Hormonal Regulation of Pheromone Biosynthesis in the Silkworm Moth, *Bombyx mori* (Lepidoptera: Bombycidae)

Rika Arima, Kasumi Takahara, Tomoko Kadoshima,
Fusako Numazaki, Tetsu Ando,[1] Masaaki Uchiyama,
Hiromichi Nagasawa,[2] Akihiro Kitamura[2] and Akinori Suzuki[2]

*Department of Applied Biological Science, Faculty of Agriculture, Tokyo University of
Agriculture and Technology, Fuchu, Tokyo 183, Japan*
*[2]Department of Agricultural Chemistry, Faculty of Agriculture,
The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan*

In the silkworm moth (*Bombyx mori* L.), the sex pheromone [bombykol, (10*E*, 12*Z*)-10, 12-hexadecadien-1-ol] became undetectable 24 hr after decapitation, but its production was restored by the injection of a brain hormone (pheromone biosynthesis activating neuropeptide, PBAN). In order to understand the mode of action of this hormone, ^{14}C-incorporation into pheromone precursors and related compounds was examined by treating the pheromone glands of normal, decapitated, and decapitated and hormone-injected virgin females with ^{14}C-hexadecanoic acid. In the decapitated female moth, conversion of the ^{14}C-acid into bombykol as well as other C_{16}-fatty alcohols was very low. The levels of ^{14}C-incorporation observed in normal females were partially recovered in the decapitated female by hormone injection. However, the amount of unsaturated C_{16}-fatty acyl moieties and their levels of ^{14}C-incorporation was not decreased as much by decapitation. This suggests that the main regulatory role of PBAN is in the reduction of an acyl moiety in the bombykol biosynthetic pathway rather than effecting unsaturation steps. Further, when the gland, which was removed from the decapitated female, was incubated in a Grace's medium with PBAN, the pheromone was again produced indicating the direct regulation of the pheromone gland by this hormone.

Key words: pheromone biosynthesis, PBAN, silkworm moth, ^{14}C-hexadecanoic acid, bombykol

INTRODUCTION

A neuropeptide hormone, which is produced in a brain and/or a suboesophageal ganglion and controls production of the sex pheromone, was first reported by Raina and Klun in the female corn earworm moth (*Heliothis zea*) in 1984. Since then, the presence of such the stimulator, pheromone biosynthesis activating neuropeptide (PBAN), has been also detected in several lepidopterous insects (Ohguchi et al., 1985; Ando et al., 1988 c; Martinez and Camps, 1988; Raina, 1988; Altstein et al., 1989; Bestmann et al., 1989; Cusson and McNeil, 1989; Tang et al., 1989; Rafaeli and

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[1] To whom correspondence should be addressed.

138   R. Arima et al.

Soroker, 1989). Recently complete amino acid sequences of PBAN from *H. zea* (Raina et al., 1989) and the silkworm moth (*Bombyx mori*) (Kitamura et al., 1989, 1990) were determined and shown to have considerable structural similarity. While the pheromone content in the gland of a decapitated or ligated female was increased by the injection of PBAN, its mode of action is still unclear. It is of interest to recognize which step of the pheromone biosynthesis is regulated by the hormone. The possible biosynthetic pathway for the female sex pheromone of *B. mori* [bombykol, (10*E*,12*Z*)-10,12-hexadecadien-1-ol] has been proposed from experiments using ^{14}C-hexadecanoic acid (Inoue and Hamamura, 1972), ^{2}H$_2$-(*Z*)-11-hexadecenoic acid (Yamaoka et al., 1984) and some ^{14}C-labeled precursor candidates (Ando et al., 1986, 1988 a, 1988 b). In this paper, we demonstrate the target step of PBAN by examining the effect of decapitation and hormone injection on the conversion of ^{14}C-hexadecanoic acid applied to the pheromone gland of *B. mori*. Furthermore, the target organ is indicated by incubation of the pheromone gland excised from decapitated *B. mori* in a medium with or without the hormone.

MATERIALS AND METHODS

Insects. Larvae of *B. mori* [hybrids of Shunrei (female) × Shogetsu (male)] were reared on a semisynthetic diet (Nippon-chlorella Co., Tokyo) and maintained under 16:8 light-dark cycles at 25°C. Females were selected at 5th larval instar.

