Terpenoid ω-hydroxylase (CYP4C7) messenger RNA levels in the corpora allata: a marker for ovarian control of juvenile hormone synthesis in Diploptera punctata

Tara D. Sutherland 1, Gopalan C. Unnithan, René Feyereisen *

Department of Entomology, The University of Arizona, Forbes 410, PO Box 210036, Tucson, AZ 85721-0036, USA

Received 18 October 1999; accepted 12 January 2000

Abstract

Ribonuclease protection assays were used to measure changes in allatal transcript levels of the CYP4C7 gene which encodes a cytochrome P450 terpenoid ω-hydroxylase thought to play a role in the metabolism of JH and its precursors. Denervation of the corpora allata does not affect the pattern of expression of the CYP4C7 gene. Transplantation experiments show that CYP4C7 mRNA levels are dependent on a humoral factor characteristic of the reproductive state of the insect. Messenger RNA levels rise substantially in mated or denervated females, or in mated or virgin females treated with hydroprene, when the follicle length is over 1.5 mm. Vitellogenic ovaries however exert a negative influence on CYP4C7 expression, as ovariectomy in mated females causes a premature rise in CYP4C7 mRNA levels. The half-life of the CYP4C7 transcript is approx. 2 h when the corpora allata are incubated in vitro. Under these conditions, coincubation with a post-vitellogenic ovary maintains high CYP4C7 transcript levels in the glands. Excess juvenile hormone or analog applied at the end of vitellogenesis blocks ovulation or causes abortion of embryos deposited in the brood sac. We conclude that expression of the CYP4C7 gene is tightly controlled by the ovary, and it coincides with the ovarian signal to turn off juvenile hormone synthesis. The role of the CYP4C7 enzyme may be to ensure the clearance of allatal juvenile hormone and its precursors at the end of the gonotrophic cycle. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Cockroach; CYP4C7; Cytochrome P450; Ribonuclease protection assay; Ovary

1. Introduction

The role of juvenile hormone (JH) in reproduction is well established but the precise molecular mechanisms controlling the endocrine activity of the corpora allata (CA) are still poorly understood. Physiological experiments linked to short-term assays for CA activity in vitro have revealed that both neural and humoral signals are involved (Feyereisen, 1985). The reproductive cycle of the cockroach, Diploptera punctata has served as a convenient model system because of its well defined cycles of CA activity that are linked to the specialized mode of reproduction of this species—viviparity. The developmental state of the basal oocytes appear to play a major role in regulating CA activity in D. punctata. Basal oocytes in rapid vitellogenesis stimulate JH production (Stay et al., 1983; Rankin and Stay, 1984) whereas those near maturity are inhibitory (Rankin and Stay, 1985). Allatostatins, isolated from the brain of D. punctata and found in the CA and neural connections to the brain, are short term, reversible inhibitors of JH synthesis (Stay et al., 1994). The sensitivity of the CA to allatostatins is tightly regulated during the reproductive cycle (Pratt et al., 1990) and acquisition of allatostatin sensitivity by the CA is determined by the endocrine milieu (Unnithan and Feyereisen, 1995). Whilst the in vitro assays of JH synthesis have been extremely useful, the study of CA regulation during the reproductive cycle has been hampered by the lack of more specific molecular tools. We have recently reported the cloning and functional expression of a cytochrome P450 expressed in the CA, CYP4C7, that metabolizes JH and JH precursors by ω-hydroxylation. The CYP4C7 mRNA levels change dramatically during a gonotrophic cycle of adult mated

* Corresponding author. Tel.: +1-520-621-9598; fax: +1-520-626-8058.
E-mail address: rfeyer@ag.arizona.edu (R. Feyereisen).
1 Present address: CSIRO, Division of Biotechnology, Canberra, ACT, Australia
females (Sutherland et al., 1998). Levels are low from the day of adult emergence and mating (day 0) to day 5 when JH synthesis is at a peak. However, one day later (day 6) CYP4C7 mRNA levels increase and then reach a maximum on day 7 just before oviposition. Levels of CYP4C7 mRNA decrease gradually during pregnancy, and are back to day 0 levels at parturition, when a new cycle of JH synthesis begins (Sutherland et al., 1998). We now report that monitoring changes in mRNA levels for this P450 is a sensitive molecular tool for the study of CA regulation. The RNase protection assay (RPA) provides a sensitive and reliable system to measure responses of CYP4C7 mRNA levels to physiological manipulation, including the effects of nerve section, the endocrine milieu, the ovary, and JH analogue on CYP4C7 expression.

