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20-HYDROXYECDYSONE SUPPRESSES YOLK PRODUCTION IN THE INDIANMEAL MOTH

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ABSTRACT As in other moths that have short-lived adult stages, egg maturation takes place during pharate adult development in the Indianmeal moth, Plodia interpunctella (Hübner). Treatment of pharate adult females with 20-hydroxyecdysone blocked vitellogenesis. Inclusion of varying concentrations of 20-hydroxyecdysone in the culture medium of ovarioles showed that maximal synthesis of yolk polypeptide-2 occurred at 10^{-8} M. Concentrations of 20-hydroxyecdysone higher than 10^{-8} M suppressed yolk polypeptide-2 synthesis. Total RNA was isolated from ovaries of pharate females that had been treated with 20-hydroxyecdysone, and the RNA was translated in a cell-free reticulocyte lysate. Cleaveland peptide mapping of the pre-yolk polypeptide-2 showed the primary translate to be similar structurally to the mature secreted form of yolk polypeptide-2. Quantitation of the relative amounts of translation products showed that 20-hydroxyecdysone suppressed the accumulation of yolk polypeptide-2 mRNAs in the ovaries to nearly 35% of normal. These data suggest that 20-hydroxyecdysone exerts a negative control over vitellogenesis and on the rate of yolk polypeptide synthesis.

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INTRODUCTION

Adults of the Indianmeal moth, Plodia interpunctella (Hübner), are non-feeding and consequently live for only a few days. To achieve sexual competency rapidly as an adult, the majority of vitellogenesis and egg maturation is completed during pharate adult development, and the females emerge with oocytes that are ready for fertilization by the time of mating. The mature eggs of P. interpunctella were found to contain two major yolk proteins that were each comprised of two unique subunits (1,2). The four major yolk polypeptide subunits (YPs) were designated YP1 (153 kDa), YP2 (69 kDa), YP3 (43 kDa), and YP4 (33 kDa) (1). Vitellogenin was comprised of two subunits, YP1 and YP3, which were synthesized by the fat body. Purified vitellin had an apparent molecular mass of 462 kDa. The yolk also contained a major protein with an apparent molecular mass of between 133 kDa and 260 kDa that was synthesized within the ovarioles and consisted of YP2 and YP4 as subunits. The vitellin was found to contribute about 40% of the protein to the yolk as did the YP2/YP4 protein.

Endocrine control of vitellogenesis in most insects has been shown to rely on the stimulation of yolk production by ecdysteroids, juvenile hormone, or a combination of these and additional hormones during the adult stage (3,4). However, inclusion of egg maturation within the format of metamorphosis rather than after the emergence of the adult should place the physiological control(s) of vitellogenesis in the Indianmeal moth under the influence of the regulatory mechanism(s) that coordinate the generation of the various adult organ systems. The rate of metamorphosis (5) and the events of eclosion (6,7) appear to be regulated by declining levels of ecdysteroids during pharate adult development in the moth, Manduca sexta (L.). Inhibition of metamorphic processes by ecdysteroids have been observed both in vitro and in vivo in several other insects as well. Cultured imaginal wing discs of P. interpunctella began the synthesis of cuticle that contained chitin only after the discs had been exposed to a 24-h pulse of 20-hydroxyecdysone (20HE) (8). However, the cultured discs would not synthesize chitin if maintained continuously in medium that contained 20HE. Similarly, continuous exposure of organ cultures of pupal cuticle (9) larval crochets (10) to

20HE prevented tanning of the epidermal structures. In addition, imaginal discs from Drosophila cultured in the presence of 20HE produced epicuticle and began deposition of procuticle, an indication of advancing metamorphosis, only when the levels of 20HE were reduced (11).

As seen in M. sexta, injection of 2-day-old pharate adult female Bombyx mori (L.) with a large single dose of 20HE (100 µg) delayed adult eclosion by as much as 8 days (12). The hormonal treatment had the additional effect of initially delaying the normal increase in ovarian weight, but when the treated females emerged as adults they deposited heavier and larger than normal eggs. A similar phenomenon appears to be operating on the control of vitellogenesis in P. interpunctella since 20HE was found to be exerting an inhibitory control on the processes of egg maturation in pharate adults (13). Egg production was inhibited in a dose responsive manner by injecting previtellogenic females twice daily throughout the final days of adult development. The females treated with 250 ng 20HE per dose had no oocytes that were at least 50% vitellogenic, whereas, females injected with saline contained an average of nine 50% vitellogenic oocytes per ovariole. Further, the total amount of protein or the total amount of radiolabeled proteins accumulating in the ovarioles was decreased to approximately 20% of normal when the moths were injected with a dose of 250 ng 20HE per treatment. The suppression of vitellogenesis did not appear to be the result of a general steroid effect since injection of 22-isoecdysone, an inactive ecdysteroid analogue (14), had no effect on the accumulation of protein in the oocytes. Although data showed that 20HE had a general inhibitory effect on vitellogenesis, the specific action of 20HE on the control of egg production was not determined. The data presented here demonstrate that 20HE had specific inhibitory actions on the synthesis of the YPs in the ovarioles.