Natural PBAN of B. mori. Crude PBAN, obtained from 5,000 heads (female and male moths) by 80% aqueous ethanol extraction as described previously (Ando et al., 1988 c), was dissolved in distilled water (5 ml) and applied to column (10 mm i.d. × 8 cm) of activated charcoal (Wako Pure Chemical Industries, Osaka). The column was sequentially eluted with 15 ml of distilled water, and 30 ml each of 20% and 80% aqueous ethanol, 100% ethanol, and 5% and 25% aqueous pyridine at a flow rate of 25 ml/hr. The activity was recovered in the 25% pyridine fraction (Nagasawa et al., 1988). After lyophilizing, this active fraction was dissolved in 0.1 M Tris-HCl buffer (pH 7.8, 10 ml) and injected into decapitated females, as the hormone source.

Synthetic PBAN-I of B. mori. PBAN-I, a carboxyl-terminally amidated peptide with 33 amino acid residues, was assembled by the solid-phase method and then the three methionine residues present were oxidized with H$_2$O$_2$ (Kitamura et al., 1989).

^{14}*C-Hexadecanoic acid and radioassay.* [16-^{14}C]Hexadecanoic acid (54 mCi/mmol) and [1-^{14}C]hexadecanoic acid (57 mCi/mmol), supplied by CEA (France), were dissolved in dimethyl sulfoxide (DMSO) to a concentration of *ca.* 50,000 dpm/μl. The latter radioactive acid was utilized only for ozonolysis to identify the double bond position of an unsaturated acyl derivative converted from this acid. The radioactivity of each fraction recovered from HPLC was quantified by liquid scintillation counting (LSC) as described previously (Ando et al., 1988 a).

HPLC. HPLC was performed by a Shimadzu LC-6A system equipped with a UV spectrometric detector (SPD-6A). Bombykol (*Rt* 11.2 min) was quantitatively analyzed at 235 nm, from a Nucleosil NO$_2$ column (5 μm, 8 mm i.d. × 15 cm; packed by Senshu-kagaku, Tokyo) with a solvent (*n*-hexane: 2-propanol=98: 2) at a 1.5 ml/min flow rate. For separation of fatty alcohols, a Senshu-gel ODS column (5 μm, 8 mm i.d. × 15 cm; Senshu-kagaku) was used with a solvent (methanol: water=93: 7) at a 1.5 ml/min flow rate. The *Rt*s of hexadecan-1-ol, (*Z*)-11-hexadecen-1-ol and

bombykol were 16.8, 11.3 and 9.3 min, respectively. The chromatographic behavior of the former two fatty alcohols was estimated with the synthetic ^{14}C-labeled standards (ANDO et al., 1988 a). Phenacyl esters of fatty acids were analyzed at UV 254 nm on an Inertsil ODS column (5 μm, 4.6 mm i.d.×25 cm; Gasukuro-kogyo Inc., Tokyo). A mixture of acetonitrile and water was served as the eluent and the ratio of the mixture was programmed from 87: 13 to 100: 0 in composition (see Fig. 4) at a flow rate of 2.0 ml/min. Ozonolysis products of an unsaturated fatty acid ester were analyzed at UV 254 nm on a Develosil ODS column (5 μm, 8 mm i.d.×15 cm; Nomura Chem. Co., Osaka) with a solvent (methanol: water=70: 30) at a 2.0 ml/min flow rate.

Decapitation of female moths and pheromone titers. Adults of *B. mori* usually emerged from the pupal stage 1–2 hr after lighting, under our rearing conditions. The virgin females were decapitated with scissors 4, 7, 12, 24, 26, 28, 30 or 31 hr after initiation of the first photophase, and the injury was coated with paraffin. For each experiment three moths were prepared. Pheromone glands were excised from females decapitated 7 hr after lighting in the second photophase and each immersed separately in ethyl acetate for 30 min. The pheromone content in each gland was quantitatively analyzed by normal phase HPLC.