2. Materials and methods

2.1. Insects

*Diploptera punctata* were reared as described previously (Meller et al., 1985). Virgin females were collected from cages free of adult males. Mated females were collected within 4 h of molting and mated status was confirmed by the presence of a spermatophore. Transplantation was performed as previously described (Unnithan and Feyereisen, 1995). For denervation of the CA, corpora cardiaca (CC) and CA of insects chilled on ice were exposed by cutting the neck membrane and the attached part of the head capsule. Connection of the CA to the brain was severed in front of the CC with fine forceps. The wound was closed with the cut portion of the head capsule, and streptomycin powder was sprinkled on the surface of the wound. Mortality after this surgery was negligible. Hydroprene (ZR512) treatment was performed by topically applying 200 µg of the compound dissolved in 2 µl acetone, to the ventral side of the thorax of one-day-old insects. Control insects were treated with 2 µl acetone. The imidazole compound TH27 (100 µg in 2 µl acetone) was topically applied to three-day-old mated females, which were assayed 24 h later. Control insects were treated with acetone. 20-hydroxyecdysone treatment was performed by injecting two-day-old mated females with 5 µg hormone dissolved in 3 µl 80% tissue culture medium M199/20% ethanol. Injections were repeated 24 and 48 h later. Control insects were injected with 3 µl 80% tissue culture medium M199/20% ethanol.

2.2. Assays

Levels of JH synthesis were determined by the radiometric assay for incorporation of label from [methyl-3H]methionine into juvenile hormone III as modified (Pratt et al., 1990). The RNase protection assay (RPA) for measuring CYP4C7 mRNA levels on single pairs of CA was performed as described previously (Sutherland et al., 1998). In all our experiments, RNA from glands of day 7 mated females (eggs after chorionation but before ovulation) serve as the arbitrary 100% control value for relative levels of CYP4C7 mRNA.

3. Results

3.1. Effect of CA denervation

We have measured the transcript levels for the CYP4C7 gene by ribonuclease protection assays performed on lysates from single gland pairs (Fig. 1). This gene is expressed within the CA during periods of intrinsic repression of JH synthesis (Fig. 1) (Sutherland et al., 1998). To understand the physiological mechanism underlying this pattern of change, we first studied the effect of severing the nervous connections between the brain and the CA. Gland denervation does not alter the pattern of JH synthesis in mated females (Stay and Tobe, Fig. 1. Changes of CYP4C7 mRNA levels during a gonotrophic cycle in mated female *D. punctata* measured by ribonuclease protection assays. The 145 bp protected fragments are shown on top for a series of individual CA. The bottom graph is adapted from (Feyereisen et al., 1981a; Sutherland et al., 1998) and shows the changes in JH synthetic activity of the glands (dots) and the levels of CYP4C7 mRNA (open squares); P, parturition. Note that there is a change in the age scale between day 8 and day 10.
When we denervated the CA on day 6 and assayed on day 8, the relative level of mRNA was 97 ± 12% (n = 3) compared to 88 ± 14% (n = 3) in the sham-operated controls, and when we denervated on day 4 and assayed on day 7 the treated insects had relative levels of message of 46 ± 12% (n = 3) compared to the controls which had 59 ± 13% (n = 3). The process of surgery invariably delays development and this presumably led to levels of CYP4C7 mRNA more typical of day 6 than of normal day 7 levels in both sham-operated and denervated animals. However these levels are significantly higher than those of day 4 (Sutherland et al., 1998). Thus denervation of the CA in six-day-old mated females did not cause a decrease in the high levels of CYP4C7 mRNA, and denervation in four-day-old mated insects did not prevent the subsequent increase in CYP4C7 message.

