In Vitro Cultures of the Endocrine Organs Secreting Melanization and Reddish Coloration Hormone in the Common Armyworm, Leucania separata (Lepidoptera: Noctuidae)

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(Received May 12, 1978)

Suboesophageal ganglia of the common armyworm, Leucania separata, maintained for 10 days in vitro continued to secrete melanization and reddish coloration hormone. The medium containing armyworm hemolymph in which suboesophageal ganglia had been cultured for 5 days induced dark pigmentation of cuticles when it was injected into isolated abdomens prepared from crowded larvae, whereas the medium free of armyworm hemolymph did not. The medium in which suboesophageal ganglia were maintained for an additional 5 days did not show the endocrine activity.

INTRODUCTION

The larvae of the common armyworm, Leucania separata, have been known to be dark when reared under crowded conditions and pale when reared under isolated conditions (Iwao, 1968). This effect of population density will appear in the color of cuticles through certain endocrine systems. The hormonal regulation of larval pigmentation in L. separata has been studied, and the secretion of hormone(s), which has been provisionally named melanization and reddish coloration hormone (MRCH), from the complex of brain-corpora cardiaca-corpora allata-suboesophageal ganglion (SG) has been proved (Ogura et al., 1971; Ogura and Saito, 1972; Ogura, 1975, 1976) In the present study, endocrine systems which secrete MRCH were maintained for some time in vitro as a preliminary study for establishing the in vitro system of cuticular pigmentation.

MATERIALS AND METHODS

Animals. Larvae of L. separata were reared on a modification of Hirai's diet (Hirai, 1976) at 25°C. Throughout the rearing period they were maintained in the crowded conditions known to produce dark coloration of cuticles. SG as well as brains, corpora cardiaca, corpora allata and muscles to be cultured were taken from the larvae 21 hr before the ecysis to the 6th instar.

Culture media. The MGM-431 medium containing 10% fetal bovine serum (Mitsuhashi, 1972) and a modified MGM-431 medium which contained 5% fetal bovine serum and 5% armyworm hemolymph were used. The armyworm hemolymph

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was collected from the final instar larvae on the 3rd day after moulting. The hemolymph was heated at 60°C for 10 min, frozen at -20°C for 24 hr, thawed and centrifuged at 1500 g for 10 min. The supernatant was incorporated into the culture medium.

Culture conditions. In each culture, 10 organs were placed together in 40–50 μl of the culture medium. Small glass dishes (10 mm in diameter and 7 mm in height) without lids were used as vessels. They were placed in petri dishes together with moistened cotton to prevent desiccation of the culture. The cultures were kept at 25°C for 10 days without changing the media in most of the experiments.

Bioassay of the endocrine activity. After the culture, cultured organs or used media were injected into isolated abdomens prepared from the 5th-instar larvae which had been reared in crowded conditions. The induction of darkening of the cuticles was examined. Because the cuticles of the crowded larvae have been known to react with MRCH more clearly than those of solitary larvae (Ogura, 1975), abdomens for bioassay were prepared from the crowded larvae.

RESULTS

Darkening of cuticles by cultured suboesophageal ganglia

SG were cultured in the modified MGM-431 medium for 10 days and then implanted into isolated abdomens. The SG cultured for 5 days and then frozen at -20°C for 5 days were also tested as a control. The muscles in the heads of larvae were cultured for 10 days and used as a non-endocrine tissue control. The results showed that SG maintained the ability to secrete MRCH throughout the culture period (Table 1). Neither control showed any endocrine activity.

Table 1. Induction of Darkening of Cuticles in Isolated Abdomens by Implantation of the Cultured Organs

<table>
<thead>
<tr>
<th>Organ</th>
<th>Duration of culture (Days)</th>
<th>Numbers of isolated abdomens used</th>
<th>Degree in darkening of isolated abdomens*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Suboesophageal ganglia</td>
<td>10</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>Suboesophageal ganglia</td>
<td>5b</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Muscles</td>
<td>10</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

* V is very dark and I is pale. For detailed classification of the degree of darkening, see Ogura and Saito (1972) or Ogura (1975).

* After 5 day cultivation, SG were frozen at -20°C for 5 days.

Darkening of cuticles by used media

SG, and muscles as a control, were cultured in MGM-431 and modified MGM-431 medium. After 5 days of cultivation, the used media were tested for the ability to induce darkening of cuticles. As is evident from Table 2, only the modified MGM-431 in which SG had been cultured showed the ability to darken the cuticles of isolated abdomens. This means that SG secreted MRCH in the modified MGM-431 medium but not in the original MGM-431 medium.