RESULTS

20HE Controls the Synthesis of YP2 in Cultured Ovarioles

To examine the specific response of an organ to 20HE, ovarioles from early vitellogenic females were cultured in vitro in the presence of varying concentrations of 20HE, and the rate of YP2 synthesis was measured. An autoradiogram showed that after 24 h of culture the rate of YP2

synthesis was greatest in 10^{-8} M 20HE (Fig. 1) and represented nearly 19% of the total proteins secreted into the medium (Table 1). The rate of YP2 synthesis was found to be similar to the rate observed for ovarioles that were incubated for only 2 h after dissection from the females (data not shown). If the concentration of 20HE were less than 10^{-8} M, the synthesis of YP2 represented only 4-7.5% of the total secreted protein. That the synthesis of YP2 occurred in ovarioles cultured without 20HE showed that the tissues had achieved a developmental state where the expression of YP2 was open. However, the tissues required the presence of 20HE to support normal levels of YP2 synthesis. When the concentration of 20HE was raised above 10^{-8} M, a progressively decreasing rate of YP2 synthesis was observed. These data suggested that the rate of YP synthesis was dependent on the presence of 20HE and was related to the concentration of the hormone. Preliminary estimations of the concentration of 20HE in P. interpunctella support this hypothesis since the levels range from 10^{-6} to 10^{-5} M at the time of pupation to 10^{-8} at adult eclosion (Brookes, unpublished).

20HE Suppresses the Accumulation of Translatable YP2 Transcripts in Ovaries

To determine what effect 20HE had on the synthesis of YP2, total RNA from ovaries of females that had been treated with 20HE was translated in a cell-free reticulocyte lysate. Poly(A) RNA isolated from ovaries contained a transcript that translated a product that was immunoprecipitable with antiserum to yolk proteins, had a molecular mass of 70 kDa (data not shown), and was considered to be the primary translate for YP2 (preYP2). PreYP2 and YP2 secreted from ovarioles (previously shown to be identical with YP2 accumulating in the oocytes (1)) were subjected to peptide mapping to determine their structural relatedness. The autoradiogram showed that the digestion patterns for the two polypeptides were nearly identical when treated with either V8 protease or α-chymotrypsin (Fig. 2). The digestion fragments for α-chymotrypsin showed the presence of a single fragment that was of slightly smaller size in the digests of YP2 than in the preYP2 digests. This suggests there may be a posttranslational cleavage of a signal peptide from the preYP2. A size shifting of some of the fragments of higher molecular mass

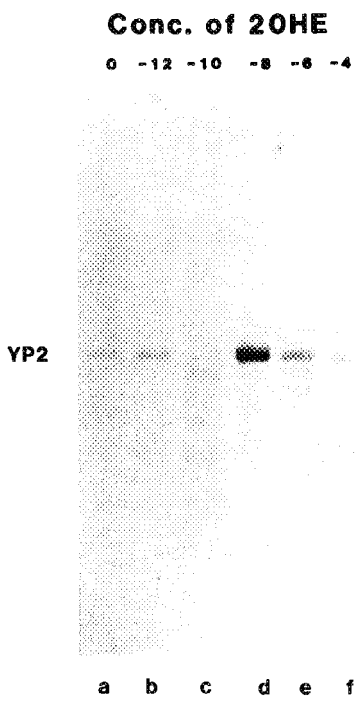

FIGURE 1. Dependence of the rate of YP2 synthesis by cultured ovarioles on the concentration of 20HE. Ovarioles from day-5 pharate adult females were placed in Grace's insect medium with varying concentrations of 20HE. After 22 h, the media were removed and replaced with media that contained 0.5 μCi/μl ^{35}S-methionine for an additional 2-h incubation. Equal amounts of TCA precipitable radiolabeled proteins were loaded on each lane of an 8-15% gradient SDS-PAGE, and the dried gel was autoradiographed. Lanes: a) no 20HE; b) 10^{-12} M 20HE; c) 10^{-10} M 20HE; d) 10^{-8} M 20HE; e) 10^{-6} M 20HE; f) 10^{-4} M 20HE.

also was seen (c.f. Fig. 2E & F), which may be accounted for by further posttranslational modifications. The autoradiogram of the translation products showed that treatment with 20HE decreased the level of YP2 transcripts specifically (Fig. 3). The amount of preYP2 produced by ovarian RNA from females treated with 250 ng 20HE

TABLE 1
20-HYDROXYECDYSONE SUPPRESSES SYNTHESIS OF YP2 BY OVARIOLES CULTURED IN VITRO AND THE ACCUMULATION OF TRANSLATABLE YP2 TRANSCRIPT IN OVARIES.[a]

20HE conc. (molar)	Synthesis of YP2 as % total secreted protein (SD)	20HE Treatment	Accumulation of YP2 transcript shown as % total translation products (SD)
0	7.3 (2.3)	Control	7.1 (1.2)
10^{-12}	5.0 (1.6)	Saline	5.5 (0.4)
10^{-10}	4.3 (1.6)	10 ng	5.0 (1.0)
10^{-8}	18.9 (4.9)	50 ng	3.4 (0.2)
10^{-6}	10.8 (4.8)	250 ng	2.5 (1.6)
10^{-4}	8.7 (2.1)		

