The role of [His\(^7\)]-corazonin in the control of body-color polymorphism in the migratory locust, *Locusta migratoria* (Orthoptera: Acrididae)

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Abstract

The effect of [His\(^7\)]-corazonin on the body color in *Locusta migratoria* was examined by varying the injected dose and the time of injection in both an albino and a normal (pigmented) strain. Albino nymphs injected with a high dose (100 pmol) of [His\(^7\)]-corazonin at the beginning of the third instar turned completely black in the following instar, whereas those injected with the same dose in the middle of the instar developed black patterns with an orange background color, the body coloration characteristic of normal gregarious (crowded) individuals. Injection at the end of the third instar induced a reddish color with few black spots. Irrespective of the time of injection of the peptide, most of these individuals became completely black after ecdysis to the fifth instar. A similar result was obtained with a lower dose (1 pmol), although the color expressed was lighter. In the normal strain, injection of 1 nmol or 100 pmol into crowded third instar nymphs also caused most of them to become completely black in the fourth and fifth instars, but a lower dose apparently had no influence. These results suggest that the temporal changes in hemolymph titer of [His\(^7\)]-corazonin are important in the expression of body color in *L. migratoria*. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Locust migratoria; Body-color polymorphism; Corazonin; Phase polymorphism

1. Introduction

Since Uvarov (1921) formulated the phase theory to explain the density-related polymorphism in locusts, much information has accumulated about various aspects of acridology (Uvarov 1966, 1977). Body-color polymorphism is one of the most conspicuous phenomena observed in some locusts. Unlike other morphological and behavioral changes related to phase polymorphism, the changes in body color are a reflection of the physiological ones, and changes in nymphal coloration are readily induced during the life of the individuals by crowding or isolation and are reversible (Uvarov, 1966). In fact, nymphal coloration is influenced not only by population density but also by temperature, humidity and background color of the habitat (Faure, 1932; Fuzeau-Braesch, 1985; Pener, 1991). Yet, there is no doubt that body-color polymorphism has received much attention in relation to phase variation. In *Locusta migratoria*, nymphs (solitary) at low population density show various body colors such as green, brown, reddish, black, etc. depending upon the background color of their habitat. At high population density, they (gregarious) are darkened and more or less similar to one another, with characteristic black patterns and an orange background color.

It is well known that the juvenile hormone (JH) is responsible for the induction of green color in locusts (Fuzeau-Braesch, 1985; Hardie and Lees, 1985; Dale and Tobe, 1990; Pener, 1991). However, little information was available about the factor inducing the other colors. Recently, our laboratory established an albino strain of *L. migratoria* by selecting for whitish individuals that had appeared by a spontaneous mutation in an Okinawa (Japan) strain (Hasegawa and Tanaka, 1994), and discovered that implantation of the corpus cardiacum or brain from normal (pigmented) individuals of the same species induced a dark color in albino nymphs under certain conditions.
(Tanaka, 1993). Using this albino strain as a novel bioassay system, we have found that a factor present in the corpus cardiacum and brain is responsible for the induction of dark color in *L. migratoria* (Tanaka, 1993; Tanaka and Pener 1994, 1995) and that a similar mechanism also exists in another plague locust, *Schistocerca gregaria* (Tanaka and Yagi, 1997). More recently, we succeeded in elucidating the chemical structure of this factor (Lom-DCIN) and found that it was [His7]-corazonin (Tawfik et al., 1999). [His7]-corazonin is a neuropeptide that was first identified from another locust, *Schi-stocerca americana* (Veenstra, 1991), but its physiological function is unknown in this locust. Although this neuropeptide has been demonstrated to induce various dark colors including the one characteristic of gregarious nymphs (Tawfik et al., 1999), no detailed information is available about how this neuropeptide induces other colors. In this paper, I examined the effects of different doses of [His7]-corazonin and timing of its administration on the induction of various body colors in albino and normal (pigmented) strains of *L. migratoria*, to understand the role of this peptide in body-color polymorphism in this locust.

2. Materials and methods

2.1. Insects

The albino strain of *L. migratoria* used in the present study was established from a laboratory colony originating from Okinawa Main Island, Japan, as previously described (Tanaka, 1993; Hasegawa and Tanaka, 1994). The normal (pigmented) strain was derived from Ishigaki Island, Okinawa. Nymphs and adults of both strains were reared at 30°C and 16 h light/8 h dark photoperiod according to the method previously described (Hakomori and Tanaka, 1992; Tanaka et al., 1993).