3.2. Effect of the endocrine milieu: CA transplantation

These results of in situ denervation experiments allowed us to study the effect of the endocrine milieu by transplanting CA from mated females forward or backwards during the cycle of JH synthesis (Fig. 2). The CA of females of day 6 (just after spermatophore release) have high levels of CYP4C7 mRNA which peak on day 7 then decline to 65% after three days (day 9). When these glands were transplanted into day 1 hosts and assayed three days later they showed only low CYP4C7 mRNA levels (9%). On the other hand CA from day 1 females, which have low levels of CYP4C7 expression, showed 34% expression when transplanted into day 1 hosts and assayed three days later. Glands from day 4 females had low levels of CYP4C7 mRNA three days after transplantation into a day 1 host, in contrast to the high levels expected if they had remained for three days in the donor endocrine environment. In all cases the transplanted glands assumed levels of CYP4C7 mRNA more closely resembling the gland from the host than levels of message normally associated with their developmental stage (Fig. 2). Thus, CYP4C7 mRNA levels in the CA are not dependent on either innervation or the intrinsic developmental age of the gland, but on a humoral factor characteristic of the reproductive state of the insect.

3.3. Correlation of CYP4C7 messenger RNA levels with physiological events

As shown earlier (Sutherland et al., 1998), CYP4C7 mRNA levels are undetectable on day 5, and almost half-maximal one day later. A more precise correlation of CYP4C7 mRNA levels and the length of the terminal follicle in a series of mated females analyzed between days 5 and 7 revealed that this increase occurs after the follicles have reached 1.5 mm in length (Fig. 3). Little or no message was observed in the CA of animals prior to this developmental stage, which is characterized by allatostatin insensitivity (Pratt et al., 1990) and message levels were high in all animals with primary follicles over 1.7 mm in length. These older animals are allatostatin-sensitive (Pratt et al., 1990). Intermediate levels of message were observed in animals with follicles between 1.55 mm and 1.60 mm in length, which corresponds to the period of transition from allatostatin insensitivity to allatostatin sensitivity (Pratt et al., 1990).

3.4. Effect of topical application of JH analog on CYP4C7 messenger RNA levels

Topical application of the JH analog hydroprene depresses biosynthesis of JH from the CA and stimulates oocyte growth (Tobe and Stay, 1979). We found that, although animals treated with hydroprene did induce
found that although JH synthesis from glands of mated females ovariectomized on day 1 was low as expected (Stay and Tobe, 1978; Rankin and Stay, 1983) and the glands small, JH synthesis in CA from ovariectomized females could be stimulated significantly by addition of the precursors farnesoic acid or mevalonolactone (Table 1).

We also studied the CA of virgin females in which levels of CYP4C7 message normally are low and do not change

Table 1

<table>
<thead>
<tr>
<th>Insects</th>
<th>JH synthesis (pmol/pair/h)</th>
<th>Spontaneous +40 μM farnesoic acid + mevalonolactone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Four-day-old ovariectomized</td>
<td>3.9±2.7 (8)</td>
<td>39.2±4.6 (8)</td>
</tr>
<tr>
<td>Four-day-old sham-operated</td>
<td>17.8±9.1 (6)</td>
<td>73.5±7.2 (6)</td>
</tr>
<tr>
<td>Six-day-old ovariectomized</td>
<td>4.0±1.4 (8)</td>
<td>77.9±3.4 (8)</td>
</tr>
<tr>
<td>Six-day-old sham-operated</td>
<td>10.6±3.4 (9)</td>
<td>22.1±2.0 (9)</td>
</tr>
<tr>
<td>Four-day-old sham-operated</td>
<td>154.6±14.8 (8)</td>
<td>208.6±12.8 (8)</td>
</tr>
<tr>
<td>Six-day-old sham-operated</td>
<td>89.4±21.6 (5)</td>
<td>100.9±20.3 (5)</td>
</tr>
</tbody>
</table>

