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Lipid and Carbohydrate Contents in the Adult Hemolymph during Flight of the Oriental Tobacco Budworm (*Helicoverpa assulta* (Guenee))

비행중인 담배나방의 혈림프내 지질과 탄수화물의 함량변화

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ABSTRACT Studies were carried out to investigate changes of lipid and carbohydrate contents in the hemolymph of the Oriental tobacco budworm(*Helicoverpa assulta* (Guenee)) adults during flight and hormonal effects on mobilization of energy sources in the hemolymph. During a few minutes after flight, both sexes showed a rapid increase in lipid content and the high level was maintained for about 2 hours. But carbohydrate content in the hemolymph during flight showed almost no change but a slight increase seen during the first 10 min of flight in males only. Synthetic adipokinetic(Lom-AKH-Ⅱ), hypertrehalosemic(Bld-HrTH) hormones and brain/corpora cardiaca extract of *H. assulta* adult elevated lipid and carbohydrate contents in hemolymph and the effect was much more pronounced for lipid. These results suggested that lipid is a main fuel for flight activity and lipid mobilization is under the hormonal control. And this study showed that both adipokinetic and hypertrehalosemic factors may exist in *H. assulta* and these factors may have similar structures to those of Mas-AKH, Hez-HrTH, Lom-AKH-Ⅱ or Pea-HrTH.

KEY WORDS *Heliocoverpa assulta*, flight, hemolymph, lipid, carbohydrate, brain/corpora cardiaca extract, adipokinetic hormone, hypertrehalosemic hormone

초 록 본연구는 비행중인 담배나방(*Helicoverpa assulta* (Guenee))성충의 혈림프내 지질과 탄수화물의 함량변화와 그들의 호르몬조절가능성을 조사하기 위하여 수행되었다. 비행초기 몇분동안에 암수 모두 빠른 지질증가반응을 보였고, 약 2시간까지 증가된 수준이 유지되었다. 비행중 혈림프내 탄수화물농도는 거의 변화가 없었지만, 수컷에서는 비행후 10분 동안 약간의 농도증가가 있었다. 합성 지질동원호르몬(Lom-AKH-Ⅱ), 당동원호르몬(Bld-HrTH), 담배나방 자신의 뇌-카디아카체 추출물들 모두 담배나방의 혈림프내 지질과 당함량을 높여주었는데, 지질이 당보다 훨씬 더 높게 나타났다.

이상의 결과로 주로 지질이 담배나방의 주 비행연료로 사용되고 혈림프내 지질함량은 지질동원호르몬의 조절을 받는 것으로 보여진다. 또한 담배나방에는 지질동원호르몬과 당동원호르몬이 같이 있을 수 있으며, 이들 펩티드들의 구조는 Mas-AKH, Hez-HrTH, Lom-AKH-Ⅱ, Pea-HrTH 등의 구조와 유사할 것이라고 유추되었다.

검 색 어 담배나방(*Helicoverpa assulta* (Guenee)), 비행, 혈림프, 지질, 탄수화물, 뇌-카디아카체 추출물, 지질동원호르몬, 당동원호르몬

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Many studies for flight energy have been mainly focused on the patterns of fuels and their hormonal controls. Of fuels for flight, are carbohydrates, lipids and amino acids. But the pattern for the utilization is different and complex depending on insect species.

In many species of Lepidoptera and Orthoptera, carbohydrate is utilized in combination with lipid. Trehalose in hemolymph is utilized during the initial period as a major fuel, but during sustained flight, diglyceride is utilized as the major fuel in *Locusta migratoria*(Jutsum & Goldsworthy 1976, Van der Horst *et al.* 1978b, 1980). But the tobacco hornworm(*Manduca sexta*) uses much more lipid from the beginning and has much higher hemolymph lipid concentration (Ziegler & Schulz 1986a, b). However, in the velvetbean caterpiller (*Anticarsia gemmatalis*), carbohydrate is the consistant fuel during the sustained flight as well as during the initial period, although lipid is the principal flight fuel in making long distance movements (Teo *et al.* 1987).

