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Induction of Precocious Adult Eclosion by the Eclosion Hormone Extracted from the Heads of *Bombyx mori* (Lepidoptera: Bombycidae) and *Samia cynthia ricini* (Lepidoptera: Saturniidae)

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In order to compare the eclosion hormone activity between two different species of lepidoptera, eclosion hormone (EH) extracted from adult heads of *Bombyx mori* or *Samia cynthia ricini* was subjected to the bioassay to induce precocious adult eclosion. By performing reciprocal injection of the EH in both species, the authors demonstrated that *Bombyx* crude EH can induce the precocious eclosion of the pharate-adult of the *Samia*, while *Samia* crude EH has the same effect to the pharate adults of *Bombyx*. The hormonal activities were not reduced throughout the several purification steps in both cases. Necessary amount of EH to induce the precocious eclosion in more than 50% of the assayed pharate-adults of *Bombyx mori* was equivalent to 3 heads in *Bombyx* and equivalent to 2.5 heads in *Samia*. The results may suggest that eclosion hormone is not species-specific, so far as two lepidopteran species used in the present experiments are concerned.

INTRODUCTION

Hormonal control of ecdysis in lepidopteran adults was first indicated by means of surgical operations carried out on Saturniid moths (TRUMAN and RIDDIFORD, 1970). Extracting an eclosion hormone (EH) in several kinds of lepidoptera such as *Hyalophora cecropia*, *Antheraea pernyi* and *Manduca sexta*, TRUMAN and his co-workers demonstrated that adult eclosion can be induced by EH contained in the brain homogenates (TRUMAN, 1971, 1973; REYNOLDS and TRUMAN, 1980).

The presence of EH in brains was confirmed in another lepidoptera, *Bombyx mori* (MOROHOSHI and FUGO, 1977; FUGO and IWATA, 1983 a, b; NAGASAWA et al., 1983).

In the present study, extraction of EH from adult heads of *Bombyx* and *Samia* and activity of EH in reciprocal assays were investigated.

MATERIALS AND METHODS

Animals. Silkworms (*Bombyx mori*) used in this experiment were F_1 hybrids of Japanese No. 106 and Daizo. The larvae were routinely fed on mulberry leaves at 25±2°C with a relative humidity of 75% under 12 hr of daily illumination. Among the mass cultured pupae those which were ecdysed within a period of five hours were selected as materials. The pupae were kept under 16 hr light–8 hr dark (16 L–8 D) photocondition at 25±1°C. In this experimental condition, the moths eclosed in 8 to 9 days after pupation.

Larvae of *Samia cynthia ricini* were reared on *Ailanthus glandulosa* leaves at 25±1°C under 18L–6D photocondition. Shortly after pupal ecdysis, the pupae were transferred to 16L–8D at 25°C. Under this condition, eclosion occurred in 15 to 16 days after pupation.

Preparation of eclosion hormone. Freshly ecdysed male adults of *Bombyx mori* (5,000 individuals) or *Samia cynthia ricini* (1,500 individuals) were frozen at −20°C and stored until use. The heads were cut off and homogenized in cold acetone (−20°C) to prepare acetone powder. After washing with 80% ethanol, 2% NaCl solution was used for extraction of the EH. The process for extraction and purification of the EH was based on the procedure for prothoracicotropic hormone (PTTH) extraction (NAGASAWA et al., 1979), as summarized in Fig. 1. Partially purified EH (termed as crude EH) was lyophilized and dissolved in distilled water for injection.

Bioassay for eclosion hormone. The *Bombyx* eclosion hormone assay was performed by the method of whole insect bioassay following FUGO and IWATA (1983 a). After pupation, male pupae were kept under a 16L–8D photocondition during the pupal-adult development up to 1 day before eclosion. When these pharate-adults were transferred into continuous lighting (LL), emergence occurred within 19 to 22 hr (FUGO, 1982). The 8-day-old pupae were subjected for the EH assay: They were injected with EH about 9 hr before the presumptive eclosion time. After injection, the assayed pharate-adults were maintained under LL condition.

The *Samia* eclosion hormone assay was also done by a whole insect bioassay. *Samia* pupae were maintained under 16L–8D photocondition throughout their pupal-adult development. Duration of the pupal stage was about 14 to 16 days at 25°C. On the 15th day of pupal-adult development, male pharate-adults of *Samia* were assayed for EH activity by injecting EH at about 9 hr before the presumptive eclosion time.

The presence of EH activity can be revealed by precocious adult eclosion within 2.5 hr after the injection. Hormone was extracted from a known number of heads and the amount of hormone injected is expressed in the number of heads per assayed animal.

One unit of the hormone corresponds to the minimum amount of EH to induce precocious adult eclosion in more than 50% of the assayed pharate-adults.

RESULTS AND DISCUSSION

It was evidenced by the works of TRUMAN (1971, 1973, 1980) using several kinds of Lepidoptera that eclosion is initiated by a neurohormone (eclosion hormone: EH). In moths this hormone was presumably produced by certain median neurosecretory cells in the brain during the pupal-adult development (TRUMAN, 1973; FUGO and IWATA, 1983 b).

Step

Adult heads (*Bombyx* or *Samia*)

1 Acetone powder

2 Washing with 80% ethanol

3 Extraction by 2% NaCl solution

4 Heat-treatment in boiling water bath for 5 min

5 Precipitation with 80% saturation of ammonium sulfate

6 Precipitation with 50–75% acetone

7 Precipitation with 90% saturation of picric acid

8 Precipitation with 80% acetone

Crude EH

Fig. 1. The procedure for purification of eclosion hormone from the adult heads of *Bombyx mori* and *Samia cynthia ricini*.