Injection of PBAN into decapitated females. Virgin females were decapitated 4 hr after lighting in the first photophase and injected with 10-head equivalents of natural PBAN (20 μl) 24 hr after the decapitation (4 hr after lighting in the second photophase). Injection was between the 3rd thoracic and the 1st abdominal sternites using a 100-μl microsyringe, and the site of injection was coated with paraffin. Maximum stimulation of pheromone biosynthesis was achieved by this amount of the hormone extract (ANDO et al., 1988 c).

Timing of ^{14}C-application and excising pheromone glands (Fig. 1). The highest level of ^{14}C-incorporation into the pheromone was achieved in a few hours after application of the ^{14}C-acid (ANDO et al., 1988 a) and pheromone biosynthesis was sufficiently activated, also in a few hours, after injection of PBAN (ANDO et al., 1988 c). Therefore, a DMSO solution of [16-^{14}C]hexadecanoic acid (1 μl, *ca.* 50,000 dpm) was applied topically to a pheromone gland 1.5 hr after the injection of natural PBAN (5.5 hr after lighting in the second photophase), above. A further 1.5 hr after the ^{14}C-application (7 hr after the lighting) the gland was excised and the radiolabeled components were analyzed (experiment C). To clarify the effect of PBAN injection, the ^{14}C-acid was applied to a normal female (experiment A) and a decapitated female without hormone injection
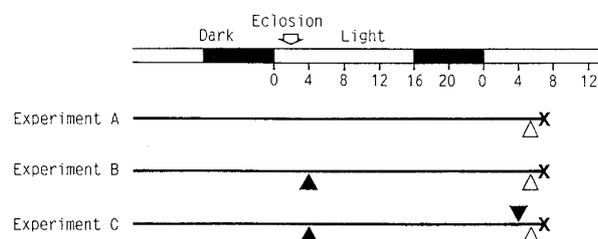

Fig. 1. Time schedule for the preparation of normal (A), decapitated (B), and decapitated and PBAN-injected females (C), and for ^{14}C-incorporation experiments with these insects. Decapitation (▲) was carried out 4 hr after lighting in the first photophase. Hormone injection (▼), ^{14}C-application (△) and extraction of the pheromone gland (×) were carried out 4, 5.5 and 7 hr after lighting in the second photophase.

(experiment B) at the same time.

Lipid extraction and analysis. Three groups of twenty glands were prepared in each of the above three experiments (A, B and C). The glands of each group were homogenized in a mixed solvent (chloroform: methanol=1: 2, 2 ml) using a Potter Elvehjem glass tissue grinder and Teflon pestle. The homogenate was centrifuged, and the precipitate was resuspended in another solvent (chloroform: methanol: water=1: 2: 0.8, 2 ml). After recentrifugation, both supernatants were combined then chloroform (1.3 ml) and water (1.3 ml) were added. The mixture was shaken thoroughly, then the chloroform layer [total lipid fraction, (Kates, 1972)] was separated from the aqueous layer by centrifugation. Chloroform was evaporated and the residue was subjected to saponification with 1 N NaOH in 80% aqueous ethanol (3 ml) at room temperature for 3 hr. The reaction mixture was acidified with 3 N HCl, and extracted with ethyl acetate (3 ml) four times. The ethyl acetate extract, which contained fatty acids and fatty alcohols, was developed on a TLC plate (silica gel $60F_{254}$, 0.25 mm thick; Merck, Germany; *n*-hexane: tetrahydrofuran: acetic acid=90: 10: 2 as solvent). The plate was autoradiographed, and then the positions corresponding to the radiolabeled alcohols (*Rf* 0.14) and acids (*Rf* 0.37) were scraped from the plate and extracted with ethyl acetate. Fatty alcohols were directly analyzed by HPLC with a reversed phase column as described previously (Ando et al., 1988 a) whereas fatty acids were first converted into the corresponding phenacyl esters (Borch, 1975). Thus, the fatty acids were reacted with phenacyl bromide (0.36 ml of an acetone solution containing 4.3 mg phenacyl bromide) and triethylamine (0.36 ml of an acetone solution containing 3.6 mg triethylamine) overnight at room temperature. Aliquots of the reaction products were run on HPLC with a reversed phase column. The eluates from the column were collected every 0.5 min during four periods (11–14, 15–18, 23–26, and 30–31 min), and every 1 min for the other periods until 35 min. The radioactivity in each fraction was measured by LSC. Authentic phenacyl esters of hexadecanoic acid, (*Z*)-11-hexadecenoic acid and (10*E*,12*Z*)-10,12-hexadecadienoic acid were recovered in fractions collected at 23.5–24.5, 16.5–17.5 and 12–13 min, respectively (see Fig. 4).