The suggestion that locusts possessed a neurosectory factor which controls lipid utilization during prolonged flight was first made by Mayer and Candy(1969). These authors showed that this active principle, which they named "adipokinetic hormone(AKH)", was located in the corpora cardiaca, and stimulated the release of diglycerides from the fat body. Since then, many similar neuropeptides have been isolated, identified, and assayed for their biological functions. In *L. migratoria* and *Schistocerca gregaria*, Stone *et al.*(1976) first identified the primary structure of a decapeptide and named it AKH, later renamed as Lom-AKH-I by a new nomenclature scheme(Raina & Gäde 1988). Primary structures of other octapeptides, Lom-AKH-II in *L. migratoria* and Scg-AKH-II in *S. gregaria*, were also reported(Carlsen *et al.* 1979, Gäde *et al.* 1986). In *M. sexta*, another octapeptide was identified by Ziegler *et al.*(1985). Even in *Heliothis zea*, two peptides, Mas-AKH(Jaffe *et al.* 1986) and Hez-HrTH(Jaffe *et al.* 1988), were identified.

On the other hand, hypertrehalosemic hormones causing an elevation of hemolymph trehalose levels have been also demonstrated in various insects species(Beenakkers *et al.* 1984). But, the presence of a hypertrehalosemic hormone in the corpora cardiaca does not necessarily imply that the insect concerned will exhibit hypertrehalosemia when injected with an extract of corpora cardiaca. For example, corpora cardiaca extracts of *Locusta* induce hypertrehalosemia in *Periplaneta americana* (Jones *et al.* 1977), but not in the locust itself (Holwerda *et al.* 1977).

The present study is concerned with the pattern for lipid and carbohydrate utilization in the hemolymph of the Oriental tobacco budworm (*Helicoverpa assulta* (Guenee)) adults during flight, and deals with the effect of neurohormones on lipid and carbohydrate mobilization from fat body into hemolymph.

MATERIALS AND METHODS

Insects

The Oriental tobacco budworm, *Heliocoverpa assulta* (Guenee), larvae were reared on an artificial diet(Park 1991). The adults were supplied with 10% sucrose solution. For a flight experiment 1 day-old adults were used, but for injection experiment of neurohormones and corpora cardiaca extracts 2 day-old adults were utilized. *Periplaneta americana* colony was maintained on powdered rabbit food(50g) supple-

mented with milk powder(30 g) and sucrose(20 g). *P. americana* male adults were used for bioassay to determine carbohydrate mobilization activity of brain/corpora cardiaca extract from *H. assulta*.

Both insects were reared at 16L/8D photoperiod, 25±1℃ and 60% RH.

Flight experiments

The back of *H. assulta* adult thorax was gently freed from scale and fixed with an instant glue to the plastic straw. For the constant flight, the tarsi were touched with brush or the animals were fanned. Control animals were treated similarly but were not flown.

Preparation and injection of brain/corpora cardiaca extract

Brain/corpora cardiaca complexes of *H. assulta* were removed in a dissection Ringer solution(Aston & Hughes 1980) and were homogenized with 95% methyl alcohol. The homogenate was centrifuged for 10 min at 2000rpm and its supernatant was stored. This procedure was performed twice. The combined supernatant was filtrated with 0.45 ㎛ membrane filter and then dried with nitrogen gas. The dried sample was dissolved in 0.15 M sodium phosphate buffer solution(PBS) (pH 7.0) containing 0.1 M NaCl amd 0.05% EDTA(Shapiro & Law 1983) and stored at −70℃ until use.

Five microliters of brain/corpora cardiaca extract at various concentrations were injected into *H. assulta* abdomen through the intersegmental membrane between 4th and 5th abdominal segments. Brain/corpora cardiaca extract was also examined for carbohydrate mobilization potency by injecting an aliquot into adult male cockroaches(Holwerda *et al.* 1977). Cockroaches were immobilized with cold temperature for handling. One hour after injection of a 10 μl aliquot of extract to be assayed into the abdomen, hemolymph was taken from the abdomen for analysis.

