**Identification of a Prothoracicostatic Peptide in the Larval Brain of the Silkworm, Bombyx mori**

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Prothoracicotropic hormone (PTTH) stimulates ecdysteroid biosynthesis in the prothoracic gland (PG) of insects. A peptide inhibiting ecdysteroid biosynthesis in the PG was isolated from the extracts of 2,000 larval brains of the silkworm, Bombyx mori, using a protocol that included four reversed-phase high performance liquid chromatography procedures. The primary structure of this prothoracicostatic peptide (Bom-PTSP) was determined to be H-Ala-Trp-Gln-Asp-Leu-Asn-Ser-Ala-Trp-NH₂. This neuropeptide has the same sequence as Mas-MIP-I, a myoinhibitory peptide previously isolated from the ventral nerve cord of the tobacco hornworm, Manduca sexta, and is highly homologous with the N-terminal portion of vertebrate peptides of the galanin family. This peptide inhibited PTTH-stimulated ecdysteroidogenesis in the PG at both the spinning and feeding stages, which indicates that Bom-PTSP interferes with PTTH-stimulated ecdysteroidogenesis.

Ecdysone is a steroid molecule synthesized by the ecdysial glands (the prothoracic, ventral, and rings glands and Y organs) and gonads of insects and crustaceans. In particular, ecdysone secreted by the prothoracic gland (PG) plays a central role in insect metamorphosis as a molting hormone (1). Since Köpec (2) suggested that the larval brain of the gypsy moth, Lymantria dispar, secretes a factor necessary for molting, attempts have been made to purify and clone this insect “brain hormone,” which was later named prothoracicotropic hormone (PTTH). It is now widely recognized that PTTH stimulates ecdysteroid biosynthesis in the PG (3).

Similar to the regulation of juvenile hormone synthesis in the corpora allata by allatotropic and allatostatin (4, 5), ecdysteroidogenesis in the PG appears to be regulated by both prothoracicotropin and prothoracostatin. Factors that inhibit ecdysteroidogenesis have been described in several insect species. Carlisle and Ellis (6) demonstrated the hormonal inhibition on the PG by the brain of locusts. Neb-TMOF, a trypsin modulating oostatic factor isolated from the ovary of the flesh fly, Neobelliteria bullata, inhibits ecdysteroid biosynthesis in the larval ring gland of the blowfly, Calliphora vicina (7, 8). Inhibitory activity toward ecdysteroidogenesis was also detected in larval extracts of C. vicina (8, 9). Injection of crude extracts from the housefly, Musca domestica, into the mosquito, Aedes aegypti, reduced ecdysteroid synthesis and inhibited egg development (10, 11). Grb-AST, a group of allatostatins isolated from the adult brain of the cricket, Gryllus bimaculatus, decreased the ecdysteroid titer in vivo (12). Other than the molt-inhibiting hormones of crustaceans (13), a neuropeptide with prothoracicostatic activity has not yet been identified in insects. In this paper, we report the results of purification and sequence analysis of a prothoracicostatic peptide (PTSP) from the larval brain of the silkworm, Bombyx mori, and its physiological functions.

**EXPERIMENTAL PROCEDURES**

**Experimental Insects**

A racial hybrid, C145 × N140, of B. mori was used. Larvae were reared at 25 ± 1 °C under a photoperiodic regime of 12 h of light and 12 h of darkness on a standard artificial diet (Nippon Nosan Co. Ltd., Yokohama, Japan). Larvae were selected by age.