*Values are means±S.E.M. of (n) measurements.
when compared to mated females (Sutherland et al., 1998). Ovariectomy does not affect CYP4C7 mRNA levels in innervated CA from virgin females (Fig. 4); the CA of these animals were small and the morphology did not apparently change after surgery. Volume and cell number of the CA were not measured in these experiments. Denervation of CA from virgins leads to a normal cycle of JH synthesis and a normal pattern of CYP4C7 mRNA levels: low levels on day 5 and high levels on day 10 (Fig. 5). Moreover, the denervated CA of virgin females responded like those of mated females to ovariectomy, showing a precocious rise in mRNA levels that was apparent on day 5 and maintained at least until day 10 (Fig. 5). The increase in CYP4C7 mRNA levels in response to ovariectomy is thus subordinated to the lifting of the tonic inhibition of the CA by the brain. The lifting of the tonic inhibition can be achieved either by mating or by denervation.

The role of the ovary was studied further by transplantation experiments. Mated females were ovariectomized on day 1, and an ovary was transplanted on day 7. CYP4C7 mRNA levels were assayed two days later, on day 9. Fig. 6 shows that implantation of a vitellogenic ovary (day 3, 1.00 mm) resulted in a decrease in CYP4C7 transcript levels and growth of the oocytes (to 1.22 mm), whereas implantation of an ovary taken just after ovulation (age day 7) did not. Implantation of a vitellogenic ovary (1.0 mm) into normal mated females on day 7 also led to a decrease in CYP4C7 levels on day 9 (12.1±6.2%, n=4; oocytes grew to 1.21 mm) when compared to normal controls of that age (60±5%), (Sutherland et al., 1998). The premature appearance of CYP4C7 transcript in ovariectomized insects (Fig. 4) can thus be ascribed to the lack of a negative influence of the vitellogenic ovary.

3.6. Effect of altered JH synthesis on CYP4C7 messenger RNA levels

To test the possibility that experimentally modified JH synthetic rates might regulate CYP4C7 expression, we used the 1,5-disubstituted imidazole, TH27, which inhibits methyl farnesoate epoxidase activity in the CA and causes both an inhibition of JH synthesis and an accumulation of methyl farnesoate (Unnithan et al., 1995). We topically applied 100 μg TH27 to day 3 mated females and measured levels of CYP4C7 mRNA in the CA 24 h later. Whilst JH synthesis was inhibited dramatically, there was no difference in levels of mRNA between treated animals and sham-treated controls (Table 2). Animals were also treated with three successive injections of 20-hydroxyecdysone on days 2, 3 and 4. Treated animals had dramatically reduced JH syn-
Table 2
Effect of altered JH synthesis on CYP4C7 mRNA levels measured by RPA a

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Follicle size (mm)</th>
<th>Relative mRNA level*</th>
<th>JH synthesis (pmol/pr/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TH27 control</td>
<td>1.0±0.12 (8)</td>
<td>2.8±0.3 (3)</td>
<td>131.2±29.3 (4)</td>
</tr>
<tr>
<td>Treated</td>
<td>1.1±0.15 (8)</td>
<td>1.0±0.5 (3)</td>
<td>44.7±7.0 (5)</td>
</tr>
<tr>
<td>20-HE control</td>
<td>1.4±0.14 (6)</td>
<td>5.0±2.8 (3)</td>
<td>177.8±17.0 (3)</td>
</tr>
<tr>
<td>Treated</td>
<td>1.1±0.13 (7)</td>
<td>3.6±2.4 (3)</td>
<td>22.9±4.1 (3)</td>
</tr>
</tbody>
</table>

* In percentage relative to day 7 controls. † The imidazole compound TH 27 was administered to three day mated females as described in Materials and methods, and the assays were performed 24 h after treatment. ‡ 20-hydroxyecdysone were administered on days 2 to 4 as described in Materials and methods and the assays were performed on day 5.

thesis and slightly delayed oocyte development as expected (Feyereisen and Farnsworth, 1987; Friedel et al., 1980), yet CYP4C7 message levels remained low and not significantly different to those found in the control animals (Table 2).