Injection of the synthetic AKH and HrTH

Synthetic locust adipokinetic hormone Ⅱ (Lom-AKH-Ⅱ) and cockroach hypertrehalosemic hormone(Bld-HrTH) (SIGMA Chemical Co.) were dissolved in PBS. An aliquot was injected as above. Time-response and dose-response were recorded.

Determination of total carbohydrate in hemolymph

Collected hemolymph samples(1-5 μl) were separated with Folch method(Folch *et al.* 1957). The upper phase was treated with 5 % TCA, and was used for determination of total carbohydrate with the anthrone-positive method (Launer 1962). To 500 μl sample were added 3.0 ml anthrone reagent(0.2 g/100 ml H_2SO_4). The mixture was heated for 10 min at 100℃ and then chilled in ice-water for 2 min. Reaction tubes were subsequently kept in the dark for 30 min. Absorption was read at 620 nm against a reagent blank mixed 500 μl of double distilled water.

In cockroaches injected with brain/corpora cardiaca extract, hemolymph samples(7-10 μl) were mixed with 1.5 ml of 98% sulfuric acid and stored at 4℃ until determination of carbohydrate content(Holwerda *et al.* 1977). To 0.15 ml sample were added 3.0 ml anthrone reagent. Absorption was read as above.

Determination of total lipid in hemolymph

The lower phase from the hemolymph sample was removed for total lipid content determina-

tion using the vanillin-positive method(Stone & Mordue 1980). Collected samples were dried with nitrogen gas, and then dissolved in 500 μl of 98% H_2SO_4. The dissolved samples were, after addition of 3.0 ml vanillin reagent(13 mM vanillin in 14 M phosphoric acid), incubated for 30min at room temperature, and then read spectrophotometrically at 547 nm against a reagent blank mixed with 500 μl of 98% sulfuric acid.

RESULTS AND DISCUSSION

Lipid mobilization

The total lipid level in hemolymph increased rapidly during the first 20 minutes of flight in *H. assulta* adults(Fig. 1). This increased level was maintained for about 2 hours of flight and then the level decreased. At resting animals(not flown), the lipid level in the hemolymph of males was about 24.5 mg/ml, which is always higher than that from females(about 15.2 mg/ml) (Figs. 1-4). The maximum level of lipid content during flight was 40.4 mg/ml in males and 39.8 mg/ml in females shown at one hour after flight, and at that time its increase rate was 65% in males and 162% in females. After 2 hrs. of flight in males and 1 hr. in females, the lipid level started to decrease. It may be due to a possibility that lipid utilization rate in the flight muscle was more than its mobilization rate from the fat body. Wingbeat frequency also became lower with time during sustained flight. The lipid level in males during flight tended to be a little higher than that of female. This means that the males may have a stronger flight activity than females. After 3 hrs. of flight, the lipid level was back almost to the resting level showing 18.5 mg/ml in males and 23.7 mg/ml in females.

Changes in the lipid content in hemolymph of

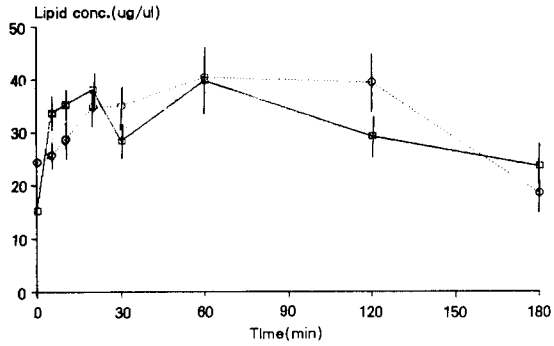

Fig. 1. Titres of total lipid content in the hemolymph during flight of adult female(□) and male (○) *Helicoverpa assulta.*