[a]The amount of radiolabeled proteins and translation products was quantified by integrating the band areas on an autoradiogram with an LKB 2222 laser microdensitometer (LKB Instruments).

represented only 2.5% of the total radiolabeled translation products whereas in normal nontreated females preYP2 was 7.1% of the total translation products (Table 1). The 20HE treatment resulted in a decrease of YP2 mRNA to 35% of the normal females. The amount of transcript for YP4 as well as several polypeptides appears to be decreased by 20HE treatment, but the changes were not quantified here. However, the 20HE treatment generally did not affect the relative percent contribution of all proteins since the percent of total translation products for a 43 kDa protein and a 96 kDa protein were found not to change with the increasing hormonal treatment.

DISCUSSION

Vitellogenesis in P. interpunctella appears to be regulated by mechanisms similar to those controlling the maturation of other organ systems in pharate adults. As observed in M. sexta (5), 20HE or some related ecdysteroid exerts an inhibitory effect on the progress of metamorphosis, and only when the levels of ecdysteroids

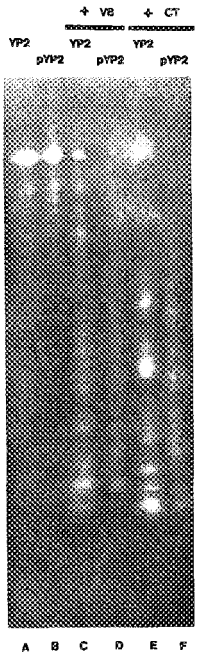

FIGURE 2. Peptide maps of the primary translation product for YP2 (preYP2) and YP2 secreted into culture medium. The immunoprecipitated radiolabeled polypeptides were treated essentially as described by Cleaveland et al. (15) with either Staphylococcus aureus V8 protease (V8) or α-chymotrypsin (CT). The digestion fragments were resolved on an 8-15% gradient SDS-PAGE, and the dried gel was autoradiographed. The presence of fragments of smaller molecular mass than YP2 or preYP2 in lanes A and B were the result of protease contamination. Lanes: A) YP2; B) preYP2; C) YP2 plus V8 protease; D) preYP2 plus V8 protease; E) YP2 plus α-chymotrypsin; F) preYP2 plus α-chymotrypsin.

decrease does organ maturation take place. The effects of 20HE on the synthesis of YP2 were shown to be specific

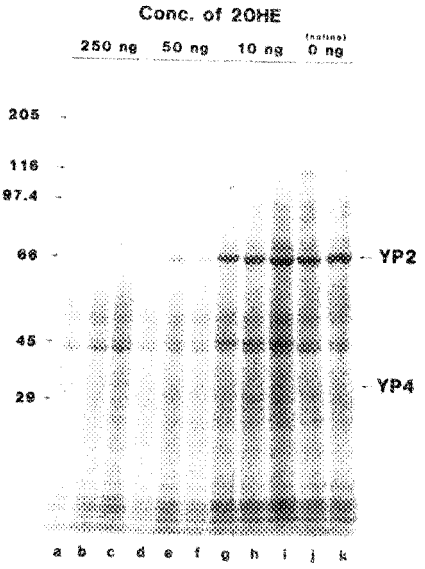

FIGURE 3. Translation products of total RNA isolated from ovaries of pharate adult females treated with 20HE. Previtellogenic females were injected with 20HE twice daily for 4 days. RNA was extracted from the ovaries with LiCl/urea (16,17) and equal A_{260} units were translated. Equal quantities of TCA precipitable radiolabeled proteins were loaded on each lane of an 8-15% gradient SDS-PAGE, and the dried gel was autoradiographed. Females were treated with 20HE at a dose of 250 ng, lanes a-c; 50 ng, lanes d-f; 10 ng, lanes g-i; and lanes j and k saline injected.

to the control of the accumulation of YP mRNA in the ovarioles. Unlike the observations on 20HE, regulation of chitin synthesis in cultured wing discs or epidermis (8-11), the synthesis of YP2 was shown to be dependent on continuous exposure of the ovarioles to 20HE and at specific concentrations. Inhibition of gene activity has been observed for other steroid hormone regulated proteins. Glucocorticoids were found to inhibit transcription of the pro-opiomelanocortin gene as measured by nuclear run-on transcription assays of nuclei isolated from hormonally treated primary cultures of rat anterior pituitary cells

(18). Similarly, the transcription of the 74 kDa serum albumin gene in Xenopus laevis was shown to be inhibited by estradiol treatment of male hepatocytes (19). However, the estradiol was found also to cause a three-fold destabilization of the albumin RNA. The combined effect of the inhibition of transcription and RNA destabilization accounted for the changes in the steady-state levels of the albumin mRNA. The nature of the effect of 20HE on the accumulation of YP mRNAs is not known. We are developing cloned copies of the genes for the YPs in order to determine the molecular action of the hormone on these genes.

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