3.7. In vitro modulation of CYP4C7 messenger RNA levels

We measured the levels of CYP4C7 mRNA in glands from day 7 mated females incubated in vitro and found that the transcript levels decreased very rapidly, with a half-life of approximately 2 h (Fig. 7). When the CA were incubated as a whole brain-CC-CA complex, the rapid decrease of CYP4C7 mRNA levels was not prevented. However co-incubation of postvitellogenic ovaries (with chorionated eggs) prevented CYP4C7 message decrease and this effect was independent of the presence of the brain in the incubation or of intact neural connections of the CA (Table 3). A vitellogenic ovary (day 4) did not prevent message decrease in vitro. Conversely, a postvitellogenic ovary did not increase CYP4C7 message level in the CA of vitellogenic females (day 4). Incubation of glands from post-vitellogenic (day 7) females with 10 nM allatostatin 1 did not prevent the decrease in message levels (results not shown).

3.8. Effects of hydroprene on ovulation and pregnancy

Possible functions of the terpenoid ω-hydroxylase have been discussed, including a role in removing JH and its immediate sesquiterpenoid precursors at the end of the gonotrophic cycle (Sutherland et al., 1998). We showed that topical treatment with JH III on day 6 caused a drop in ovulation rate and an increase in spontaneous abortion in females that had ovulated (Sutherland et al., 1998). We extended these studies by using hydroprene, a more metabolically stable JH analog, to allow a dose–response analysis of this effect. Fig. 8 shows that hydroprene caused little or no change in ovulation rate when applied on day 4, but caused a dose-dependent abortion of the embryos from the brood sac within two weeks after ovulation. When applied two days later, the effects of hydroprene were more marked. Ovulation was severely curtailed in a dose-dependent manner, and those females that did ovulate subsequently aborted.

4. Discussion

The ribonuclease protection assay (RPA) for CYP4C7 mRNA levels used in this study and in our previous

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Relative mRNA level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 7 CA</td>
<td>5.0±2.7 (3)</td>
</tr>
<tr>
<td>Day 7 Brain-CC-CA complex</td>
<td>2.4±3.0 (3)</td>
</tr>
<tr>
<td>Day 7 Brain-CC-CA complex+day 7 ovary*</td>
<td>92.8±28.0 (3)</td>
</tr>
<tr>
<td>Day 7 CA+day 7 brain+day 7 ovary*</td>
<td>103.5±35.0 (3)</td>
</tr>
<tr>
<td>Day 7 CA+day 7 ovary*</td>
<td>97.1±12.5 (6)</td>
</tr>
<tr>
<td>Day 7 Brain-CC-CA complex+day 4 ovary</td>
<td>16.6±54.8 (5)</td>
</tr>
<tr>
<td>Day 4 CA</td>
<td>Und. † (3)</td>
</tr>
<tr>
<td>Day 4 CA+day 7 ovary*</td>
<td>Und. † (3)</td>
</tr>
</tbody>
</table>

* Day 7 ovary with chorionated eggs. † Und., Ribonuclease-protected CYP4C7 band was undetectable. Values are mean±S.D. of three to six assays, relative to mRNA levels in freshly excised CA.
description of the CA terpenoid \( \omega \)-hydroxylase (Sutherland et al., 1998) has proven to be a dependable tool in the study of CA physiology. First, because of its inherent specificity, the RPA results offer insights in the transcript levels of a single gene. Although mRNA levels represent a balance between transcriptional activity of the gene and messenger decay, this measurement is more refined than a global assessment of gland activity such as JH synthetic rates or allatostatin sensitivity. Second, the sensitivity of the RPA has allowed us to measure CYP4C7 mRNA levels in individual pairs of CA. Third, our results show that CYP4C7 mRNA levels consistently reflect an important facet of CA physiology, namely the role of the ovary in the control of JH synthesis. CYP4C7 mRNA levels can thus be proposed to serve as a marker for ovarian regulation of JH synthesis. Thus while the role of the \( \omega \)-hydroxylase itself is still uncertain, measurement of its transcript levels can lead to a molecular approach in CA physiology.