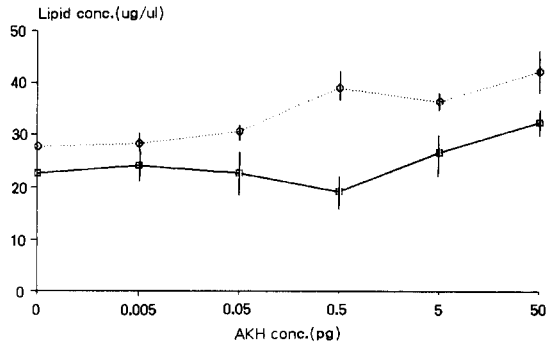

Fig. 2. Effect of synthetic Lom-AKH-Ⅱ injection on lipid mobilization in adult female(□) and male (○) *Helicoverpa assulta*(measured 1 hr. after the treatment).

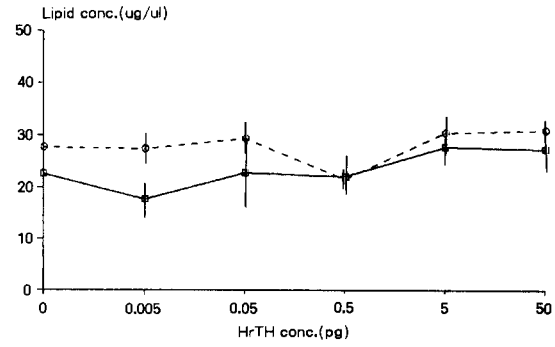

Fig. 3. Effect of synthetic Bld-HrTH injection on lipid mobilization in adult female(□) and male (○) *Helicoverpa assulta*(measured 1 hr. after the injection).

H. assulta during flight reflect changes in lipid utilization. The initial increase is similar to that reported for *L. migratoria*(Jutsum & Goldsworthy 1976, Van der Horst *et al.* 1978a, 1980) and *A. gemmatalis*(Teo *et al.* 1987). This initial increase in lipid content was reported to be due to release of octopamine from neurosecretory cells in insects such as *L. migratoria*(Goosey & Candy 1980). Octopamine also stimulate lipid oxidation in the flight muscle besides lipid mobilization from the fat body, and the release of AKH(Orchard & Lange 1983). It is not clear yet whether octopamine is also involved in lipid mobilization in *H. assulta*.

However, in *M. sexta*, the lipid content in hemolymph rapidly decreased during a few minutes after flight and reached a steady state about 30 min after the flight start(Ziegler & Schultz 1986b). In male *H. assulta*, the steady state of lipid level in hemolymph lasted for about 1 hour from 1 hour to 2 hours after the beginning of flight. This indicates that the utilization rate of lipid in *H. assulta* flight muscle is the same as the mobilization rate from the fat body and also that this continuous mobilization may be due to a control by a certain hormone. This assumption is supported by injection experiments of synthetic Lom-AKH-Ⅱ, Bld-HrTH and brain/corpora cardiaca extract of *H. assulta*.

One hour after injection of synthetic Lom-AKH-Ⅱ, lipid was mobilized in the hemolymph. Synthetic Lom-AKH-Ⅱ was effective in lipid mobilization at 0.5 pg/individual or higher in males and at 5 pg or higher in females(Fig. 2). The increase rate was about 42% in males at the concentration of 0.5 pg and about 18% in females at the concentration of 5 pg. And the increase rate was 53% in males and 43% in females, respectively, at the level of 50 pg.

Time-response for Lom-AKH-Ⅱ injection showed, when tested at three different concentrations, 0.5, 5 and 500 pg per individual, that the lipid content reached the maximum level in males at 60 min and in females 30 to 60 min after injection(Jung 1991). Thereafter the lipid content rapidly decreased to reach the concentration at or lower value than the preinjection level 3 hours after the injection. This decrease may be due to metabolism of the neurohormone injected. When the time-response for Lom-AKH-Ⅱ injection is compared between the two sexes, the concentration change(up and down) is much more dynamic in males than in females. For example, the highest level was 1.7-2.6 times higher than the resting level in males but less than 1.7 times higher in females. In either sex the response is somewhat abnormal when injected with 500 pg per individual, the highest level tested in the present study(Jung 1991).