**In Vitro Bioassay for Determination of the Presence and Level of Prothoracicostatic Activity**

A sample was tested for the presence of prothoracicostatic activity by testing whether it inhibited ecdysteroid biosynthesis in the PG of larvae in an in vitro assay combined with ecdysteroid RIA. The bioassay was a modification of the method of Bollenbacher and Granger (14). Briefly, the left and right PGs of a fifth instar B. mori larva were dissected and placed in Ringer’s solution (0.75% NaCl, w/v). The left and right PGs were precultured separately in 20 μl of Grace’s insect medium (Sigma-Aldrich) in a covered microtiter plate well at 26 °C for 30 min. The test sample was then added to the medium of the left PG. The left PG (“experimental” condition) and right PG (“control” condition) were further incubated in the covered microtiter plate wells at 28 °C for 3 h, at which time methanol was added to stop the ecdysone synthesis. The left and right PGs were removed from their respective media, and each medium was taken and lyophilized. The amount of ecdysteroids secreted by the PG into the medium was determined by ecdysteroid RIA as described below. An inhibition ratio was calculated for the left and right PGs of each larva with the formula, (amount of ecdysteroid in experimental/amount of ecdysteroid in control) × 100. Student’s t test was used to evaluate the significance of the difference in the biological response to the treatment. A p value of <0.05 was considered to be statistically significant.

**Ecdysteroid RIA**

The ecdysteroid RIA was performed as described by Takeda et al. (15). The cross-reactivity ratio between ecdyson and 20-hydroxyecdysone is 1:2.6 (15). The results are expressed as ecdysone equivalents.
Prothoracicostatic Peptide from B. mori Brain

Larval Brain Extraction

Approximately 2,000 brains (100 brains/batch) were dissected from B. mori larvae on day 2 or 3 of the fifth instar and stored in a precooled 90:9:1 mixture of methanol/water/acetic acid. The brains were homogenized with an ultrasonicator at room temperature and then centrifuged at 10,000 × g at 4 °C for 10 min. The pellet was re-extracted twice. The methanol in the supernatant was allowed to evaporate, and the remaining aqueous residue was re-extracted with the same volume of ethyl acetate and n-hexane as the aqueous residue, to remove the bulk of the lipids. After the organic solvent layer was decanted, the aqueous solution was diluted 5-fold with aqueous 0.1% trifluoroacetic acid (TFA). Diluted solution containing roughly 500 brains was loaded onto a C18 Sep-Pak cartridge (Waters, Milford, MA), which had previously been treated with acetonitrile/TFA and which had afterward been rinsed with aqueous 0.1% TFA. The cartridge was then eluted with 5 ml of each of water and 13, 35, and 60% acetonitrile in 0.1% TFA. Prothoracicostatic activity was detected in the 35% acetonitrile fraction. Four of these Sep-Pak separations were required to process all of the brain material.

Purification of B. mori Prothoracicostatic Peptide (Bom-PTSP) from the Brain Extracts

We isolated the prothoracicostatic peptide from the 35% acetonitrile fraction using a protocol which included four reversed-phase high performance liquid chromatography (RP-HPLC) procedures using a Hitachi HPLC L-7100 instrument (Hitachi, Hitachi City, Japan). In subsequent steps, an increasing number of brain equivalents (10, 15, 20, and 40 brain equivalents per PG in steps 1, 2, 3, and 4, respectively) was used in the assays due to loss of material during purification.

Step 1: Pegasil-300 Octadecylsilyl Column—Pegasil-300 octadecylsilyl column (4.6 × 250 mm, 5 μm, Senshu Kagaku Co. Ltd., Tokyo, Japan) was used. The 35% acetonitrile Sep-Pak fraction (2,000 brain equivalents) was evaporated to reduce the concentration of acetonitrile, diluted 5-fold with aqueous 0.1% TFA and then loaded onto the Pegasil-300 column through a pump. The column was eluted with a linear gradient of 0–50% acetonitrile in aqueous 0.1% TFA over 50 min at a flow rate of 1 ml/min. Fractions were collected at 1-min intervals and subjected to the in vitro bioassay. Prothoracicostatic activity was detected in Fraction 11.

Step 2: Vydac C4 Column—Vydac C4 column (4.6 × 250 mm, 5 μm, The Separations Group, Inc., Hesperia, CA) was used. Pooled Fraction 29 from the first HPLC separation was dried to a small volume, dissolved in 500 μl of 0.1% TFA in water, and loaded onto the second column. The column was eluted with 15% acetonitrile for 5 min, followed by a gradient of 15–30% acetonitrile in 0.1% TFA over the next 45 min at a flow rate of 1 ml/min. Fractions were collected at 1-min intervals, and each fraction was subjected to the in vitro bioassay. Prothoracicostatic activity was consistently detected in Fraction 29, and this fraction was used for further purification.