4.1. Regulation of CYP4C7 transcript levels

Transplanted CA assumed levels of CYP4C7 mRNA that closely reflected levels in the CA from the host. They did not maintain the levels of CYP4C7 mRNA normally associated with their developmental stage. Thus, in mated females CYP4C7 expression is not dependent on either innervation or the intrinsic properties of the gland, but on a humoral factor(s) characteristic of the reproductive state of the insect. The finding that timing of CYP4C7 expression is correlated to follicle length in both normal and hydroprene-treated mated and virgin insects, where the timing of follicle growth is accelerated, strongly suggests that the ovary is a major regulator of CYP4C7 mRNA levels.

In vitro experiments lend support to this idea. CYP4C7 mRNA levels in CA from post-vitellogenic insects cultured in vitro drop to less than 10% within 6 h. Co-incubation of post-vitellogenic ovaries completely prevents the loss of message from these CA. This protection is independent of the presence of the brain, with or without neural connections. The ovarian signal is also developmentally regulated as ovaries from day 4 females do not prevent disappearance of mRNA from CA of day 7 females. The signal of the ovary does not appear to be 20-hydroxyecdysone as this compound has dramatic effects on JH synthesis but does not lead to an increase in CYP4C7 mRNA levels.

Levels of CYP4C7 mRNA are apparently not under the influence of neural regulation. Denervation of CA in mated insects had no significant effect on levels of CYP4C7 mRNA compared to control insects suggesting that the nervous connections are not required for the normal profile of mRNA levels in mated insects. After denervation, virgins had the same pattern of CYP4C7 mRNA as mated females. It has been well documented that virgin CA undergo a normal cycle of JH synthesis when denervated on day 0 (Stay and Tobe, 1977). The pattern of expression in denervated virgins suggests CYP4C7 is under similar neural constraint. However, this constraint is overridden by maturing follicles as indicated by the increased expression of CYP4C7 in post-vitellogenic, hydroprene-treated virgins.

4.2. CYP4C7 expression in ovariectomized insects

CYP4C7 gene expression is apparent in females after the removal of the ovaries. This occurs only in mated females, with ovariectomised virgins showing continued low levels of CYP4C7 expression. This implies that mating lifts a neural constraint of CYP4C7 expression. Although CA from ovariectomised mated females insects have greatly repressed JH synthesis they can be stimulated 20-fold by the inclusion of 40 \( \mu M \) farnesoic acid and two-fold by the inclusion of mevalonalactone in the incubation media (Table 1). Despite having the appearance of ‘low activity’ glands (Johnson et al., 1993) these glands have the cellular machinery required for much higher rates of synthesis. A similar result has been described in Blattella germanica where mevalonolactone stimulates ovariectomised females (Maestro et al., 1994).