Ozonolysis of an unsaturated fatty acid ester. Thirty normal females were treated with [1-^{14}C]hexadecanoic acid in the same manner as experiment A, and the phenacyl esters of the radio-labeled acids were prepared. Among them, the phenacyl ester of a monounsaturated acid, which eluted from the above HPLC column during 16.5–17.5 min, was purified and utilized to the following ozonolysis experiment. The ester was dissolved in dichloromethane (1 ml) containing a trace of pyridine (20 μl), the solution cooled to −70°C and a stream of ozone was bubbled in for 5 min. Excess ozone was dispelled with a stream of nitrogen, dimethyl sulfide (200 μl) added and the solution stirred for 15 min at −70°C and 1 hr at room temperature. This solution was subjected to HPLC analysis with a reversed phase column. Fractions at 4–11 min were collected every 0.5 min and the radioactivity profile determined by LSC. The HPLC *Rt*s of the aldehydes from ozonolysis of the phenacyl esters of synthetic (*Z*)-9-, (*Z*)-10-, (*Z*)-11-, and (*Z*)-12-hexadecenoic acids were as follows; 9-oxononanoate (*Rt* 5.1 min), 10-oxodecanoate (*Rt* 6.5 min), 11-oxoundecanoate (*Rt* 7.8 min) and 12-oxododecanoate (*Rt* 9.5 min).

Incubation of pheromone glands and pheromone titers. The 0-day-old virgin females were decapitated 4 hr after lighting. The terminal abdominal segments were excised from the abdomens 24 hr after decapitation, washed in insect Ringer's solution, wiped

with filter paper, then incubated in Grace's medium (80 μl) with synthetic PBAN-I solution [0, 20 or 40 ng in 0.1 M Tris-HCl buffer (pH 7.8, 20 μl)] at 25°C for 3 hr with continuous shaking. Two glands each were used per incubation and the experiment repeated in triplicates. The glands and medium were extracted with n-hexane (250 μl) three times, and the pheromone content was measured by normal phase HPLC. The incubation was repeated with the pheromone glands excised from 1-day-old intact females 4 hr after lighting, and their pheromone titer was obtained by the same manner.

RESULTS

Bombykol titer of decapitated females

The just-emerged female possesses bombykol, and the amount present fluctuates thereafter depending on the light-dark cycle (ANDO et al., 1988 a). The pheromone is more abundant in the photophase. A large increase in the pheromone titer is observed 4–7 hr after lighting in 1-day-old virgin females, indicating that the pheromone bio-synthesis may be activated by PBAN most easily in this period. Figure 2 shows phero-mone titers of some decapitated 1-day-old females. They were decapitated at various times and the pheromone was extracted 7 hr after the initiation of the second photo-phase. Pheromone content quickly decreased after decapitation, but almost one day was necessary for its complete disappearance from the gland. To examine the ac-celeration of pheromone biosynthesis by PBAN, the hormone should be injected into the female which no longer contains the pheromone. Therefore, we decided to de-capitate the 0-day-old females 4 hr after lighting, and to inject the hormone 24 hr after the decapitation.

Effect of decapitation and PBAN injection on conversion of ^{14}C-hexadecanoic acid into fatty alcohols