Step 3: Vydac C18 Column—Vydac C18 column (4.6 × 250 mm, 5 μm, The Separations Group, Inc.) was used. Fraction 11 from the second HPLC separation was dried to a small volume, dissolved in 500 μl of 0.1% TFA in water, and loaded onto the third column. The column was eluted in a gradient of 15–30% acetonitrile in aqueous 0.1% TFA over 45 min at a flow rate of 1 ml/min. Fractions were collected manually and assayed for prothoracicostatic activity.

Step 4: Vydac C4 Column—Vydac C4 column (4.6 × 250 mm, 5 μm) was used. Prothoracicostatic material from the third step (the fraction between 24.6 and 25.8 min) was dried to a small volume, dissolved in 500 μl of 0.1% TFA in water and loaded onto the C18 column. The column was eluted in a gradient of 10–20% acetonitrile in aqueous 0.1% TFA over 40 min at a flow rate of 1 ml/min. Individual peaks were collected manually and assayed for prothoracicostatic activity.

Molecular Mass and Amino Acid Sequence Analysis—The molecular mass of the purified peptide was determined by matrix-assisted laser-desorption ionization mass spectrometry (Voyager Biospectrometry Workstations, PerSeptive Biosystems, Inc., Framingham, MA). The acceleration voltage was set to 11.25 kV in the linear mode. A saturated solution of α-cyano-4-hydroxycinnamic acid in water/acetonitrile (6:4) was used as the matrix. The spectrum was the average of 256 single-shot spectra.

Results

Peptide Synthesis

A peptide of the C-terminal amide of Bom-PTSP was synthesized on a 9050 Plus Pepsynthesizer System (PerSeptive Biosystems, Inc.) using Fmoc (N-(9-fluorenyl)methoxycarbonyl) chemistry according to the manufacturer’s instructions. The crude synthetic peptide was purified by HPLC to a purity exceeding 95%. One of the pair of PGs of a larva was incubated in medium containing various concentrations of the synthetic Bom-PTSP peptide, whereas the other PG was incubated in medium that lacked Bom-PTSP to study the inhibitory effect of Bom-PTSP on edysteroidogenesis.

Recombinant PTTH Preparation

Recombinant Bombyx PTTH was expressed in Escherichia coli and purified by the method described by Ishibashi et al. (16), which involves two ion exchange chromatography steps followed by reversed-phase HPLC. One of the pair of PGs of a larva was incubated in medium containing various concentrations of PTTH and synthetic Bom-PTSP peptide, whereas the other PG was incubated in medium that contained neither PTTH nor Bom-PTSP to study the effect of combined PTTH and Bom-PTSP treatment on edysteroidogenesis.

Results

Purification of Bom-PTSP—Fractions that had been eluted with 35% acetonitrile from the C18 Sep-Pak cartridge showed strong prothoracicostatic activity in the in vitro bioassay, which was completely eliminated by Pronase treatment (data not shown); this indicated that the biological material is a peptide. Therefore, we combined these fractions and applied them to reversed-phase HPLC. In the first HPLC, prothoracicostatic activity was detected in Fraction 29 (28.4% acetonitrile) (Fig. 1A). The second and third HPLC runs each yielded one major peak with prothoracicostatic activity, in Fraction 11 (16.7% acetonitrile) and Fraction 26 (24.8 to 25.8 min, 23.4% acetonitrile), respectively (Fig. 1B and C). A single, apparently homogenous peak showing prothoracicostatic activity, which was eluted at 23.05 min with 35% acetonitrile, was manually collected from the final HPLC (Fig. 1D). This peptide with prothoracicostatic activity was named Bom-PTSP.