2.2. Injections of [His7]-corazonin

[His7]-corazonin was synthesized by Yamazaki Industries, Co. (Tokyo, Japan) and dissolved in 80% acetonitrile with 0.1% trifluoroacetic acid (TFA). Various amounts of the peptide in the acetonitrile solution were dried and mixed with pure rape seed oil (Hayashi Chemical Co., Tokyo, Japan) for injection. In the present study, rape seed oil was used instead of peanut oil, because the former is less viscous than the latter, although neither of them has dark-color-inducing activity by itself. In preliminary tests, no significant difference was observed in the dark-color-inducing activity of [His7]-corazonin when mixed with any of the oils including the above two oils, soy bean oil, olive oil and sesame oil (Tanaka, unpublished work). On different days of the third instar, nymphs were injected with 4 μl of oil containing different amounts of the peptide using a microsyringe (Ito, Shizuoka, Japan) between the second and third abdominal sternites (Tanaka, 1996). Some individuals were injected with oil alone as controls. Most third instar nymphs (>80%) took 5 or 6 days to ecdyse to the following instar and the rest 4 or 7 days. The group of nymphs treated on day 4 did not include those that had ecdysed to the fourth instar (10–20%). In experiments to examine the effect of multiple injection of the peptide, injection was made twice, on days 0 and 3, during the third instar. In this case, each injection consisted of 2 μl of rape seed oil containing different doses of the peptide. In each experiment, 10–17 individuals were used for each treatment and kept under crowded conditions.

2.3. Body-color types

The effect of injection was assessed by observing the visual colors of the body. Because the variation in body coloration was rather continuous, fourth instar albino nymphs were categorized into 13 types, as shown in Fig. 1, according to the darkness, color and pattern. Likewise, fifth instar albino nymphs were divided into six types, as shown in Fig. 2. Nymphs of the normal strain injected with the peptide were categorized into three types: their body color was normal (F in Fig. 1), darker than normal (like B-1 in Fig. 1) or completely black (like A-1 in Fig. 1).

3. Results

3.1. Effects of different doses of [His7]-corazonin and timing of the injection in albino nymphs

Albino nymphs injected with 100, 1 or 0.1 pmol of [His7]-corazonin during the third instar showed various body colors in the following (fourth) instar depending not only on the dose but also on the timing of the injection (Figs. 1 and 3). In general, the higher the dose the darker the color induced. As shown in Fig. 3, all nymphs injected with 100 pmol on day 0, 1 or 2 became practically black (type A-1 in Fig. 1) except for 60% of individuals injected on day 2 in which a dark orange color appeared on the coxae and mandible (Fig. 3; type A-2 in Fig. 1). As the time of injection was further delayed, individuals with black patterns and an orange background color (B-1 and B-2 in Fig. 1) appeared. This type of body coloration is similar to that of normal individuals under crowded conditions (F in Fig. 1). Twenty per cent of those injected on day 4 developed a reddish brown color along the ridge of the thorax (C-1 in Fig. 1; Fig. 3). When the dose was reduced to 1 or 0.1 pmol [Fig. 3(B) and (C)], the nymphs injected on day 0 or 1 showed a mixture of black, purple and brownish spots all over
Fig. 1. Photographs showing various body colors induced in albino fourth instar nymphs of *L. migratoria* by injection of [His<sup>7</sup>]-corazonin (A–D) during the third instar. A-1 to A-4, uniformly pigmented; B-1 to B-4, black patterns with orange or brown background color and the ridge of the thorax black; C-1 to C-3, some black patterns with orange or brown background color and the ridge of the thorax not black; D, uniformly reddish. Photographs showing the body colors for an untreated albino (E) and a normal (F) individual are also included.

the body (A-3 and A-4 in Fig. 1) and none of them turned very dark. Some individuals injected with 1 pmol on day 2 developed black patterns with an orange background color. This type of body coloration was not manifested when nymphs were injected with the higher dose at this age. Injection on day 3 or 4 induced black patterns (B-3, B-4, C-1 and C-2 in Fig. 1), and the color tended to be lighter when the dose was lower. Among those injected with 1 pmol on day 4, 20% developed a reddish or dark pink color without conspicuous black patterns (D in Fig. 1). They were those that ecdysed to the next instar within 24 h after injection. These results indicated that more or less uniformly pigmented individuals (A-1 to A-4 and D in Fig. 1) appeared when they were injected with the hormone at the beginning and the end of the previous instar, whereas black patterns with orange background color occurred in those injected in the middle of the instar.

The right panels of Fig. 3 show the proportions of individuals with various body colors observed after ecdysis to the fifth nymphal instar in the above experiment. Most nymphs injected with 100 pmol of [His<sup>7</sup>]-
corazonin became black (A-1 in Fig. 2) no matter when the injection was made during the third instar. It appeared that a high hormonal titer created by an injection of 100 pmol of [His\textsuperscript{7}]-corazonin persisted during the fourth instar and caused the nymphs to turn completely black. Some individuals injected on day 3 of the third instar, which developed black patterns and orange color in the fourth instar, remained in a similar body coloration in the fifth instar. When injected with 1 or 0.1 pmol, the individuals showing a uniform body color were all light brown or purple in color (A-3 in Fig. 2). All nymphs injected with 1 pmol on day 3, which showed black patterns and orange color after ecdysis to the fourth instar (B-3 and C-1 in Fig. 1), developed a similar coloration in the fifth instar (B-2 in Fig. 2). These results indicated that a lightly pigmented uniform body coloration was induced by injection of a relatively low dose of the hormone and that the black patterns expressed on the upper part of the thorax in the fourth instar tended to persist in the following instar in some individuals.