Analysis of the alcohol fraction from the pheromone gland treated with ^{14}C-hexadecanoic acid indicated that ^{14}C-label was incorporated into hexadecan-1-ol (16: 0_{OH}), (Z)-11-hexadecen-1-ol (16: 1_{OH}), and bombykol (16: 2_{OH}) (ANDO et al., 1988 a). Figure 3 shows the ^{14}C-incorporation into the total alcohols and distribution to the three
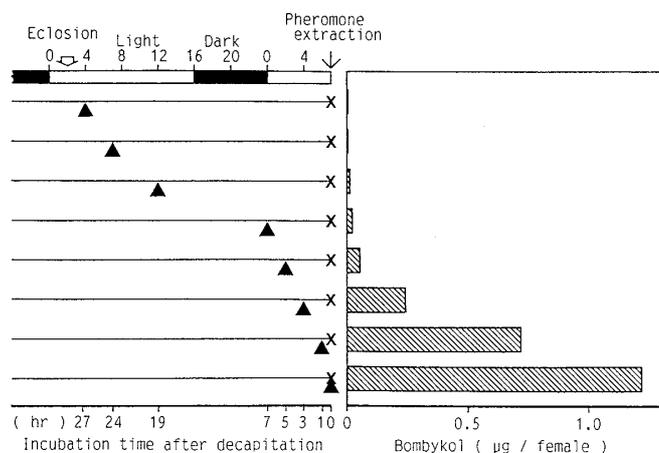

Fig. 2. Bombykol titer of *Bombyx mori* decapitated at various times. The pheromone was extracted 0, 1, 3, 5, 7, 19, 24 or 27 hr after decapitation (▲) of the virgin females. Each extraction (×) was carried out 7 hr after lighting in the second photophase.
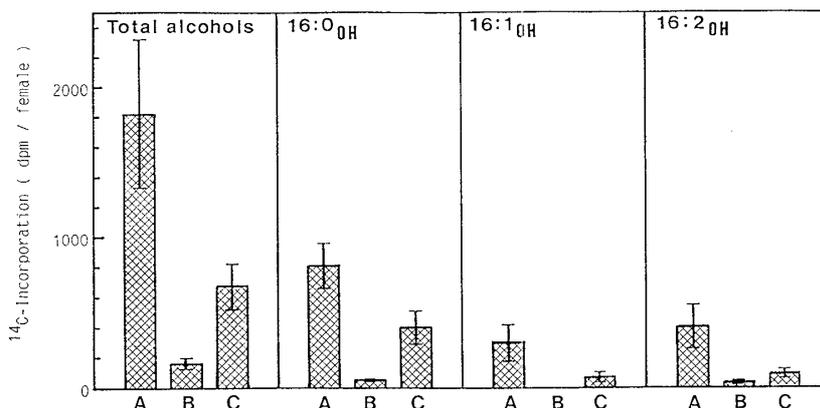
Fig. 3. ^{14}C-Incorporation into fatty alcohols [total alcohols, hexadecan-1-ol (16:0$_{OH}$), (Z)-11-hexadecen-1-ol (16:1$_{OH}$), bombykol (16:2$_{OH}$)] in the sex pheromone glands of normal (A), decapitated (B), and decapitated and PBAN-injected females (C). Each 1-day-old female was treated with [16-^{14}C]hexadecanoic acid (50,000 dpm) 5.5 hr after lighting and the gland was excised 1.5 hr after the ^{14}C-application.

components in normal (A), decapitated (B), and decapitated and PBAN-injected females (C). The incorporation is represented by dpm per female. About 0.8% radioactivity of the ^{14}C-acid treated at the gland was incorporated into bombykol in the normal female. Furthermore 1.8 and 0.6% of ^{14}C-incorporation was observed in the saturated and monounsaturated alcohols, respectively. In the decapitated females (B), bombykol was scarcely detected (<5 ng/female) and the ^{14}C-incorporation into each alcohol fraction was remarkably diminished. When the decapitated female was injected with PBAN (C), the pheromone was biosynthesized again (ca. 200 ng/female) and the levels of ^{14}C-incorporation relative to the normal female showed some recovery. Although the recovery shown in the unsaturated alcohols is much smaller than that in the saturated alcohol, the ^{14}C-incorporation into the unsaturated alcohols was certainly stimulated by the PBAN injection.