However, lipid was almost not mobilized in hemolymph of *H. assulta* adults when injected with synthetic Bld-HrTH, regardless of the sex (Fig. 3). At the concentration of 50 pg, the lipid level in the hemolymph was only 12 % higher in males and 21% higher in females than those before the injection. These results show that Lom-AKH-Ⅱ has a much higher activity on lipid mobilization than Bld-HrTH in *H. assulta*, even though an opposite experimental result was reported in *L. migratoria*(Gäde 1990).

When methanolic extract of its own brain/corpora cardiaca complex was injected into 2-day old adults of *H. assulta*, lipid content in hemolymph was elevated at the level of 0.1 pair equivalent or higher(Fig. 4). The increase rate was about 84% in males and about 69% in females at the level of 1 pair equivalent. This response of *H. assulta* is lower than that(400%) for locust(Holwerda *et al.* 1977), but higher

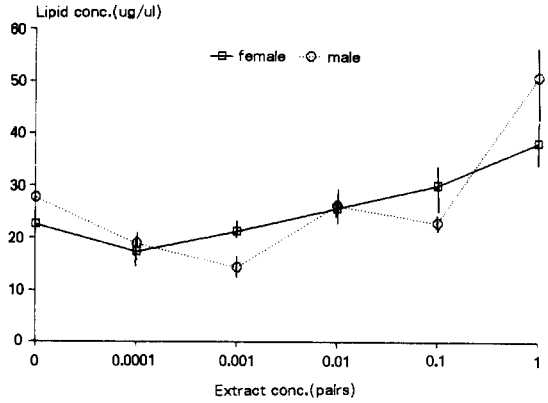

Fig. 4. Dose-response curves for lipid mobilization by methanolic extract of *Helicoverpa assulta* brain/corpora cardiaca complex(Measured 1 hr. after the injection).

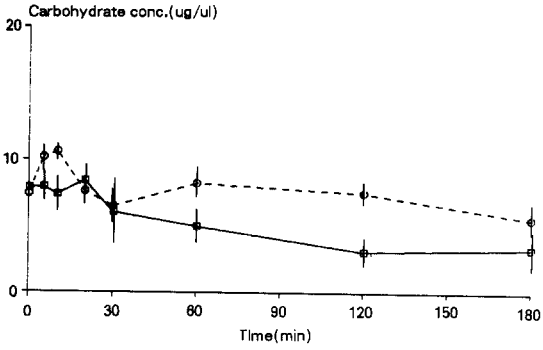

Fig. 5. Titres of total carbohydrate content in the hemolymph during flight of adult female(□) and male(○) *Helicoverpa assulta.*

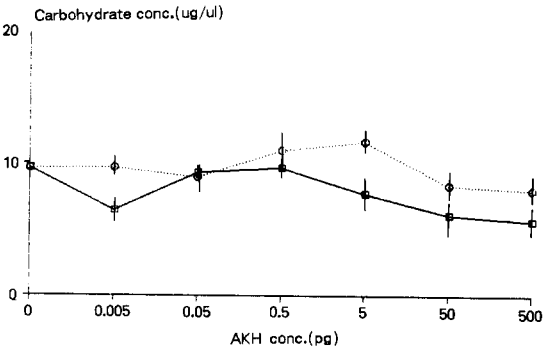

Fig. 6. Effect of synthetic Lom-AKH-Ⅱ injection on carbohydrate mobilization in adult female(□) and male(○) *Helicoverpa assulta*(measured 1 hr. after treatment).

than those for *M. sexta*(40%) (Ziegler & Schultz 1986a) or *Heliothis zea*(63%) (Jaffe *et al.* 1986). These results indicate that an adipokinetic factor may exist and control mobilization and utilization of lipid during the prolonged flight of *H. assulta* adults.