Molecular Mass and Amino Acid Sequence Analysis—The molecular mass of the purified peptide was determined by matrix-assisted laser-desorption ionization mass spectrometry. An m/z of 1089.78 (M+H)+ was obtained. Automated Edman degradation of the purified peptide in a protein sequencer showed that its primary structure is a nonapeptide with the...
sequence Ala-Trp-Gln-Asp-Leu-Aaa-Ser-Ala-Trp, which corresponds to a calculated m/z of 1089.50 ((M+H)+) for an amidated peptide. This agrees well with the mass analysis data. We calculated that the four-step purification protocol of the 2000 B. mori larval brains yielded approximately 200 pmol of pure Bom-PTSP.

**Dose Response of Synthetic Bom-PTSP**—A synthetic peptide of Bom-PTSP was prepared as a C-terminal amide and purified by HPLC. That the synthesized peptide is identical with the native peptide was confirmed by coelution with the native peptide on RP-HPLC, automated Edman degradation, and mass spectrometry. These results indicate that the amided peptide is the naturally occurring form of the peptide in B. mori larvae (data not shown).

Various concentrations of the synthetic peptide were subjected to the *in vitro* bioassay, and a dose response curve was constructed. As a quantitative measure of the level of prothoracostatic activity, the prothoracostatic effect of the peptide was expressed as an inhibition ratio, similar to the activation ratio used for *in vitro* PTTH measurement (14). Bom-PTSP inhibited ecdysteroid biosynthesis in the PG in a dose-dependent manner (Fig. 2). A concentration of $1 \times 10^{-11}$ M of Bom-PTSP significantly inhibited ecdysteroid biosynthesis in the PG of day 8 fifth instar larvae, 1 day after spinning. Maximal inhibition (56%) was achieved with $1 \times 10^{-9}$ M Bom-PTSP, and half-maximal inhibition (which differs from 50% inhibition because ecdysteroid biosynthesis in the PG could not be completely inhibited) was reached at a concentration of approximately $5 \times 10^{-9}$ M. The level of prothoracostatic activity of the synthetic peptide was equivalent to that of the purified natural product at each concentration as assessed by the *in vitro* bioassay, confirming that the synthetic and natural peptides are identical.

**Change in the Sensitivity of the PG to Bom-PTSP during the Fifth Instar**—The inhibitory effect of synthetic Bom-PTSP on ecdysteroid synthesis in PGs that were obtained from *B. mori* at different stages in the fifth instar (days 3, 5, 8, and 9), was studied. Activated PGs secreted a large amount of ecdysone and were more sensitive to Bom-PTSP than the PGs of larvae in the feeding stage. That is, Bom-PTSP at a concentration of $1 \times 10^{-9}$ and $1 \times 10^{-7}$ M significantly inhibited ecdysone biosynthesis in the PG of larvae in the spinning stage (days 8 and 9) (up to 70% inhibition), whereas Bom-PTSP at a concentration of $1 \times 10^{-7}$ M significantly inhibited ecdysone biosynthesis in the PG of larvae in the feeding stage (days 3 and 5) (Fig. 3).

**Reduction of PTTH-stimulated Ecdysteroidogenesis in PGs by Bom-PTSP**—The combined effect of synthetic Bom-PTSP and recombinant *Bombyx* PTTH on ecdysteroid synthesis in the PG was examined. PGs were dissected from day 3 fifth instar larvae. The PGs of a larva were preincubated in Grace's medium (days 3, 5, and 7) and were more sensitive to Bom-PTSP than the PGs of larvae in the feeding stage. That is, Bom-PTSP at a concentration of $1 \times 10^{-9}$ and $1 \times 10^{-7}$ M significantly inhibited ecdysone biosynthesis in the PG of larvae in the spinning stage (days 8 and 9) (up to 70% inhibition), whereas Bom-PTSP at a concentration of $1 \times 10^{-7}$ M significantly inhibited ecdysone biosynthesis in the PG of larvae in the feeding stage (days 3 and 5) (Fig. 3).

