for maximum lipid mobilisation. It would be interesting to see whether Lom TK-I and CCAP could act synergistically in *L. migratoria* to produce a larger release of AKH. To see if the effect of trehalose and glucose is mediated through enhanced energy production in the CC, non-metabolisable analogues, such as L-glucose, could be used in the incubation media.

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