Carbohydrate mobilization

On the other hand, the carbohydrate level in the hemolymph of *H. assulta* adults was not altered significantly during flight(Fig. 5). The carbohydrate level in resting animals was about the same between two sexes with about 7.5 mg/ml in males and 7.9 mg/ml in females. This level is similar to that from locust(9.8 mg/ml), but much lower than that from *M. sexta*(20.3 mg/ml) and *A. gemmatalis*(27.4 mg/ml) (Teo *et al.* 1987, Van der Horst *et al.* 1978b, Ziegler & Schultz 1986b). The carbohydrate level during the first 10 min of flight slightly increased only in males reaching the maximum level of 10.6 mg/ml(Fig. 5). The increase rate at this maximum level was about 43%. After this time the carbohydrate level was maintained more or less at the resting level in males but gradually decreased in females.

In males, the increase of the carbohydrate content during the first 10 min of flight may be due to its mobilization stimulated by flight activity itself or octopamine. In *L. migratoria*, the carbohydrate utilization during the sustained flight is only 23% of preflight level(Van der Horst *et al.* 1978b) and *M. sexta* and *A. gemmatalis* also showed similar aspects(Teo *et al.* 1987, Ziegler & Schultz 1986b). And, in females, the carbohydrate level was maintained more or less at the resting level during first 20 min of flight and then began to decrease. This means that the carbohydrate may be also utilized for flight activity but not replenished

enough due to an exhaustion of carbohydrate store in the fat body during the sustained flight.

When Lom-AKH-Ⅱ was injected into *H. assulta* adults at the concentration of 0.5 and 5 pg per individual, the carbohydrate content reached the maximum level, about 1.4 times higher than the resting level between 10 min and 30 min after injection in both sexes(Jung 1991). And one hour after the injection, carbohydrate mobilization was slightly effective only in males when injected with 0.5 or 5 pg(Fig. 6). The increase rate in males was about 21% when 5 pg was injected. Synthetic Bld-HrTH was also effective in elevating carbohydrate level only in the hemolymph of male *H. assulta* at the concentrations of 5 pg or higher per indivual 1 hour after the injection(Fig. 7). The increase rate was about 29% at 5 pg. But females showed almost no response to synthetic Bld-HrTH injection. Response one hour after 5 pg of Lom-AKH-Ⅱ or Bld-HrTH was injected into *H. assulta* showed that the hypertrehalosemic activity of Bld-HrTH was higher than that of Lom-AKH-Ⅱ. But Gäde (1990) reported the opposite data when both hormones were injected into *P. americana*. This may be due to species specificity on the effects of neurohormones for carbohydrate mobilization.

Smaller amount of carbohydrate was mobilized when its own brain/corpora cardiaca extract was injected into *H. assulta* in comparison to lipid(Fig. 8). Carbohydrate content in the hemolymph from similarly treated adults was elevated by only 23% in males and 6% in females at the concentration of 1 pair equivalent. And this value is very low in comparison to that(73 %) of *H. zea*(Jaffe *et al.* 1988). This small value suggests a possibility that there is no hypertrehalosemic factor or much lower titre in

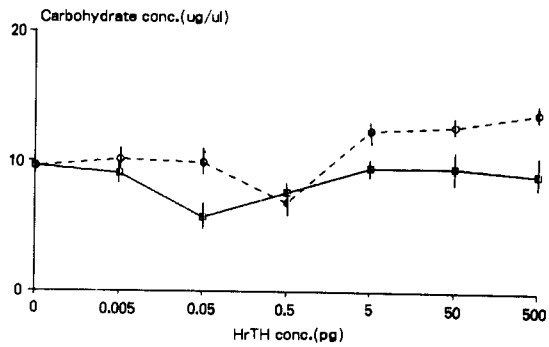

Fig. 7. Effect of synthetic Bld-HrTH injection on carbohydrate mobilization in adult female(□) and male (○) *Helicoverpa assulta*(measured 1 hr. after the injection).