**Reduction of PTTH-stimulated Ecdysteroidogenesis in PGs by Bom-PTSP**—The combined effect of synthetic Bom-PTSP and recombinant *Bombyx* PTTH on ecdysteroid synthesis in the PG was examined. PGs were dissected from day 3 fifth instar larvae. The PGs of a larva were preincubated in Grace's medium for 30 min and then transferred to fresh medium containing Bom-PTSP ($1 \times 10^{-7}$ M) and/or PTTH ($1 \times 10^{-9}$ M) for further incubation for 3 h. PTTH significantly up-regulated ecdysone production in PGs ($1.16 \pm 0.41$ ng/PG for experimental; $0.05 \pm 0.02$ ng/PG for controls). Bom-PTSP did not significantly reduce ecdysone production when applied alone ($0.02 \pm 0.01$ ng/PG for experimental; $0.03 \pm 0.01$ ng/PG for controls). In the presence of both PTTH and Bom-PTSP, however, the level of ecdysone production in PGs was nearly the same as that in the controls ($0.05 \pm 0.03$ ng/PG for experimental; $0.04 \pm 0.01$ ng/PG for controls) (Fig. 4). These data indicate that Bom-PTSP and PTTH have opposing effects on ecdysteroidogenesis.

PGs obtained from day 3, 5, and 7 fifth instar larvae were treated with $1 \times 10^{-10}$ M PTTH and different concentrations of Bom-PTSP ($1 \times 10^{-10}$ and $1 \times 10^{-7}$ M), Bom-PTSP reduced PTTH-stimulated ecdysone biosynthesis in a dose-de-
Prothoracicostatic Peptide from *B. mori* Brain

**Table I**

Reduction of PTTH-stimulated ecdysteroidogenesis in PGs obtained from day 3 (V3), day 5 (V5), and day 7 (V7) fifth instar larvae by Bom-PTSP

The left and right PGs were dissected from day 3, 5, and 7 fifth instar larvae and preincubated in Grace’s insect medium for 30 min. One of the two PGs of a larva was transferred to fresh medium that contained either 10^{-2} M PTTH (except where indicated) and Bom-PTSP (0 M, or between 10^{-3} and 10^{-7} M) for a 3-h incubation (treatment group). The other PG of a larva was incubated in medium that contained neither PTTH nor Bom-PTSP (control group). The amount of ecdysteroids secreted by the PG into the incubation medium was determined by RIA. The activation ratio (Ar) of ecdysteroidogenesis of the PGs of a larva was calculated as [amount of ecdysteroid released by PG in treatment group]/[amount of ecdysteroid released by PG in control group]. Each value in the Treatment, Control, and Ar columns represents the mean ± S.D. (n = 5). The Ar of PGs that had been incubated in media that contained 10^{-10} M PTTH (except where indicated), and the indicated concentration of Bom-PTSP was significantly lower than the Ar of age-matched PGs that had been incubated in the presence of PTTH alone.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Concentration of 3cm-PTSP</th>
<th>Ecdysome/PG</th>
<th>Ar</th>
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<tr>
<td></td>
<td>µg</td>
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<tr>
<td>V3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.64 ± 0.14</td>
<td>0.14 ± 0.02</td>
<td>4.72 ± 1.23</td>
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<td>10^{-10}</td>
<td>0.23 ± 0.07</td>
<td>0.12 ± 0.02</td>
<td>1.58 ± 0.75</td>
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<td>10^{-9}</td>
<td>0.26 ± 0.06</td>
<td>0.13 ± 0.03</td>
<td>1.50 ± 0.71</td>
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<tr>
<td>10^{-8}</td>
<td>0.20 ± 0.07</td>
<td>0.11 ± 0.02</td>
<td>1.82 ± 0.62</td>
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<tr>
<td>10^{-7}</td>
<td>0.15 ± 0.06</td>
<td>0.13 ± 0.05</td>
<td>1.22 ± 0.31</td>
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<td>10^{-7} (without PTTH)</td>
<td>0.09 ± 0.02</td>
<td>0.12 ± 0.03</td>
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<td>V5</td>
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<td>15.31 ± 4.28</td>
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<td>10^{-10}</td>
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<td>8.82 ± 2.98</td>
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<tr>
<td>10^{-7} (without PTTH)</td>
<td>0.12 ± 0.05</td>
<td>0.19 ± 0.06</td>
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<td>V7</td>
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<td>4.05 ± 2.00</td>
<td>2.17 ± 0.42</td>
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<tr>
<td>10^{-7} (without PTTH)</td>
<td>0.99 ± 0.42</td>
<td>2.02 ± 0.52</td>
<td>0.48 ± 0.15</td>
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</table>