Desaturation of ^{14}C-hexadecanoic acid in the pheromone gland

Phenacyl derivatives of fatty acids in the gland of the normal female were separated on a reversed phase HPLC column as shown in Fig. 4 (II). The *R*ts of peaks 1-8 were identical with those of the phenacyl derivatives from authentic (10*E*,12*Z*)-10,12-hexadecadienoic acid (16:2), (9*Z*,12*Z*,15*Z*)-9,12,15-octadecatrienoic acid (18:3), tetradecanoic acid (14:0), (*Z*)-11-hexadecenoic acid (16:1), (9*Z*,12*Z*)-9,12-octadecadienoic acid (18:2), hexadecanoic acid (16:0), (*Z*)-9-octadecenoic acid (18:1) and octadecanoic acid (18:0), respectively [see Figs. 4 (I) and (II)]. This acyl composition as analyzed by the HPLC technique showed good coincidence with that obtained by GC measurement (BJOSTAD and ROELOFS, 1984). Figure 4 (III) shows that radioactivity was primarily incorporated into the 16:0 and 16:1 compounds and little into 16:2. These appeared as peaks 6, 4 and 1, respectively. Radiolabeled peak 6 arises from unincorporated free ^{14}C-hexadecanoic acid (ca. 65%) and from direct incorporation of the ^{14}C-acid into lipids (ca. 35%). The latter two peaks, 4 and 1, arise from desaturation of the ^{14}C-acid. Ozonolysis followed by reductive cleavage of the ^{14}C-labeled 16:1 phenacyl ester produced only a radioactive ester of 11-oxoun-
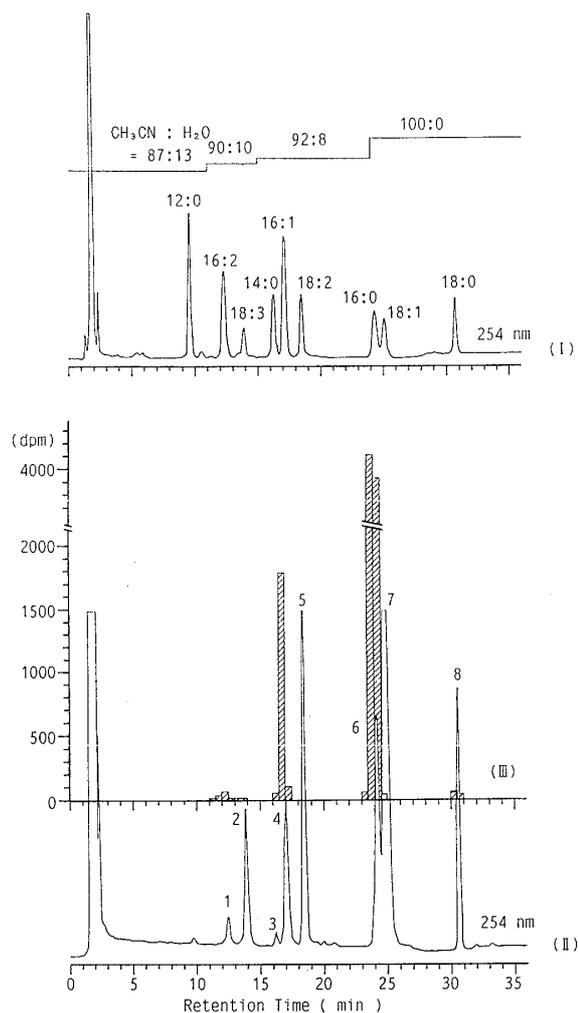
Fig. 4. HPLC-elution profiles of authentic fatty acids (I) and of the acids in a pheromone gland of the normal female treated ^{14}C-hexadecanoic acid (II), and ^{14}C-incorporation into the acids in the gland (III). Each fatty acid was converted to its phenacyl ester for analysis on an ODS column at UV 254 nm.

decanoic acid, indicating the double bond to be at 11-position in the starting material. Aldehydes of other chain lengths were not detected. While the level of ^{14}C-label incorporated into the 16: 1 acyl moiety was much greater than that into the 16: 2 acyl moiety, the ^{14}C-label incorporated into the 16: 1$_{OH}$ was nearly equal to that into the 16: 2$_{OH}$ (see Figs. 3 and 5). This result suggests that the 16: 1 acyl compound was more easily accumulated in the glands than the 16: 2 acyl compound.

