Cyclic Nucleotides and Hormonal Control of Cuticular Melanization in the Armyworm Larva, *Leucania separata* (Lepidoptera: Noctuidae)

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Injection of cyclic AMP, dibutyryl cyclic AMP, cyclic GMP or dibutyryl cyclic GMP into isolated abdomens prepared from 5th instar armyworm larvae, *Leucania separata*, caused darkening in body color. Injection of melanization and reddish coloration hormone (MRCH) plus theophylline caused more intense melanization in isolated abdomen than did injection of MRCH. Injection of dopamine failed to cause melanization in cuticle. These findings imply that cyclic AMP may play a role as an intercellular messenger of MRCH.

INTRODUCTION

Cuticular melanization and reddish epidermal pigmentation in the armyworm larva, *Leucania separata*, are caused by the hormone(s) originating from the brain-corpora cardiaca-corpora allata complex and the suboesophageal ganglion, and the hormone(s) has been temporarily named as melanization and reddish coloration hormone (MRCH) (Ogura, 1975). From the viewpoint of some chemical natures, MRCH is thought to be peptidal and different from bursicon, one of the cuticular hardening and darkening hormones in insects (Matsumoto et al., in preparation). In the action of peptide and amine hormones of vertebrate, adenosine 3',5'-cyclic monophosphate (cAMP) is an intercellular messenger (see Butcher et al., 1972) and, up to now, the intermediary of this nucleotide has also been suggested in the action of several insect hormones; e.g. ecdysone (Applebaum and Gilbert, 1972), prothoracicotropic (brain) hormone (Vedeckis et al., 1976), eclosion hormone (Truman et al., 1976), bursicon (Vandenberg and Mills, 1974), diuretic hormone (Maddrell et al., 1971) and puparium tanning factor (Seligman et al., 1977). These situations promoted us to examine the effect of some nucleotides on cuticular melanization in *L. separata* as a preliminary study on the mode of action of MRCH.
MATERIALS AND METHODS

Bioassay of chemicals was made on isolated abdomens of 5th instar larvae reared under crowded condition because the integument of crowded larva was more likely to form melanine than that of solitary larva (Ogura, 1975). Method of injection and classification of melanization caused by injection have been described in previous papers (Ogura, 1975; Suzuki et al., 1976).

The biological activity of various nucleotides was investigated by use of adenosine 5'-monophosphate (5'-AMP) (Kohjin Co. Ltd.), cAMP (Kohjin Co. Ltd.), dibutyryl adenosine 3',5'-cyclic monophosphate (dB-cAMP) (Yamasa Shoyu K.K.), guanosine 5'-monophosphate disodium salt (5'-GMP) (Kohjin Co. Ltd.), guanosine 3',5'-cyclic monophosphate sodium salt (cGMP) (Kohjin Co. Ltd.) and dibutyryl guanosine 3',5'-cyclic monophosphate sodium salt (dB-cGMP) (P. L. Biochemicals). Each nucleotide was dissolved in sterilized distilled water to give appropriate concentrations and 10 μl of each solution was injected. The effect of theophylline (Tokyo Kasei Co. Ltd.) and partially purified MRCH extracted from the heads of silkworm moths, Bombyx mori, and dopamine (Nakarai Chemicals Ltd.) was also examined in the same manner.

RESULTS

Effect of exogenous nucleotides on melanization

400 μg of 5'-AMP, cAMP, dB-cAMP, 5'-GMP, cGMP or dB-cGMP was injected

Table 1. Induction of Cuticular Melanization in Isolated Abdomens by Injection of Various Nucleotides

<table>
<thead>
<tr>
<th>Chemicals* injected</th>
<th>No. of isolated abdomens used*</th>
<th>No. of isolated abdomens showing following types*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>5'-AMP</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>cAMP</td>
<td>8 (7)</td>
<td></td>
</tr>
<tr>
<td>dB-cAMP</td>
<td>8 (4)</td>
<td></td>
</tr>
<tr>
<td>5'-GMP</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>cGMP</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>dB-cGMP</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Distilled water</td>
<td>10</td>
<td>1</td>
</tr>
</tbody>
</table>

* 400 μg of the respective chemicals dissolved in 10 μl of distilled water was injected in to each isolated abdomen. cAMP was suspended in distilled water due to its low solubility.

* Numerals in parentheses indicate no. of isolated abdomens necrosed.

* Types of melanization were graded from pale (I) to most intense darkening (V), according to Ogura (1975).