3.2. Effects of multiple injection in albino nymphs

Albino nymphs injected with 10 pmol of [His\textsuperscript{7}]-corazonin on day 0 of the third instar were injected again with 0 (oil only), 10 or 100 pmol on day 3 of the same instar to examine how the first injection would influence the effect of the second injection on the induction of body color (N=12 each). As described above [Fig. 3(A)], a single injection of 100 pmol on day 3 caused albino nymphs to develop black patterns with orange background color in the following instar. In multiple injections, however, all individuals injected with 10 pmol on day 0 turned black completely in the following instar irrespective of the dose injected on day 3. These results indicated that the orange background color was not manifested when a high dose of the peptide (10 pmol) prevailed during the initial stage of the previous instar.

A similar experiment was carried out in which albino nymphs were injected with 1 pmol on day 0 and then injected again with 0, 1 or 10 pmol on day 3 of the third instar. In this case, those injected with oil alone on day 3 became a mixture of A-3 (23.0\%) and A-4 (77\%) types of fourth instar (N=13), whereas all individuals (N=18) injected with 1 pmol on days 0 and 3 became type A-3 and no individual developed an orange color. This result indicated that an injection of 1 pmol on day 3 of the third instar failed to develop an orange color if a low titer of the peptide was present during the initial stage of the third instar [cf. Fig. 3(B)]. Some orange or reddish color appeared among fourth instar nymphs that had been injected with 1 pmol on day 0 and 10 pmol on day 3 of the previous instar. In this case (N=14), 78.6\% of nymphs were similar to type B-1 of the fourth instar and 14.3\% type A-3. One individual (7.1\%) developed a dark reddish color all over the body without black spots.
These results indicated that an orange or reddish color could be induced even among those injected with 1 pmol of the peptide at the beginning of the third instar, if the dose injected three days later was high (≥10 pmol).

### 3.3. Effects of different doses of [His⁷]-corazonin in normal nymphs

Nymphs of the normal strain were injected with 1 nmol, 100 pmol, 1 pmol or 0.1 pmol of [His⁷]-corazonin on day 0 of the third instar and their body color was checked in the fourth and fifth instars. The highest dose caused all individuals to turn completely black like A-1 in Fig. 1 (Fig. 4). Twenty-seven per cent of those injected with 100 pmol turned completely black and the rest also became black with a dark orange color on the lower part of the body (like B-1 in Fig. 1). The lowest two doses had no apparent effect on the body color, as in those (F in Fig. 1) injected with oil alone (Fig. 4; data not shown for 0.1 nmol). After ecdysis to the fifth instar, all individuals injected with the highest two doses became completely black, whereas those nymphs injected with a lower dose were indistinguishable from oil-injected controls.

Two doses, 1 and 100 pmol, of [His⁷]-corazonin were used to observe the effect of the timing of injection on body color in normal nymphs. All third individuals injected with 100 pmol became either completely black (like A-1 in Fig. 1) or black with some dark orange color (B-1 in Fig. 1) in the following instar. The proportion of nymphs with the former body coloration was 27.3, 81.8, 28.6, 35.7 and 8.3% in those injected on day 0, 1, 2, 3 and 4, respectively (N=11–14). It was the highest in those injected on day 1 and the lowest on day 4. In the fifth instar, all individuals injected during the first two days of the third instar became completely black, and the proportion of such black individuals was 66.7 and 91.7% in those injected on days 3 and 4, respectively. None of the injected individuals showed the normal gregarious body color (F in Fig. 1) except for the
oil-injected controls and 22.2% of those injected on day 3, the time when injection of a high dose of the peptide induced black patterns with an orange background in albino nymphs (Fig. 3).