Effect of decapitation and PBAN injection on the desaturation

Figure 5 shows the effect of decapitation and PBAN-injection on the amount of C_{16}-fatty acyl moieties in the pheromone gland and on the ^{14}C-incorporation. Approximately equal amounts of the 16: 0 or 16: 1 acyl moiety were observed in the females in the three experiments A, B and C, and the level of ^{14}C-incorporation was also similar. Interestingly, while the 16: 2 acyl moiety was more abundant in a decapitated female

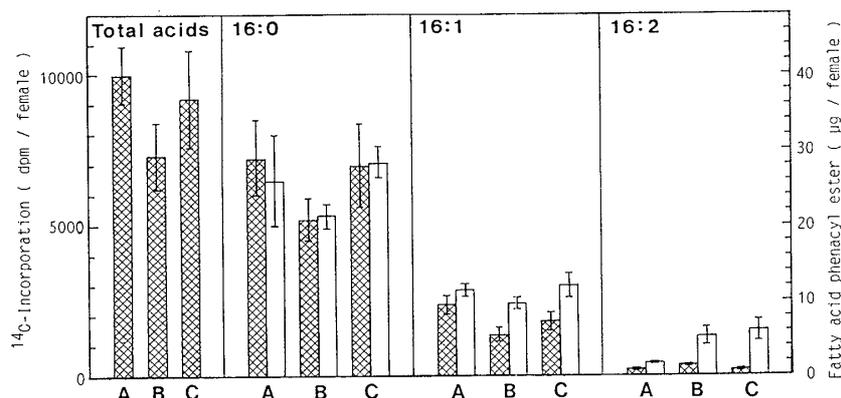144   R. Arima et al.

Fig. 5. Amount of fatty acyl moieties (white histograms) [total acyl moieties, hexadecanoate (16: 0), (Z)-11-hexadecenoate (16: 1), hexadecadienoate (16: 2)] and the levels of ^{14}C-incorporation (cross-striped histograms) into these moieties in the sex pheromone glands of normal (A), decapitated (B), and decapitated and PBAN-injected females (C). Each 1-day-old female was treated [16-^{14}C]hexadecanoic acid (50,000 dpm). These acyl moieties were analyzed as phenacyl esters.

Table 1. Bombykol titer after *in vitro* incubation of pheromone glands with or without synthetic PBAN-I

	Bombykol (ng/gland)[a]	
	Decapitated	Normal
Buffer[b]	0.0 ± 0.0	19.6 ± 7.2
PBAN-I[c] (20 ng)	22.0 ± 6.8	76.5 ± 21.1
(40 ng)	20.1 ± 4.3	—

[a] Values are means$\pm$SE based on four replicates of two glands.

[b] Incubated in Grace's medium (80 μl) with 0.1 m Tris-HCl buffer (20 μl) for 3 hr at 25°C.

[c] Incubated in Grace's medium (80 μl) with synthetic PBAN-I (20 or 40 ng) in 0.1 m Tris-HCl buffer (20 μl) for 3 hr at 25°C.

(B) and in a decapitated and PBAN-injected female (C) than in a normal female (A), the ^{14}C-incorporation in the three experiments was small and almost the same.

Bombykol biosynthesis in pheromone gland organ culture with PBAN

The pheromone glands were excised from normal and decapitated females and incubated in Grace's medium in the absence and presence of synthetic PBAN-I for 3 hr. Table 1 shows the results. Bombykol was not detected in the pheromone gland of the decapitated female incubated without PBAN, but the pheromone was produced in the gland incubated with the hormone. Two different doses (20 and 40 ng) of the hormone induced similar amounts of bombykol, indicating their maximum activation. The pheromone gland of the intact female incubated without PBAN possessed about 20 ng of bombykol. When this gland was incubated with the hormone, pheromone content increased about four-fold.

DISCUSSION

Lepidopterous sex pheromones are mainly C_{12}–C_{16} straight chain alcohols, acetates and aldehydes with one or two double bonds. Biosynthesis studies in several species demonstrates a common pathway including the desaturation of a saturated fatty acyl intermediate before the formation of its terminal functional group (BJOSTAD et al., 1987). In *B. mori* females one probable biosynthetic pathway for the sex pheromone is as follows; acetyl CoA → hexadecanoate (16: 0) → (Z)-11-hexadecenoate (16: 1) → (10E,12Z)-10,12-hexadecadienoate (16: 2) → bombykol (16: 2_{OH}) (YAMAOKA et al., 1984; ANDO et al., 1988 b). Bombykol disappears 24 hr after decapitation, and incorporation of radioactivity into the bombykol fraction from ^{14}C-hexadecanoic acid is barely observed. When the biosynthesis is stimulated by PBAN injection, the decapitated female reproduces the pheromone, as indicated by incorporation of the ^{14}C-label. On the other hand, the amount of the 16: 1 acyl moiety and its ^{14}C-incorporation is not decreased substantially by decapitation. Rather, the 16: 2 acyl moiety increases. These results strongly suggest that PBAN regulates reduction of the unsaturated acyl moiety rather than the desaturation steps.