Instead of isolated SG, brain–corpora cardiaca–corpora allata–SG complexes were cultured in the same manner as in the preceding experiments, because the melanization of cuticles has been known to be regulated not only by SG but also by this complex. Here again, muscles were cultured as a non-endocrine tissue control. The
Table 2. Induction of Darkening of Cuticles in Isolated Abdomens by Injection of the Media in Which Suboesophageal Ganglia (SG) were Cultured

<table>
<thead>
<tr>
<th>Culture media</th>
<th>Cultured organ</th>
<th>Duration of culture (Days)</th>
<th>Dose of injection (μl)</th>
<th>Numbers of isolated abdomens used</th>
<th>Degree in darkening of isolated abdomens*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGM-431</td>
<td>SG</td>
<td>5</td>
<td>15</td>
<td>18</td>
<td>I 2 8 7</td>
</tr>
<tr>
<td>MGM-431</td>
<td>Muscles</td>
<td>5</td>
<td>15</td>
<td>14</td>
<td>I 4 4 6</td>
</tr>
<tr>
<td>Modified MGM-431</td>
<td>SG</td>
<td>5</td>
<td>15</td>
<td>26</td>
<td>I 1 7 9 9</td>
</tr>
<tr>
<td>Modified MGM-431</td>
<td>Muscles</td>
<td>5</td>
<td>15</td>
<td>30</td>
<td>I 7 16 6 1</td>
</tr>
</tbody>
</table>

* See caption to Table 1.

Table 3. Induction of Darkening of Cuticles in Isolated Abdomens by Injection of the Media in Which Endocrine Complex were Cultured

<table>
<thead>
<tr>
<th>Culture media</th>
<th>Cultured organ</th>
<th>Duration of culture (Days)</th>
<th>Dose of injection (μl)</th>
<th>Numbers of isolated abdomens used</th>
<th>Degree in darkening of isolated abdomens*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGM-431</td>
<td>Br-CC-CA-SG^b</td>
<td>5</td>
<td>15</td>
<td>3</td>
<td>I 2 1</td>
</tr>
<tr>
<td>MGM-431</td>
<td>Muscles</td>
<td>5</td>
<td>15</td>
<td>4</td>
<td>I 3 1</td>
</tr>
<tr>
<td>Modified MGM-431</td>
<td>Br-CC-CA-SG^b</td>
<td>5</td>
<td>15</td>
<td>3</td>
<td>I 3</td>
</tr>
<tr>
<td>Modified MGM-431</td>
<td>Muscles</td>
<td>5</td>
<td>15</td>
<td>4</td>
<td>I 4</td>
</tr>
</tbody>
</table>

* See caption to Table 1.

^b Complex of brain, corpora cardiaca, corpora allata and suboesophageal ganglion.

Table 4. Induction of Darkening of Cuticles in Isolated Abdomens by Injection of the Media Used Only for the Latter Half Period of the Culture*^a

<table>
<thead>
<tr>
<th>Organ</th>
<th>Duration of culture (Days)</th>
<th>Dose of injection (μl)</th>
<th>Numbers of abdomens used</th>
<th>Degree in darkening of isolated abdomens*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suboesophageal ganglia</td>
<td>5+5^e</td>
<td>15</td>
<td>7</td>
<td>I 4 3</td>
</tr>
<tr>
<td>Muscles</td>
<td>10</td>
<td>15</td>
<td>8</td>
<td>I 2 4 2</td>
</tr>
</tbody>
</table>

*^a Modified MGM-431 medium was used.

^b See caption to Table 1.

^c Suboesophageal ganglia were cultured for 5 days and the medium was renewed. The culture was continued for further 5 days.

ability of the used media to induce darkening of cuticles was tested after 5 days of cultivation. The results were similar to those of the preceding experiments (Table 3). Only the complex cultured in the modified MGM-431 secreted MRCH during cultivation.

In order to examine the secreting activity of SG during the later period of cultivation, SG were cultured in the modified MGM-431 medium for 5 days and then the medium was renewed. The cultures were continued for an additional 5 days. This last medium was tested for the ability to induce darkening of cuticles. The muscles
from heads were also cultured for 10 days as a control. The results shown in Table 4 indicate that the secretion of MRCH did not occur in the latter half of the 10 day cultivation period.

DISCUSSION

Several endocrine organs, such as brains, corpora allata, and prothoracic glands, have been cultured by many investigators. In the present study, SG, which is known as an endocrine organ secreting MRCH in L. separata as well as the diapause hormone in Bombyx mori, were cultured to examine the stability of the secreting activity of the ganglia when placed in the culture media.

The SG cultured in the modified MGM-431 medium for 10 days showed secreting activity when it was implanted into an isolated abdomen. This indicates synthesis of the hormone in the cultured SG when it was implanted, because the hormone accumulated in the ganglia before the culture had been released during the first 5 days of the culture, and during the following 5 days no hormone was released from the ganglion (Tables 2 and 4).

The hormone already present in the SG seemed to be released into the culture media at an early stage in the cultivation (Tables 2 and 3). The hormonal activity of the used media, however, could be obtained only when the modified MGM-431 medium was used. This suggests the important role of the hemolymph of this species in the culture media. The hemolymph probably promoted the secretion of the hormone and also protected the secreted hormone from degradation. On the other hand, in the media without the hemolymph, the hormone was not secreted or, if secreted, the amount was very small. It may also be possible that the secreted hormone was unstable and decomposed in the absence of the hemolymph.

The fact that no hormonal activity was found in the culture media used for the last 5 days suggests that synthesis of the hormone did not take place under the culture conditions. To maintain the synthesis activity and secretion of the hormone in vitro, further improvement of the culture conditions is necessary.

ACKNOWLEDGEMENTS

The authors wish to express their thanks to Dr. S. YAGI, Associate Professor of Tsukuba University, for his overall cooperation.

REFERENCES