4. Discussion

The present results demonstrated that the dose of [His\(^7\)]-corazonin and the timing of the injection of this peptide determine the kind of body color induced in albino nymphs of \(L.\ \textit{migratoria}\). In general, the higher the dose the darker the body color induced. Injection of this peptide during the early or the very late stage of the third nymphal instar induced different colors in the fourth instar. That is, injection of a high dose during the early stage of the third instar caused albino nymphs to turn completely black in the following instar. A lower dose, however, induced a brown or purple color. Nymphs injected at the end of the third instar developed a reddish color in the following instar. On the other hand, injection of the peptide in the middle of the instar induced black patterns with an orange background color. Because the body colors induced by injections of the peptide in this study are quite similar to some of those seen in normal individuals of this species, it is likely that a similar variation in hemolymph titer of [His\(^7\)]-corazonin occurs in normal individuals. For example, nymphs with a uniformly black, brown, purple or reddish body color can be found in the field (Tanaka, 1993), and they are often referred to as “homochromy” (adaptation to the background color) (Fuzeau-Braesch, 1985). Black patterns with an orange background color are characteristic of gregarious (or crowded) nymphs of this locust (Faure, 1932). One may wonder whether [His\(^7\)]-corazonin plays any role in other phase-related phenomena in locusts. However, so far, there has been no study to investigate such a role.

In terms of the pattern of response by albino nymphs, the present results are similar to the results of a previous study (Tanaka, 1996) in which corpora cardiaca (CC) taken from the two-spotted cricket, \textit{Gryllus bimaculatus}, were implanted into albino locusts on different days during the fourth instar and the effect was evaluated in the following instar. Cricket CC induce various dark colors in albino locusts depending on the time of implantation, and the relationship between the time of implantation and the kind of body color induced is basically the same as in the present study using [His\(^7\)]-corazonin. Our recent study (Hua et al., 2000) has elucidated that the cricket CC contain [Arg\(^7\)]-corazonin, a corazonin isomer slightly different from [His\(^7\)]-corazonin: the arginine has been substituted for a histidine residue (Veenstra 1989, 1991). Both peptides show a similar dark-color-inducing activity in \(L.\ \textit{migratoria}\) (Tawfik et al., 1999) and other acridids including \textit{S. gregaria}, \textit{Nomadacris succincta}, \textit{Gastrimargus marmoratus}, \textit{Atractomorpha lata}, \textit{Acrida cinerea} and \textit{Oxya yezoensis} (Tanaka, unpublished observations).

The half-life of hormones is sometimes fairly short. For example, injected juvenile hormone has a half-life of 25 min to several hours (Tobe and Stay, 1985). No information is available about how long the injected [His\(^7\)]-corazonin remains in the body of locusts. In the present study, no attempt was made to collect this type of information directly. However, the injected peptide appears to remain in the hemolymph for a long time, because normal nymphs injected with 100 pmol of this peptide during the third instar became darker in the fourth instar, but they became even darker and most turned completely black in the fifth instar irrespective of the time of injection. Albino nymphs injected with the same dose developed various colors in the fourth instar depending on the time of injection in the previous instar, but most of them turned completely black after ecdysis to the fifth instar. As described above, albino nymphs with a completely black color can be obtained even in the fourth instar if they have been injected with a high dose of the peptide at the beginning of the previous instar. Therefore, the fact that fifth instar nymphs were completely black would indicate that a high titer of the peptide was present at the beginning of the fourth instar.
My unpublished observations indicated that oil droplets were still found in the hemolymph a week after 4 μl of oil were injected into locusts. Therefore, it is likely that the peptide injected in the oil also stays long in the hemolymph and exerts its dark-color-inducing effect over many days.

Results of the multiple injections indicated that the dose of [His7]-corazonin injected at the beginning of the third instar influenced the effect of a second injection of the peptide applied later in albino nymphs. The orange or reddish color, which is otherwise manifested in the fourth instar upon injection of the peptide late in the third instar, did not appear if a high dose (10 pmol) had been injected at the beginning of the third instar. If a low dose (1 pmol) was used for the first injection, a reddish or orange color appeared only when a second injection had a high dose. Although the present study shed no information on the pigments involved, it is possible that the black pigment(s) induced simply made the orange or reddish pigment(s) difficult to detect by visual inspection.

In the present study, the gregarious body coloration was observed among fourth instar nymphs if they had been injected with a high dose of [His7]-corazonin on day 3 of the third instar. It is interesting to note that some of them retained the orange color in the lower part of the body in the fifth instar, while the rest and those injected before day 3 of the third instar turned completely black. So far there is no explanation for this phenomenon, but it was observed both in the albino and normal strains.

Although JH may not be a major factor in the control of body-color polymorphism in locusts (Pener et al., 1992), it functions to induce the solitary green color, as demonstrated by many workers (Dale and Tobe, 1990; Pener, 1991; Pener and Yerushalmi, 1998). This was also confirmed with albino nymphs of *L. migratoria* using JH analogs (Hasegawa and Tanaka, 1994). In this case, however, the green individuals obtained after administration of JH analogs do not exactly look like solitary individuals normally observed in the field, because the former do not develop the dark color normally shown by the latter on the ventral side of the body. Therefore, it is likely that both JH and [His7]-corazonin are necessary for the expression of some solitary body coloration. Studies to test this hypothesis are in progress.

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References


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