In addition to bombykol (16: 2_{OH}), other fatty alcohols (16: 0_{OH} and 16: 1_{OH}) are also distributed in the pheromone gland. These are not direct precursors for bombykol biosynthesis but incorporate radioactivity from ^{14}C-hexadecanoic acid administered to the gland. It has been suggested that this ^{14}C-incorporation may be caused by low substrate selectivity of the reductase involved in the pheromone biosynthesis (ANDO et al., 1988 b). Decapitation and PBAN-injection also affected on the ^{14}C-incorporation into 16: 0_{OH} and 16: 1_{OH}. Incorporation is very low in the decapitated female but recovered on PBAN injection. This result provides another proof for the regulation site of PBAN, if we assume that the enzyme reduces three C_{16}-fatty acyl moieties (16: 0, 16: 1 and 16: 2) to the corresponding three C_{16}-fatty alcohols unselectively.

The precise forms of acyl precursors relative to the desaturation steps, are not known, even now. We analyzed the phenacyl esters of fatty acids obtained from the total lipids in the pheromone gland after the hydrolysis and derivatization with phenacyl bromide. Although the total lipids include fatty acids which are not concerned with the pheromone biosynthesis, the ozonolysis experiments revealed that the ^{14}C-labeled 16: 1 acyl ester had a double bond only at the characteristic 11-position. This monoene acid was detected only in the pheromone gland as an important precursor of the biosynthesis (YAMAOKA and HAYASHIYA, 1982). The 16: 2 acyl moiety is a characteristic component of the lipids from *B. mori* female (BJOSTAD and ROELOFS, 1984). By analyzing the 16: 1 and 16: 2 acyl esters with ^{14}C-label, we could speculate whether the desaturation steps were regulated by PBAN or not.

The mode of action of PBAN has been already investigated with some other lepidopterous females. BESTMANN et al. (1989), using $^2H_9$-hexadecanoic and $^2H_9$-(Z)-11-hexadecenoic acids, reported that PBAN regulated a desaturation step of the pheromone biosynthesis in the cabbage armyworm moth (*Mamestra brassicae*). Experiments with the tomato looper moth (*Chrysodeixis chalcites*) by ALTSTEIN et al. (1989) supported this suggestion. However, in contrast, TANG et al. (1989), using ^{14}C-acetate and $^2H_3$- hexadecanoic acid, demonstrated that in the redbanded leafroller moth (*Argyrotaenia velutinana*) PBAN regulated the biosynthesis by activating synthesis of saturated

fatty acyl intermediates. From our experiments using *B. mori* we conclude that another mechanism for the regulation by PBAN is possible. Notwithstanding this, because the sex pheromones of these females are similar unsaturated aliphatic compounds, the hormone might be expected to activate a similar step in their biosynthesis.

Recently, Teal et al. (1989) showed that brain-suboesophageal ganglion extract did not induce biosynthesis of *H. zea in vitro* when it was applied directly to the excised terminal abdominal segments possessing the pheromone glands, and concluded that the target site of PBAN was not the pheromone gland but the terminal abdominal ganglion. In our experiment with the terminal abdominal segments of *B. mori*, however, pheromone production *in vitro* was successfully induced by the synthetic PBAN-I, and Yamamoto (1990) also obtained the similar result. Soroker and Rafaeli (1989) also reported pheromone production in the incubated gland of the cotton bollworm moth (*Heliothis armigera*) in the presence of PBAN extract. This *in vitro* incubation reveals that, at least in *B. mori* and *H. armigera*, the target organ of PBAN is the pheromone gland.

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