The implantation of a vitellogenic ovary suppresses the expression of CYP4C7 in ovariectomized insects, and CYP4C7 expression declines in CA from postvitellog-
of sesquiterpenoid precursors of JH III and of JH III itself appears to be one of the plausible functions of CYP4C7 in the CA. The decrease in JH titer that occurs on day 6/7, must occur rapidly to allow ovulation and prevent abortion (Fig. 8), (Sutherland et al., 1998). Inhibition of the biosynthetic enzymes through allatostatin (Sutherland and Feyereisen, 1996) or other mechanisms is apparently not sufficient to prevent the release of residual JH-active sesquiterpenoids from the CA at that time. Indeed, the glands have high steady-state levels of methyl farnesoate and other precursors at the peak of JH synthesis, and these precursors have a low turnover or clearance. Methyl farnesoate for instance is present on day 5 at levels several hundred-fold higher than its critical micelle concentration (about 10 μM, Hammock and Mumby, 1978), and is therefore likely to be present in CA membranes in high concentration (Feyereisen et al., 1981b). This precursor, if slowly released from the CA at inopportune times could easily be epoxidized to JH III by fat body P450 enzymes, as was shown for CYP6A1 in the house fly (Andersen et al., 1997) or CYP9E1 in D. punctata (our unpublished results). The membrane-bound CYP4C7 in the CA is thus ideally suited to dislodge methyl farnesoate from the membranes by ω-hydroxylation, possibly as a first step to the formation of soluble, non JH-active dicarboxylic acids (Sutherland et al., 1998). We note that the hemolymph JH esterase which is inducible by JH, increases too late (day 7, basal oocytes >1.7 mm) to fulfill this important catabolic role (Rotin et al., 1982). Whether the products of CYP4C7 metabolism in postvitellogenic insects have, additionally, an intrinsic physiological function remains to be seen, but the expression of the CYP4C7 gene is now established as a faithful molecular response of the CA to the ovarian signal. It should therefore become a helpful tool in the identification of this signal.

5. Conclusion

The important physiological message from the ovary to the CA indicating the completion of vitellogenesis and the impending chorionation and ovulation is a humoral message (or series of messages) that leads to the decrease in JH synthesis, to the expression of allatostatin sensitivity and to the expression of the CYP4C7 gene. Suppression of JH synthesis itself (experimentally—hydroprene, TH27, 20-hydroxyecdysone) is not sufficient to induce CYP4C7 expression. Furthermore, expression of the CYP4C7 gene is suppressed by either a vitellogenic ovary (which also stimulates IH synthesis) or by tonic inhibition from the brain (in virgin females with quiescent CA). CYP4C7 is therefore a P450 enzyme under tight physiological control, as shown also by the short half-life of its transcript when removed from influences of the internal milieu. Omega hydroxylation

Acknowledgements

This work was supported by NIH grants DK34549 and ES 06694.

References


Feyereisen, R., Friedel, T., Tobe, S.S., 1981a. Farnesoid acid stimulation of C16 juvenile hormone biosynthesis by corpora allata of enic insects implanted into young mated females. This implies that the young ovary has a dual role: stimulating JH synthesis and repressing CYP4C7 expression; these roles are reversed in the post-vitellogenic ovary.

4.3. CYP4C7 induction and allatostatin sensitivity—are they controlled by the same mechanism?

In mated females, levels of CYP4C7 mRNA are related to the biosynthetic activity of the CA. A more detailed study of glands from five- to seven-day-old insects showed that the gene is induced when the growing follicles exceed 1.5 mm in length (Fig. 3), which is correlated with preparation for choriogenesis and is remarkably similar to the timing of the acquisition of allatostatin sensitivity (Pratt et al., 1990). CA transplanted from animals with high levels of message to an animal with low levels of message, or vice versa, showed levels of CYP4C7 mRNA more closely associated with those of the recipient than of the donor, suggesting control by a humoral factor. Likewise, it is the interaction of the CA with its endocrine milieu which is responsible for the acquisition of allatostatin sensitivity (Unnithan and Feyereisen, 1995). The similarity between the two events is further extended to the finding that denervation per se does not lead to, or prevent, either CYP4C7 gene expression or the acquisition of allatostatin sensitivity (Unnithan and Feyereisen, 1995). However, when ovariectomy is performed during early vitellogenesis the CA do not acquire allatostatin sensitivity (Unnithan and Feyereisen, 1995) whereas CYP4C7 is induced. Therefore, as the two events can be separated by physiological manipulation it is unlikely that they are the result of a cascade of responses from one regulatory mechanism but rather are parallel results of different primary responses.


Hammock, B.D., Mumby, S.M., 1978. Inhibition of epoxidation of methyl farnesoate to juvenile hormone III by cockroach corpus allatum homogenates. Pesticide Biochemistry and Physiology 9, 39–47.