* p < 0.01.

**Table II**

Activation ratio of ecdysteriogenesis in the PG of *B. mori* day 5 fifth instar larvae treated with various concentrations of PTTH and Bom-PTSP

The left and right PGs of a larva were preincubated in Grace’s medium for 30 min and then transferred to fresh medium that contained the indicated concentrations of PTTH and Bom-PTSP for further 3-h incubation. The control condition was considered to be incubation in medium that contained neither PTTH nor Bom-PTSP. The amount of ecdysteroids secreted by the PG into the incubation medium was determined by RIA. Each value in the table represents the mean ± S.D. of the activation ratio (Ar), which was calculated as [amount of ecdysteroid released by PG in treatment group]/[amount of ecdysteroid released by PG in control group] for each larva (n = 5).

<table>
<thead>
<tr>
<th>PTTH</th>
<th>Concentration of 3cm-PTSP</th>
<th>Ecdysome/PG</th>
<th>Ar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg</td>
<td></td>
<td></td>
</tr>
<tr>
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<td></td>
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</tr>
<tr>
<td>10^{-7} M</td>
<td>3.31 ± 0.51</td>
<td>7.76 ± 2.02</td>
<td>12.64 ± 2.96</td>
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<tr>
<td>10^{-3} M</td>
<td>3.22 ± 0.48</td>
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<td>9.71 ± 1.52</td>
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<tr>
<td>10^{-1} M</td>
<td>2.62 ± 0.30</td>
<td>2.05 ± 0.36</td>
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<td>10^{-11} M</td>
<td>0.93 ± 0.38</td>
<td>0.91 ± 0.25</td>
<td>0.93 ± 0.38</td>
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* The activation ratio is significantly different from that of the respective control (without PTSP); p < 0.01.

**Discussion**

Bom-PTSP, a neuropeptide that inhibits ecdysteriogenesis in the PG of *B. mori*, was isolated from 2,000 *B. mori* larval brains using a protocol that included four RP-HPLC procedures. The results of a sequence homology search indicated that Bom-PTSP does not have sequence similarity with the molt-inhibiting hormones of crustaceans (13) nor with Neb-TMOR, a prothoracicostatic peptide that had been isolated from the ovary of the gray fleshfly, *N. bullata* (17). To our surprise, Bom-PTSP has the same amino acid sequence as Mas-MIP-I, a myoinhibitory peptide that was isolated from the nerve cord of the adult tobacco hornworm, *Manduca sexta* (18), and is highly homologous to Lom-MIP, the first insect peptide of the MIP family that was isolated from the ovary of the gray fleshfly, *Sarcophaga bullata* (19). To our knowledge, Bom-PTSP is identical with that of Mas-MIP-I, is similar to that of Grb-AST-B1, and shares sequence similarity with the highly conserved N terminus of vertebrate galanins (sequences according to Bersani et al. (22) and Tatemoto et al. (23)).
Mas-MIP I on ecdysteroidogenesis in digestive processes or excretion (18). However, the effect of Mas-MIP I on ecdysteroidogenesis in M. sexta has not been reported, and no additional information on the cellular localization of Mas-MIP I is available. Bom-PTSP also shows high sequence homology with a group of allatostatins, Grb-AST-B (Fig. 5). Grb-AST-B that were isolated from the brain of the adult cricket, G. bimaculatus, inhibit juvenile hormone biosynthesis (20). Grb-AST-B1 of G. bimaculatus has been shown to inhibit ovarian ecdysteroidogenesis in an in vitro study (21) and to decrease the ecdysteroid titer in an in vivo study (12); this evidence indicates that this peptide is multifunctional in G. bimaculatus. Bom-PTSP also shares sequence similarity with the highly conserved N-terminal region of galanins (Refs. 22 and 23 and Fig. 5), a family of vertebrate neuropeptides that has an inhibitory and hyperpolarizing action on numerous hormone-secreting cells (24). Galanin has been shown to inhibit corticosterone secretion after injection into the paraventricular nucleus (25). Our results strongly suggest that a galanin-like neuropeptide is involved in suppressing steroidogenesis in insects.