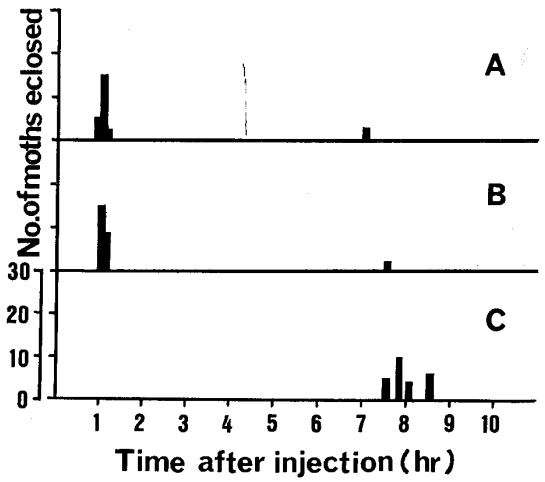

Fig. 2. Precocious eclosion by injecting the eclosion hormone (EH) into the *Bombyx* pharate-adult. A: EH obtained from adult heads of *Bombyx mori*. B: EH obtained from adult heads of *Samia cynthia ricini*. C: control (20 μl of 0.1 M Tris-HCl buffer (pH 7.8) was injected). Each animal received the amount of EH equivalent to 4 heads.

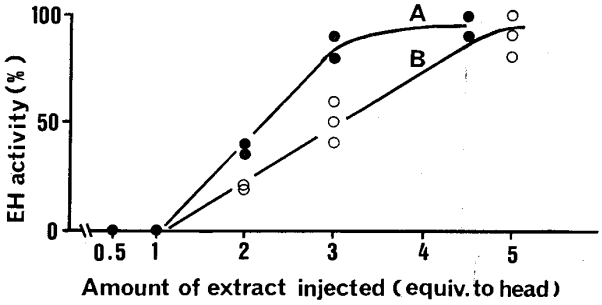

Fig. 3. Dose response curve for eclosion hormone (EH) in the *Bombyx* EH assay. A: EH obtained from *Samia* adult heads. B: EH obtained from *Bombyx* adult heads.

Table 1. Purification of eclosion hormone from *Bombyx* heads and *Samia* heads

Purification step (species)	Weight[a] (mg)	Total activity[b] (unit)	Specific activity (mg/unit)
(*Bombyx mori*)			
3 Saline extract	8,560	1,390	6.15
8 Crude EH	150	1,200	0.13
(*Samia cynthia ricini*)			
3 Saline extract	3,240	680	4.8
8 Crude EH	49	550	0.1

[a] Dry weight.

[b] EH activity was estimated by *Bombyx* eclosion hormone assay. One unit of the hormone corresponds to the amount of EH to induce precocious eclosion in more than 50% of the assayed *Bombyx* pharate adults.

Table 2. Relationship between the injection of *Bombyx* EH or *Samia* EH and the induction of precocious eclosion in *Samia cynthia ricini*

Samples (dose: equiv. to heads)	No. of animals used	No. of eclosed moths up to 3 hr after injection (%)	No. of eclosed moths after 3 hr (%)
Samia crude EH (5)	10	10 (100)	0 (0)
Bombyx crude EH (8)	19	17 (89.5)	2 (10.5)
Control (D. W. 20 μl)	10	0 (0)	10 (100)

One important piece of evidence that the EH triggers adult ecdysis is the demonstration of artificial induction of early ecdysis by hormone injection.

By using the method for extraction and partial purification of EH as summarized in Fig. 1, the authors obtained the EH from adult heads of *Bombyx mori* or *Samia cynthia ricini*. As shown in Fig. 2, the extracts of the adult heads of *Bombyx* or *Samia* contained the neurohormone to induce the precocious eclosion of the silkworm, *Bombyx mori*. The results of purification of EH are summarized in Table 1. Saline extract at step 3 possessed 1,390 units (0.3 unit/head) in *Bombyx* and 680 units (0.45 unit/head) in *Samia*. Specific activity of the crude EH was elevated to 0.13 mg/unit in *Bombyx* and 0.1 mg/unit in *Samia*, and about 50-fold purification was achieved in both cases. Since hormonal activity was not reduced throughout the several purification steps (Table 1), EH seemed to be stable against heat-treatment, acetone precipitation and repeated lyophilization. Futhermore, when the active materials were stored at $-20°C$ for more than 8 months, hormonal activities were still retained (FUGO, unpublished data).

Both EHs showed significant activity in reciprocal assays: *Samia* extracts have a potency to induce the precocious eclosion of *Bombyx mori* (Figs. 2 and 3), while *Bombyx* EH accelerated the adult eclosion of *Samia cynthia ricini* (Table 2). Dose response relationships in *Bombyx* eclosion hormone assay were exhibited in both EHs (Fig. 3). The minimum amount to induce the precocious eclosion in more than 50% of the assayed *Bombyx* pharate-adults was equivalent to 3 heads (ca. 0.3 unit/head) in *Bombyx* and 2.5 heads (0.4 unit/head) in *Samia*. Since EH is released from the brain-corpora cardiaca-corpora allata into the haemolymph at the time of adult eclosion of *Bombyx* (FUGO, in preparation), the activity in adult heads seemed to be lower than that of

pharate-adult heads. It was reported that in *Bombyx mori* the pharate-adult heads possessed 5 to 10 times as much activity as the adult heads (NAGASAWA et al., 1983). Eclosion hormone seems not to be species-specific, so far as two lepidopteran species used in the present experiments are concerned. However, positive effects of both EHs of *Bombyx* and *Samia* to adult eclosion of *Bombyx* (Figs. 2 and 3) do not necessarily supply the proof that the same action in both extracts is due to the same hormone.

The further purification of EH from *Bombyx mori* and *Samia cynthia ricini* is under investigation and details of the chemical properties will be reported in the near future.

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