The partially purified MRCH was prepared from the male adult heads of the silkworm, Bombyx mori, and could induce intense melanization (Type V) in the isolated abdomens of Leucania separata at a dose of 12 μg/abdomen. The method of preparation of the partially purified MRCH will be reported elsewhere (Matsumoto et al., in preparation). In brief, the acetone powder of heads was extracted with 80% ethanol and the extract was processed through concentration, heating, removal of precipitates with 50% acetone, chromatography on SP-Sephadex C-25, ultrafiltration with Amicon UM-2, gel-filtration with Sephadex G-25 and chromatography on DEAE-Sepharose CL-6B to give partially purified MRCH.
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Fig. 1. Cuticular melanization caused by the injection of various nucleotides; a, cAMP; b, dB-cAMP; c, cGMP; d, dB-cGMP. Bars represent standard errors of the means. See Table 1 for degree of melanization.

into each isolated abdomen of 5th instar crowded larvae. As shown in Table 1, isolated abdomens into which 5'-AMP or 5'-GMP was injected showed slight melanization similar to ones applied with distilled water. Seven out of eight isolated abdomens treated with cAMP were necrosed and the remaining one showed darkening in body color (type IV). Injection of dB-cAMP, cGMP or dB-cGMP caused intense melanization (type IV-V). The biological activity of four nucleotides was examined at various dosages where eight to nine isolated abdomens were used in respective doses. Four nucleotides showed similar effectiveness. The results are summarized in Fig. 1.

Effect of theophylline and MRCH on melanization

Theophylline, an inhibitor of cyclic nucleotide phosphodiesterase, was injected into isolated abdomens at each dose of 5, 10 and 20 μg. As shown in Table 2, the resulting abdomens showed pale color similar to ones injected with distilled water (type I-III).

The following experiment was conducted to elucidate the effect of theophylline on MRCH action. As shown in Table 2, injection of 3 μg of MRCH (about 36 μg/g weight) caused slight melanization in isolated abdomens (one out of four abdomens showed type II; two, type III; one, type IV). Injection of 3 μg of MRCH plus 20 μg of theophylline (about 240 μg/g weight) caused more intense melanization in isolated abdomens (all four abdomens showed type IV). Treatment with 6 μg of MRCH caused intense melanization in isolated abdomens (six, type IV; one, type V) and all isolated abdomens showed most intense dark color (type V) when 6 μg of MRCH plus 20 μg of theophylline were injected.

Effect of exogenous dopamine on melanization

Since dopamine has been known to be a precursor of melanine, it was injected into each isolated abdomen at doses of 100, 200 and 400 μg in order to examine its
Table 2. Induction of Cuticular Melanization in Isolated Abdomens by Injection of Theophylline and Partially Purified MRCH

<table>
<thead>
<tr>
<th>MRCH and chemicals injected</th>
<th>No. of isolated abdomens used&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. of isolated abdomens showing following type&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Theophylline 5 µg</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Theophylline 10 µg</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Theophylline 20 µg</td>
<td>7 (3)</td>
<td>1</td>
</tr>
<tr>
<td>Distilled water</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>MRCH 3 µg</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>MRCH 6 µg</td>
<td>8 (1)</td>
<td>6</td>
</tr>
<tr>
<td>MRCH 20 µg</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Theophylline 20 µg</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Numerals in parentheses indicate no. of isolated abdomens necrosed.

<sup>b</sup> See Table 1 for types of melanization.

effect on cuticular melanization. Even at a dose of 400 µg/abdomen, dopamine failed to cause melanization.

DISCUSSION

The cuticular melanization and reddish epidermal coloration in the armyworm larva, *Leucania separata*, are caused by neurosecretory hormone(s) termed MRCH, which is thought to be peptidal because MRCH is inactivated by the treatment with some proteolytic enzymes (Suzuki et al., 1976; Matsumoto et al., in preparation). The results in this paper revealed that both cAMP and dB-cAMP could induce cuticular melanization in the absence of MRCH, which are analogous phenomenon with various peptide and amine hormones in vertebrate (see, Butler et al., 1972). Simultaneous injection of MRCH and theophylline enhanced the activity of the former, which further supports the possibility that cAMP may play a part as an intercellular messenger in the action of MRCH.

Bursicon is known to control tanning firstly by activating membrane adenyl cyclase to produce cAMP, which facilitates the formation of dopamine, a precursor of melanine, by increasing the blood cell permeability to tyrosine (Mills and Whitehead, 1970; Vandenberge and Mills, 1974). The failure of dopamine to induce melanization in the armyworm larva even at high dosages indicates that the action of MRCH may not be restricted to formation of dopamine, even if MRCH is related to the formation of dopamine.

The cuticular melanization was also induced by cGMP and dB-cGMP, which might mimic the action of MRCH by affecting its cAMP-dependent system. In the silkmoth, *Hyarophora cecropia*, pre-eclosion behavior of isolated abdomens of pharate adults was caused by cGMP as well as cAMP, although levels of endogenous cGMP in the central nervous system did not change after injection of eclosion hormone (Truman et al., 1976). In the tobacco hornworm, *Manduca sexta*, levels of endogenous cAMP in the prothoracic gland varied during growth, whereas cGMP was not detected in the gland (Vedeckis et al., 1976). At least in these two species, cGMP seems
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not to play a role as an intercellular messenger of the hormones. The following explanation as to cGMP action has been given; exogenous cGMP can penetrate cells more rapidly than cAMP, and affect the activity mediated by cAMP. In fact, at high concentration cGMP activates cAMP-dependent protein kinases and inhibits hydrolysis of cAMP (see, TRUMAN et al., 1976). This can be attributable to the present results.

Taking into account these facts, to clarify the mechanisms of cuticular melanization further, it will be necessary to elucidate the changes of cyclic nucleotide contents during the tanning process in target organ(s).

ACKNOWLEDGEMENTS

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REFERENCES