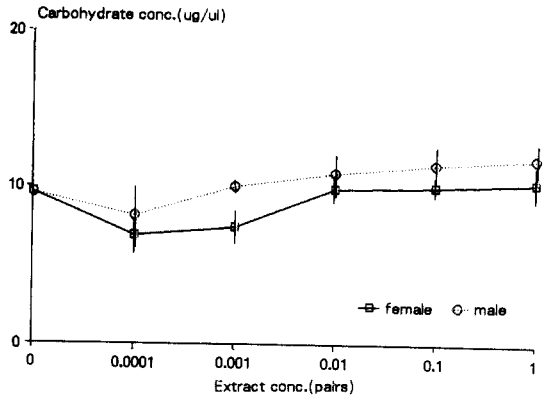

Fig. 8. Dose-response curves for carbohydrate mobilization by methanolic extract of *Helicoverpa assulta* brain/corpora cardiaca complex(measured 1 hr. after the injection).

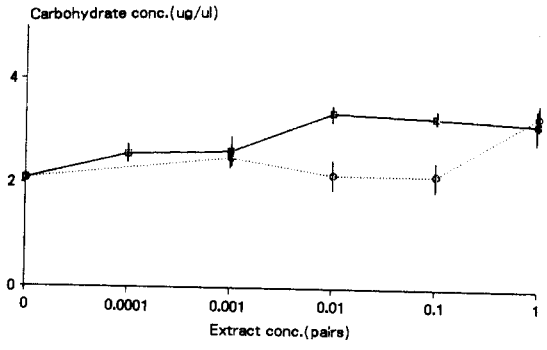

Fig. 9. Effect of methanolic extract of *Helicoverpa assulta* female(□) and male(○) brain/corpora cardica complex on carbohydrate mobilization in *Periplaneta americana*(measured 1 hr. after the injection).

H. assulta. In *M. sexta*, corpora cardiaca extract could not activate glycogen phosphorylase in the fat body, although the glycogen phosphorylase was activated during flight(Ziegler & Schultz 1986b).

However, brain/corpora cardiaca extract of *H. assulta* male and female activated the carbohydrate mobilization in *P. americana* when one pair equivalent was injected(Fig. 9). At this concentration, the difference for the activity between both sexes was not found. This result suggests that a hypertrehalosemic factor or an adipokinetic factor of *H. assulta* may have a similar structure to Pea-HrTH and be included in AKH/PRCH family. The extract of males did not show significant activities when 0.1 and 0.01 pair equivalents were injected into the cockroach. This may be experimental error or due to the loss of the activity during extraction, dilution and/or storage.

Therefore, from these results, it is concluded that *H. assulta* utilizes lipid as the major fuel and carbohydrate as a minor, even if it is utilized. Lipid utilization in *H. assulta* during the sustained flight must be under the control of adipokinetic hormone. And, there is a possibility that a hypertrehalosemic factor, besides an adipokinetic factor, may also exist because of the results on the carbohydrate mobilization. Regardless of the question about existence of these two factors, the adipokinetic factor is much more active than the hypertrehalosemic factor in this insect. This is demonstrated indirectly in the fact that brain/corpora cardiaca extract had a relatively superior effect in lipasemia than hypertrehalosemia(Table 1). Jaffe(1986, 1988) isolated both adipokinetic(Mas-AKH) and hypertrehalosemic neuropeptide(Hez-HrTH) from *H. zea*, and reported that Hez-AKH and Hez-HrTH existed in a proportion of 3 : 1. Therefore, if adipokinetic and hypertrehalosemic factors both exist in *H. assulta*, they may have similar primary structure to Mas-AKH, Hez-HrTH, Lom-AKH-Ⅰ, Ⅱ and Pea-HrTH, but in a disproportional ratio.

Table 1. Effect of methanolic extract from *Helicoverpa assulta* brain/corpora cardiaca complex(1 pair equiv.) on the relative mobilization of carbohydrates and lipids

		Famale	Male	Combined
Carbohydrate	Control	9.6	9.6	9.6
(μg/μl)	Treated	10.3	11.9	10.9
	Increase(%)	107.3	124.0	113.5
Lipid	Control	22.6	27.6	24.6
(μg/μl)	Treated	38.2	50.8	44.8
	Increase(%)	169.0	184.1	182.1
Mobilization ratio(lipid/carbohydrate)		9.5	3.5	6.1

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