The physiological role that Bom-PTSP plays in controlling insect development is not yet clear. We demonstrated that Bom-PTSP inhibits ecdysteroidogenesis, and this suggests that this peptide plays a role in decreasing the ecdysteroid titer that is responsible for controlling insect development. PTTH significantly enhanced ecdysteroidogenesis in the PG of day 3 fifth instar larvae in vitro, whereas Bom-PTSP almost completely inhibited PTTH-stimulated ecdysteroid biosynthesis (Fig. 4). This suggests that Bom-PTSP may play a role in keeping the ecdysteroid titer low in larvae during the feeding stage. Bom-PTSP inhibited ecdysteroidogenesis in the PG of larvae in the spinning stage to a greater extent than in the PG of larvae in the feeding stage (Fig. 3), which suggests that Bom-PTSP plays a role in PTTH-stimulated ecdysteroidogenesis rather than in basal ecdysteroidogenesis. Therefore, this peptide may be involved in shutting down ecdysteroid biosynthesis.

The biosynthesis and release of ecdysone are mainly controlled by PTTH in insects (3) and by molt-inhibiting hormone in crustaceans (13). Our discovery of Bom-PTSP provides a new perspective on the regulation of the biosynthesis and release of ecdysteroids and raises an important question as to the functional relationship between Bom-PTSP and PTTH. Our results, however, strongly support our working hypothesis that both activating and inhibiting factors are involved in the regulation of the biosynthesis and release of ecdysteroids in insects. The integration of these opposing factors in stereoidogenic tissue leads to the complex secretory pattern observed in vivo.

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REFERENCES

Additions and Corrections


Identification of a prothoracicostatic peptide in the larval brain of the silkworm, Bombyx mori.

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Page 31172: Table II is incorrect. The title of the table should be “Activation ratio of ecdysteroidogenesis . . . .” The corrected table is shown below.

**TABLE II**

<table>
<thead>
<tr>
<th>PTTH Concentration</th>
<th>PTSP Concentration</th>
<th>Activation Ratio (Ar)</th>
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<tbody>
<tr>
<td>10⁻⁹ M</td>
<td>2.02 ± 1.85</td>
<td>4.32 ± 1.56</td>
</tr>
<tr>
<td>10⁻⁸ M</td>
<td>3.82 ± 1.30</td>
<td>2.02 ± 0.36</td>
</tr>
<tr>
<td>10⁻⁹ M</td>
<td>3.31 ± 0.51</td>
<td>7.76 ± 2.02</td>
</tr>
<tr>
<td>10⁻¹⁰ M</td>
<td>0.69 ± 0.25</td>
<td>2.03 ± 0.34</td>
</tr>
<tr>
<td>0 M (without PTSP)</td>
<td>0.36 ± 0.34</td>
<td>0.93 ± 0.38</td>
</tr>
</tbody>
</table>

The activation ratio is significantly different from that of the respective control (without PTSP); *p* < 0.01.

The activation ratio is significantly different from that of the respective control (without PTSP); *p* < 0.05.

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*Bombyx mori*

Yue-Jin Hua, Yoshiaki Tanaka, Keiji Nakamura, Mika Sakakibara, Shinji Nagata and Hiroshi Kataoka

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